Fruit and vegetable processing

Improving quality

Edited by
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Introduction

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Fruit and vegetables are both major food products in their own right and key ingredients in many processed foods. Consumers increasingly require food products that preserve their nutritional value, retain a natural and fresh colour, flavour and texture, and contain fewer additives such as preservatives. These requirements pose new challenges for fruit and vegetable producers and processors. There has been a wealth of recent research both on the importance of fruit and vegetable consumption to health and on new techniques to preserve the nutritional and sensory qualities demanded by consumers. This book reviews these developments.

Eating fruits and vegetables has long been associated with health benefits, though some of the ways in which these foods enhance health have only become clear in recent decades. Part 1 looks at this recent research. Chapter 2 considers the epidemiological evidence linking increased fruit and vegetable consumption with health benefits, the constituents of these foods which may be responsible for these benefits and the factors influencing their modes of action and efficacy. As well as being rich in micronutrients, plant foods also contain an immense variety of biologically-active, non-nutritive secondary metabolites known as phytochemicals. Chapter 3 discusses one of the most important groups of phytochemicals, antioxidants, which are thought to play an important role in the body's defence against cardiovascular disease, certain (epithelial) cancers, visual impairments, arthritis and asthma. Against the background of these two chapters, Chapter 4 looks at the impact of processing on both key nutrients and antioxidants, taking tomato as a case study to demonstrate how the nutritional quality of fruits and vegetables may be preserved and even enhanced during processing.

Fruit and vegetable production and processing involves a complex supply chain from the farm to the point of consumption. One of the central themes of

recent research has been the importance of strengthening each link in the chain and improving the integration of the supply chain as a whole if consistent and high fruit and vegetable quality is to be maintained. Part 2 considers how safety and quality can be better managed in the supply chain. Chapter 5 looks at the increasing use of mathematical modelling techniques to better understand and control cultivation, again using tomato as a case study. Such techniques help to make more efficient use of resources with both economic and environmental benefits valued by the consumer, and are increasingly being applied to improving sensory and nutritional quality. Chapter 6 describes how the Hazard Analysis and Critical Control Point (HACCP) system, originally developed for the food processing sector, is being applied on the farm to cultivate safer fresh produce free of contamination from pathogens or other contaminants such as pesticides.

Once harvested, fruits and vegetables must be handled carefully if they are not to deteriorate before they reach consumers as fresh retail products or manufacturers for further processing. This critical stage in the supply chain is reviewed in Chapter 7 which defines quality criteria in freshly-harvested produce, describes the principal causes of quality deterioration and the main storage and packaging techniques used to maintain quality. At each stage in the supply chain there is a need for effective measurement of product quality. Chapters 8 and 9 describe some of the advanced instrumental techniques that are now being developed to measure quality and spot defects so that they can be remedied quickly. The development of rapid, non-destructive on-line instrumentation is a critical weapon in maintaining quality at all stages in the supply chain. The final two chapters in Part 2 look at the processing stage in the supply chain, discussing how to better understand and control the thermal processing of fruits and vegetables, and ensure the safety of cooked chilled foods containing vegetables.

Against the background of Part 2, the final part of the book considers the range of new techniques that are being developed to improve quality at the various stages of the supply chain. The first two chapters consider ways of improving quality during cultivation and immediately after harvesting, discussing ways of improving the natural resistance of fruit and the genetic modification of plants to improve shelf-life. The following three chapters build on the overview provided by Chapter 7 in describing techniques for maintaining the postharvest quality of fresh fruit and vegetables. Chapter 14 looks at minimal processing methods whilst the following two chapters consider developments in modified atmosphere packaging (MAP) and the development of edible coatings. The final two chapters then consider two new technologies in processing fruit and vegetables: high pressure processing and vacuum technology.

Part 1

Fruit, vegetables and health

2

Health benefits of increased fruit and vegetable consumption

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2.1 Introduction

Although life expectancy of the 'average' European citizen has increased since 1990, many populations are living with higher levels of chronic disease and disability, and governments have to cope with spiralling social and health care costs. There is evidence that diets rich in vegetables and fruits can decrease this burden of chronic disease. This chapter presents the strength and consistency of evidence for the health benefits of diets rich in fruits and vegetables and introduces, briefly, the putative contribution of the microconstituents of these foods to their beneficial properties. The emphasis on the microcomponents of fruits and vegetables in no way implies that the macronutrients lack importance with regard to human health and well-being. It reflects the keen interest that currently exists in possible relationships between the content and profile of the minor constituents in food plants and the prevention of chronic disease. The important issue of the health significance of whole foods, as compared to isolated components of those foods, is debated, as is the need to define the extent of (i) release of biologically active compounds from the complex food plant matrix (bioaccessibility) and (ii) absorption, metabolism and tissue dispersion (bioavailability). The concepts of bioaccessibility and bioavailability are extremely important, since the types and quantities of biologically active microcomponents contained in fruits and vegetables may have very little health impact unless they are effectively delivered to target sites within the human body. Finally, some initiatives to increase fruit and vegetable intake and suggested future trends in research on the public health significance of fruits and vegetables are presented.

2.2 Evidence of benefit

Of the 2.8 million deaths each year in the European Union (EU) countries, 1.9 million are from potentially mutable chronic diseases (for example, 767 000 from cancers; 1 111 000 cardiovascular and cerebrovascular disease; and 52 000 diabetes mellitus). Of the current total EU population of about 375 million, 78 million are living with the disability of chronic disease. In addition to cancers, cardiovascular disease (CVD), stroke and diabetes, disability is also associated with osteoporosis, digestive disorders, cataract, age-related macular degeneration and dementia, to name but a few of the debilitating conditions to which humans are prone. Thus, although life expectancy of the 'average' EU citizen has increased by about 2 years since 1990, in many regions Europeans are living with more disability and governments are having to cope with increasing social and health care costs.[1]

There is consistent evidence, primarily from epidemiology, that diets high in vegetables and fruits can decrease this burden of chronic disease, with the evidence for reduced risk of many cancers being particularly strong. The evidence that such diets decrease the risk of mouth and pharyngeal, oesophageal, lung, stomach and colon cancers is convincing. They probably also protect against laryngeal, pancreatic, breast and bladder cancers, and possibly protect against ovarian, cervical, endometrial, thyroid, primary liver, prostate and renal cancers. The choice of the terms 'convincing', 'probable' and 'possible' reflects the present strength of evidence for a particular relationship (Table 2.1).[2]

At least 37 cohort, 196 case-controlled and 14 ecological studies have investigated the relationship between vegetable and fruit consumption and the risk of cancer. Overall, when studies of all cancer sites are taken together, 78% have shown a significant decrease in risk for higher intake of at least one vegetable and/or fruit category examined. The general picture is not altered when allowance is made for the fact that some apparently significant protective associations may be due to chance alone and that some studies have reported non-significant protective trends. It is recognised that measurement of food intake is a problem (especially fruit and vegetable intake where there is a tendency towards overestimation of self-reported intakes) and that other lifestyle factors are known, or have the potential, to confound diet–health relationships. Nevertheless, the strength of evidence for the relationship between fruits and vegetables and reduced cancer risk, provided from over 200 epidemiological studies conducted in diverse populations, is impressive. The literature on vegetables, fruits and the prevention of cancer has been reviewed extensively.[3–6]

Current scientific evidence also suggests a protective role for fruits and vegetables in prevention of cardiovascular disease and evidence is accumulating for a protective role in stroke. Fruit and vegetable consumption has been linked to reduced cardiovascular disease and stroke. In addition, a new scientific base is emerging to support a protective role for fruits and vegetables in prevention of cataract formation, age-related macular degeneration, chronic obstructive pulmonary disease, diverticulosis and other digestive disorders, and possibly hypertension.

Table 2.1 Analysis of the level of evidence of protection provided by studies on fruit and vegetables and cancers

Cancer sites	CNERNA (France 1996)	World Cancer Research Fund (USA 1997)	COMA Food and Nutrition Policy (UK 1998)
Mouth and pharynx	consistent	convincing	fruit: weakly consistent vegetables: inconsistent
Larynx	consistent	probable	moderately consistent
Oesophagus	consistent	convincing	strongly consistent
Lung and respiratory tract	consistent	convincing	fruit: moderately consistent vegetables: weakly consistent
Stomach	consistent	convincing	moderately consistent
Colon-rectum	vegetables: moderately consistent	vegetables: convincing	vegetables: moderately to weakly consistent
Pancreas	consistent	probable	consistent but limited
Liver	ND	vegetables: possible	ND
Breast	inconsistent	green vegetables: probable	green/yellow vegetables: moderately consistent
Ovary	inconsistent	possible	inconsistent
Endometrium	inconsistent	insufficient	inconsistent
Cervix	ND	possible	consistent but limited
Prostate	inconsistent	vegetables: possible	vegetables: moderately consistent but limited
Kidney	ND	vegetables: possible	ND
Bladder	ND	probable	moderately consistent but limited
Thyroid	ND	possible	ND

ND = not determined. Reproduced with permission from '*The Antioxidants in Tomatoes and Tomato Products and their Health Benefits*', ed AMITOM. EU Concerted Action FAIR CT 97-3233.[2]

Estimates of the proportion of cancer cases and cardiovascular deaths that could be prevented by increasing fruit and vegetable consumption, particularly in northern Europe, are 7–28% for cancers (depending on the site), 20–40% for coronary heart disease and 0–25% for stroke, with the risk of ischaemic heart disease being about 15% lower at the 90th compared to the 10th centile of fruit and vegetable consumption.[7]

Of 100 expert reports published between 1961 and 1991, 66 recommended higher consumption of fruits and vegetables, with none disagreeing. In fact, increasing fruit and vegetable consumption is regarded as the second most

important strategy for cancer prevention after reducing smoking. In 1990, The World Health Organisation recommended a goal of at least 400 g of vegetables and fruits daily (in addition to potatoes) including, within that, at least 30 g of legumes, nuts and seeds.[8] This report, together with other reports from expert bodies, has been translated into a recommendation for the consumption of at least five portions of fruits and vegetables per day. The World Cancer Research Fund and American Institute for Cancer Research go a little further by recommending that diets should be based primarily on foods of plant origin, provided that such diets are nutritionally adequate and varied.[9] This recommendation is similar to, but broader than, those of other expert reports concerned with the prevention of cancer or other chronic diseases.

Available evidence provides support for the health benefits of a wide variety of fruits and vegetables, however specific claims are most prolific for many of the coloured-fleshed fruits and vegetables, in particular, dark-green leafy, cruciferous and deep-yellow-orange vegetables, and a wide variety of fruits, in particular, citrus and deep-yellow-orange-red fruits. Such foods are particularly rich in vitamin C, pro- and nonpro-vitamin A carotenoids, folates and a range of bioactive (so-called) phytonutrients. However, despite the passing of three decades since the emergence of epidemiological evidence of a strong link between diet and health, diet–health associations, including those relating to fruit and vegetable consumption, remain elusive. Whilst the experimental evidence available largely supports epidemiological observation, the experimental science is still very much in its infancy, especially in the 'whole-food' area. The individual components of fruits and vegetables have attracted far more research attention than their food sources, although the mixture and balance of the micro- and macro-constituents of these foods is far more likely to be responsible for their health benefits than any single compound.

The constituents of fruit and vegetables and their purported role in health promotion and maintenance are outlined in the next section.

2.3 Fruits and vegetables: their constituents and modes of action

A considerable amount of effort has been invested in identifying biologically active components within fruits and vegetables. Much of this work has related to the development of chemical analyses to quantify composition, and development of experimental models (animals and *in vitro* systems) to assess the functional consequences of supplementation with single compounds, or simple mixtures. The extent to which data produced from supplementation studies in animal or cell culture models can be extrapolated to humans consuming complex diets is not certain, but such studies have provided insight into putative mechanisms of health protection and promotion.

Thousands of biologically active phytochemicals have been identified in food plants. Of these food plant groups, fruits and vegetables are the most botanically

Table 2.2 Richest fruit and vegetable sources of specific compounds

Substance	Richest source
Vitamin C	Citrus (and other) fruits, green vegetables, potatoes
Vitamin E	Vegetable oils, avocado
Folates	Green leafy vegetables, potatoes, oranges
Vitamin K	Green leafy vegetables
Calcium, iron, magnesium	Green vegetables
Potassium	Bananas, vegetables and fruits generally
Fibre, NSP, pectin	Fruits and vegetables generally
Mono-unsaturated fatty acids	Olive oil
Alpha and beta-carotene	Carrots, green leafy vegetables, yellow/orange fleshed fruits
Beta-cryptoxanthin	Oranges and related fruits
Lutein	Yellow/green vegetables
Lycopene	Tomatoes
Flavonoids	Onions, apples, green beans
Flavanoids	Peach, strawberry
Anthocyanins	Red/purple berries
Glucosinolates	Brassicas
Alkenyl cysteine sulphoxides	Alliums
Glycoalkaloids	Potato, aubergine
Furanocoumarins	Parsnip, celery
Cyanogenic glycosides	Cassava, *Prunus* species, butter beans

NSP = non-starch polysaccharides

diverse, represented in the Western diet by more than 40 botanical families. Table 2.2 lists the richest fruit and vegetable sources of specific compounds. However, apart from one or two exceptions, these compounds are also present (in varying amounts) in most other fruits and vegetables.

There are several biologically plausible reasons why the consumption of fruits and vegetables might slow, or prevent, the onset of chronic diseases. They are a rich source of a variety of vitamins, minerals, dietary fibre and many other classes of bioactive compounds collectively called phytochemicals. Experimental dietary studies in animals, cell models and humans demonstrate the capacity of some of these constituents of fruits and vegetables to modify antioxidant pathways, detoxification enzymes, the immune system, cholesterol and steroid hormone concentrations, and blood pressure, and their capacity to act as antioxidant, antiviral and antibacterial agents.

There has been extensive focus on antioxidant effects, as oxidative damage to biomolecules has been hypothesised to be responsible for CVD, cancer initiation, cataract formation, inflammatory disease and several neurological disorders. Our

antioxidant defence system prevents the formation of damaging free radicals, removes radicals before damage can occur or repairs damage that has occurred. Several trace elements, such as manganese, copper, zinc, iron and selenium, are essential constituents of the antioxidant metalloenzymes: superoxide dismutase, glutathione peroxidases and catalase. Vitamins C and E and the carotenoids, which have received most attention with respect to their antioxidant capability, can interrupt free radical initiated chain reactions of oxidation, or scavenge free radicals before they damage cellular components. The antioxidant effects of several other groups of compounds, such as the flavonoids, have been studied mainly *in vitro*, but their metabolism is complex and effects *in vivo* may be different in type and extent from those observed in *in vitro* model systems. Some of the same factors that contribute to oxidative damage can also lead to the production of reactive, potentially carcinogenic, nitrogen species. Vitamins C and E, and polyphenols, have been shown to inhibit *N*-nitroso compound formation by destroying nitrosating agents.

Compounds in fruits and vegetables have been shown to attenuate the formation of carcinogens from non-toxic precarcinogens *in vitro*, by affecting their metabolism by the phase I enzymes (such as cytochrome P450 (CYP)-dependent monooxygenases) which catalyse oxidation, hydroxylation and reduction reactions, and/or by the induction of phase II biotransformation enzymes (such as UDP-glucuronosyltransferases, sulphotransferases and glutathione transferases) that accelerate the detoxification of the active carcinogenic metabolite. Studies *in vivo* are hampered by lack of knowledge of the normal range of expression or activity of these enzymes in human populations, the influence of other environmental factors and the influence of genetic polymorphism on phenotype.

It is also known that many of the constituents of fruits and vegetables have the ability to influence the immune system, which in turn is known to be intimately involved in both the prevention and promotion of chronic disease. Enhanced immune and inflammatory responses are central to our ability to deal with unwanted and potentially dangerous foreign particles such as bacteria and play a major role in tumour surveillance and cancer prevention. However, abnormal activation of the immune system has the potential to promote debilitating disorders such as gout and rheumatoid arthritis, and suppression of pro-atherogenic inflammatory responses have been suggested as one mechanism for the association between fish oil consumption (and specific n-3 fatty acids) and reduced CVD. Dietary strategies need to optimise rather than maximise immune reactivity and this will depend very much on individual susceptibility.

Several vitamins are associated with improved delayed-type hypersensitivity skin responses; some nutrients and phytochemicals modulate the activity of natural killer cells (NKC, a component of the antitumour host defences); vitamins C and E supplementation has been shown transiently to increase cytokine production (which assists in T cell and NKC activation); and beta-carotene enhances the expression of functionally associated molecules on human monocytes. The complexities of the immune system and its interaction with nutrients have been reviewed comprehensively.[10,11]

Garlic and garlic extracts appear to reduce risk factors for cardiovascular disease by decreasing platelet aggregation and reducing cholesterol and triacylglycerol concentrations in a variety of conditions. Specific dietary fibres from fruit and vegetable sources also show hypocholesterolaemic effects. Results from studies with other foods and beverages (for example, carrots and spinach, red wine and the polyphenols it contains) are less clear-cut in terms of influence on platelet function and cholesterol metabolism. However, it has been shown that replacing animal products with vegetable products in the diet can reduce blood pressure in both normotensive and hypertensive volunteers. Trials using components isolated from fruits and vegetables have reported inconsistent results.

The ability of fruits and vegetables and their constituents to stave off or relieve the symptoms of bacterial and viral infection tends to rely on anecdote rather than science. However, studies reported in the literature in the 1990s indicate that perhaps some credence should be given to the folklore. There is evidence from a double-blind, randomised, placebo controlled trial that cranberry juice positively influences the microflora of the urinary tract and that its use as a treatment for urinary tract infection may be well founded. Garlic too has a long history of use, as an antibiotic, antiviral and antifungal agent, which appears to be borne out by results obtained in a number of *in vitro* studies; however, verification of this activity *in vivo* is required. The health effects of vegetables and fruits and possible mechanisms of action in humans have been reviewed.[12]

There is a large literature on the effects of specific compounds in model animal and cell systems, relatively less in humans and a much smaller literature on the effects of fruit and vegetable interventions. Table 2.3 provides examples of some general and specific fruit and vegetable intervention studies and their outcome. A point to note is that the 'doses' used in most studies, particularly those using a single food item, are beyond what could be introduced reasonably into the day-to-day diet without distorting that diet in terms of the variety of fruits and vegetables, or other foods, consumed.

2.4 Health benefits of whole foods over isolated components

There are many claims made in the media and promotional literature about the qualities and benefits of specific (or groups of) compounds found in fruits and vegetables. We are told that wrinkles, absentmindedness, cancer and clogged arteries (among many other disorders) can be prevented, or alleviated, by consuming these compounds in the form of isolates or concentrated extracts. In such claims the words 'tested', 'effective', 'safe', 'essential' and 'proven' are freely used. In the world of nutritional science, however, the picture is not so clear. The following two quotes provide an example of this apparent contradiction. The first relates to a study of antioxidant vitamins and risk factors for cardiovascular disease, 'These results back-up the findings of previous studies and point to a positive role for antioxidant supplementation among those suffering from coronary artery disease'.[25] The second statement is again related to antioxidants and chronic

Table 2.3 Selected general and specific fruit and vegetable (F&V) interventions and their outcome

Food type	Study period	Outcome	Reference
F&V providing 325 mg vitamin C	1 meal	Urinary *N*-nitrosoproline reduced	13
F&V (1170 g)	5 weeks	Serum total cholesterol reduced by 4%	14
F&V mean number of servings, 8.5 and 3.6	8 weeks	Systolic and diastolic pressures lowered with 8.5 servings, particularly in hypertensives	15
F&V	2 weeks	Oxidation resistance of low-density lipoprotein increased	16
Raw apple (350–400 g)	1 month	Plasma cholesterol reduced by >10%	17
Prunes (100 g)	4 weeks	Low density lipoprotein (LDL) cholesterol decreased in mildly hypercholesterolaemic males	18
Guava (500–1000 g)	4 weeks	Serum total cholesterol, triglycerides and systolic and diastolic pressures all decreased	19
Raw carrots (200 g)	3 weeks	Serum cholesterol reduced	20
Carrots providing 15 g fibre	3 weeks	No effect on serum cholesterol	21
Brussels sprouts (300 g)	3 weeks	Detoxification enzyme activity increased	22
Broccoli (500 g)	10 days	Detoxification enzyme activity increased	23
Cranberry juice (300 ml)	6 months	Bacteriuria and pyruria decreased	24

disease, 'Current evidence is not strong enough to recommend antioxidant vitamin pills'.[26] These quotes, apart from highlighting apparently contradictory views of scientists on a very similar point, also serve as an example of the fact that, whilst the public health significance of fruits and vegetables has arisen largely from observations of people eating traditional diets rich in these foods, research and information are dominated by the potential benefits of isolated compounds.

Recent surveys in Europe indicate that far more people are concerned about their food and their health than in the past. However, while consumers say they want to eat in a healthier manner, the reality is that they want to eat more easily, hence the claim of an enormous market potential for dietary supplements, nutrient enriched and functional foods, each of which contains perhaps one, or just a

few, of the hundreds of components present in a diet containing a variety of fruits and vegetables.[27] Compounds isolated from plant foods, or synthetic copies of compounds that can be found in these foods, are promoted and used for their putative medicinal or health promoting properties. The literature that accompanies their sale can be very convincing to those who want to stay healthy. For those with a diagnosed condition, these compounds can appear a more natural and safer alternative to drug therapy and certainly a much easier option than trying to change the dietary habits of a lifetime.

Epidemiological data reveals that diets rich in particular foods are associated with reduced risk of a chronic disorder. At this stage, however, the association between diet and health is merely an observation. This observation needs to lead to some reasonable hypotheses, possibly supported by earlier experimental evidence. These hypotheses then need to be tested in a wider range of experimental systems, often *in vitro* and/or animal and cell-line model systems, followed by smaller studies involving human volunteers, perhaps leading to very much larger trials. As part of this process a risk–benefit analysis of any dose is a vital consideration, as exemplified by β-carotene.

The predominant carotenoids in blood and tissues are β-carotene found in carrots, some orange coloured fruits and green vegetables, β-cryptoxanthin found in oranges; lycopene found in tomatoes and lutein found in yellow/green vegetables. These compounds have significant antioxidant activity, at least *in vitro*, and are therefore thought to be capable of protecting the cells and tissues of our body against the ravages of living in a world full of potentially toxic oxygen. Carotenoids also have a range of other biological activities. They modulate immune and inflammatory response and have long been known to influence cell–cell communication, which is a vital part of our ability to control the activity of individual cells within a tissue. *In vitro* and animal studies strongly support some carotenoids as natural anticancer agents and populations consuming higher amounts of carotenoid-rich foods have lower rates of CVD, cancer and other chronic diseases. There are convincing hypotheses about why this should be, but little is known about what dose provides optimum protection or how this may vary depending upon individual sensitivity.

Human trials were undertaken. Volunteers were given relatively high dose supplements of β-carotene for several years, which substantially raised plasma and, presumably, tissue concentrations. These studies showed one of two things, either supplementation with β-carotene was not effective with regard to CVD, cancer or all-cause mortality or, in susceptible individuals like smokers and asbestos workers, the mortality rate from lung cancer was significantly increased. On the other hand, plasma β-carotene concentration (reflecting the consumption of carotenoid-rich foods) before supplementation was inversely and significantly associated with lower cancer rate.

As with the carotenoids, epidemiology has implicated vitamin E as protective, particularly with respect to cardiovascular disease. Human intervention studies, involving high dose supplementation in ‘at-risk’ individuals, however, have not consistently demonstrated a role for vitamin E but perhaps too much is expected.

Research has concentrated on the potential for single food components to reverse existing disease, whilst the primary role of these components (in the balance and amounts found in diets rich in fruits and vegetables) is, arguably, in the prevention or slowing of initiating events.

Evidence to date, albeit largely observational, remains heavily in favour of the protective effects of specific foods or food groups consumed as part of a traditional diet and the role of any one component of fruits and vegetables in isolation from all others remains to be established. As part of this process, demonstration that the component of interest is released from the (often) complex food matrix and is effectively delivered to its putative site of action within the human body, is obviously essential.

Reviews debating these issues, with appropriate reference to the scientific literature, have been published.[28,29]

2.5 Influence of cell structure on nutrient delivery

With the advent of present-day analytical techniques and instrumentation, it is possible to describe the complex chemical nature of our foods with ever more accuracy and sensitivity. However, the types and quantities of either the nutrient or non-nutrient components of fruits and vegetables may have very little bearing on their potential contribution to our nutrient or 'health' status. The reason for this is that only a proportion of these food components can be absorbed and utilised. This proportion may be highly variable depending upon the physiological state of the consumer, the food matrix, dietary mix, process history and storage. Determination of the extent of the release of bioactive compounds from different types and forms of fruits and vegetables during human digestion (recently defined as the bioaccessibility of the compound) and the extent to which that nutrient is absorbed and targeted to sites of action within body tissues (defined as the bioavailability of the compound) is essential knowledge for those involved in food production and nutritional assessment.[30]

The influence of plant food structure on the bioaccessibility and subsequent bioavailability of many of the potentially bioactive components of foods is an area that has been poorly researched, particularly with respect to the lipid soluble compounds, so that there is only a small diffuse literature. However, the bioaccessibility of lipophilic microconstituents of fruits and vegetables (especially carotenoids) was an area of focus in a European collaboration and the key issues examined in this project are outlined below.[30] The carotenoids have been chosen for special focus because they serve as an excellent example of where too little understanding of the complexity of their behaviour in foods and human tissues has confounded interpretation of their role in the putative health benefits of specific food plants.

There are two main mechanisms by which nutrients are released from the cell matrix of food plant tissue. First, if the plant cells are broken open, the digestive

enzymes have free access to the contents and it would be predicted that this would allow rapid and efficient digestion. Second, if the cells are not broken open the rate of digestion will be modulated by the permeability of the cell wall (pore size) that regulates the rate of penetration of the cell by digestive enzymes and the rate of diffusion of the products from the cell. Small mobile hydrophilic molecules, for example sugars, fatty acids, amino acids and mineral ions, will diffuse easily but the diffusion of larger hydrophilic molecules, for example complex phenolic compounds, may be severely impaired. For large hydrophobic molecules that need to be dissolved in a lipid structure for transport, for example the carotenoids, the situation is more complex, since the cell wall is unlikely to be permeable to lipid emulsions or micelles, and the presence of lipases will strip away the solvating lipid.

Plant cells are compartmentalised membrane-bound structures contained within a semi-rigid cell wall composed mainly of cellulose and pectic substances. The main features of the cell are the vacuole, cytoplasm, nucleus and a range of sub-cellular organelles. This compartmentalisation is an essential mechanism for separating the various biochemical and physical functions of the cellular processes. Disruption of this physical separation, as in bruising, leads to metabolic chaos, resulting in cell death and the production of undesirable colours (enzymic or non-enzymic browning) and flavours (lipid oxidation), and destruction (vitamin C) or production (isothiocyanates, cyanide) of bioactive compounds. Cellular compounds are not free to move about within the cell and are bound to specific structures (for example, lipoproteins, glycoproteins) or associated with particular domains (for example, carotenoids associated with lipid membranes). The carotenoids are very hydrophobic and are normally associated with the lipid structures of the sub-cellular organelles. In green leafy vegetables, the main carotenoids, lutein and β-carotene, are bound to lipoproteins in the light-harvesting complex of the chloroplasts (organelles responsible for photosynthesis). In the carrot and tomato, the carotenoids may be present as membrane bounded semi-crystalline structures or present in lipid droplets. In fruits, the carotenoids are more frequently present in oil droplets, although the solubility of carotenoids in oil is low. The different types of plant tissue (leaf, root, fruit, seed) and the environment and physical nature of the cellular carotenoids have implications for the ease with which they are made available for absorption through processing (thermal or physical), mastication and digestion.

To be absorbed the carotenoids need to be released from the constraints of the gross physical structure of the plant tissue and from the plant cells and transferred to the free lipid phase of the processed product or digesta. In general, carotenoids in plant structures are stable and they will survive quite aggressive processing and intense light exposure with a minimum of loss or isomerisation. However, once released from the structure, they are more prone to degradation by heat, light and atmospheric oxygen. There is, therefore, a trade-off between maximising release and retention during storage. It should be noted that aggressive processing may result in conversion of the native all-*trans* carotenoids to their

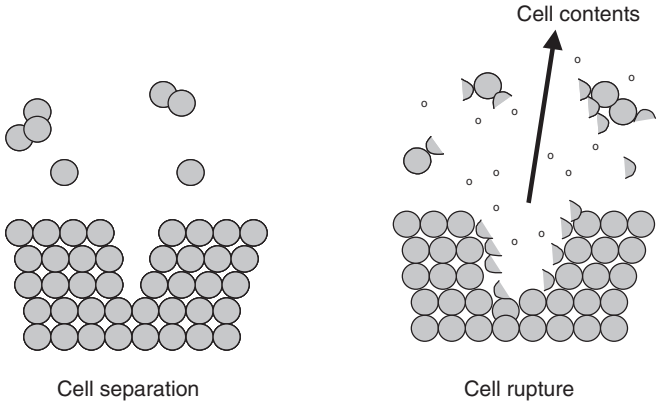

Fig. 2.1 Food processing promotes cell separation but cell rupture, which is associated with the greatest release of plant cell constituents, does not always occur.

cis-isomers and the production of highly reactive species that can continue to degrade the carotenoids after processing is complete.

As a general rule, cooking and processing sterilises and softens the plant tissue leading to cell separation, which is the primary mechanism of tissue disintegration. In contrast, mastication of raw fruit and vegetables causes crushing and shearing of the tissue and tears the cells open (Fig. 2.1). Both mechanisms of particle size reduction will contribute to increased release, so it is not a foregone conclusion whether the raw or cooked tissues will provide more bioaccessible carotenoid. This is clearly demonstrated by examination of grated carrot strips fed to ileostomy patients. Carrot recovered from the terminal ileum (having passed through the gastrointestinal tract) shows loss of the carotenoid from only the fractured surface cells. There is no evidence for loss of carotenoid from deeper plant tissue.

It will be appreciated that the delivery of nutrients from foods is attenuated by the structure of the food and the way in which it is digested. Thus, delivery from the food structure occurs over the same timescale as gastric emptying. Carotenoids and other compounds isolated from the food structure are generally emptied from the stomach and absorbed more rapidly. These different rates of delivery may have profound effects on subsequent metabolism.

There are proven health benefits from 'slow release' carbohydrate foods; they do not stimulate the oversecretion of insulin, undesirable large excursions in blood glucose or unnecessary glycosylation of proteins. By analogy, the slower delivery of other food components may maximise health benefits by not overloading transport systems or causing undesirable excursions in plasma concentration. The fact that some portion of nutrients escape absorption in the ileum and are 'lost' to the colon should not automatically be interpreted negatively, since they may contribute positively to colon health and the production of beneficial products of colonic fermentation.

The complex nature of the mass transfer of carotenoids to absorbable lipid species, the diversity of raw and processed foods consumed and individual variations in the degree of mastication will lead to differences in the amount of carotenoid that becomes bioaccessible and potentially available for absorption. By understanding the underlying mechanisms of these processes, for a wider range of fruit and vegetable constituents, it will become possible not only to recommend 'five portions' a day but also to suggest domestic and commercial processing practice to maximise the potential health benefits.

2.6 Absorption, metabolism and tissue targeting

Many of the microconstituents of vegetables and fruits have been hypothesised to play an active role in the prevention or delay of many chronic debilitating diseases. However, in order to prove that any individual compound, or group of compounds, contributes to the beneficial effects of a fruit and vegetable-rich diet, it is necessary to demonstrate and measure absorption and the subsequent processes of distribution to target tissues and to characterise metabolism, because downstream metabolic products may have a different degree of, or entirely different, bioactivity from the parent compound. Understanding factors controlling the bioavailability of the constituents of fruits and vegetables is a necessary step for providing informed food choice and designing commercial processes that provide desired levels of bioavailability in food products.

The absorption and transport processes of many of the potentially bioactive components of fruits and vegetables are not fully understood; thus, prediction of their bioavailability is problematic. If this is coupled with too little understanding of the complexity of their behaviour in food systems and human tissues and the use of inappropriate methods for the assessment of absorption and tissue distribution, then confusion can abound in the literature.

Native compounds and those resulting from degradation by endogenous enzymes (glucosinolates/myrosinase) or digestive enzymes (glucose, amino acids, fatty acids) form the pool of bioaccessible compounds that may be absorbed and metabolised. The term 'bioavailability' embraces elements of absorption, distribution, metabolism and excretion (ADME) and yet it is frequently used simply to describe the plasma response to acute or chronic feeding of foods or isolated compounds. Although a simple plasma response may provide some useful indication of the relative absorption of a component delivered from different foods, it cannot describe absorption in absolute terms. Research needs to focus on the provision of experimental approaches that are able to quantify absorption in humans, for example the use of isotopic labelling methods, measurement of response in appropriate blood and/or tissue pools and metabolic modelling to discriminate between different components of response.

So, whilst there is strong and irrefutable evidence that the consumption of vegetables and fruits is correlated negatively with chronic disease rates, proof of which of the dietary components may be the active principle is dependent on demonstrating that it is absorbed, dispersed to putative sites of action and that

there is a dose-related response linked to the aetiology of the disease under consideration. The absorption and metabolism of many of the bioactive substances present in food plants (carotenoids, vitamins C and E, folates, simple and complex phenols, glucosinolates, phytosterols and certain trace elements) have been critically reviewed.[31]

2.7 Increasing consumption: what is being done?

The strength of the scientific base for the health benefits of diets rich in fruits and vegetables has also guided national policy making in diet and health issues and facilitated community and local programmes that address national dietary goals to increase fruit and vegetable consumption. An example of this is the '5-A-Day for Better Health' programme in the USA that aims to increase consumption of fruits and vegetables to an average of five or more servings a day. The aim is to improve the health of Americans through a partnership among the health community, government agencies, the fruit and vegetable industry and other private sectors. According to this programme, consumer awareness of the '5-A-Day' message increased from 8 to 39% between 1991 and 1997, promotion activities increased fruit and vegetable sales in stores and average fruit and vegetable consumption increased by half a serving from 3.9 servings a day in 1991 when the 5-A-Day programme began, to 4.4 servings by 1994 (www.5aday.com). Links to initiatives in many other regions of the world can also be found at this 5-A-Day website.

In several programmes, emphasis is placed on the education and involvement of children, because many of the processes linked to the development of chronic disease begin in childhood. Evidence from the Bogalusa Heart Study, tracking early risk of heart disease among American children, suggests that eating habits in childhood have a potential lifelong effect on cholesterol levels and on adult coronary heart disease.[32] A study of British schoolchildren found that children who ate fruit more than once per day had better lung function compared with those who did not. The difference was evident even after controlling for possible confounding factors such as social class and passive smoking.[33] A further study in Italy found that even low intakes of fruit can reduce wheezing and asthma with effects being most noticeable in children with a history of respiratory problems.[34] Continued attention to increasing fruit and vegetable consumption in children is viewed as a practical and important way to optimise nutrition and maximise good health throughout life, and reduce disease risk in older age.

The National School Fruit Scheme in the UK is an example of dietary guidelines for children being put into practice. The plan is that by 2004 every child in nursery, and aged four to six in infant schools, will be entitled to a free piece of fruit each school day. The practicalities of the scheme are being examined through pilot studies before the scheme is introduced nationally. Issues relating to distribution and how best to encourage the children to eat and enjoy the fruit provided

are part of the preliminary studies. If such schemes are to succeed they need to be positive and fun, making fruit and vegetables part of the children's culture. The scheme will run alongside new nutritional standards for school meals and community projects aimed at improving access to 'healthy' foods, increasing involvement in physical activities and tackling the growing problem of obesity (one in ten 6-year-olds in the UK are classified as obese, which represents a doubling since 1990).

2.8 Future trends

The World Cancer Research Fund and American Institute for Cancer Research recommend plant-based diets consisting of a variety of fruits and vegetables, pulses and minimally processed starchy foods that are low in energy. Their report states that these diets may prevent a variety of cancers (and other chronic diseases) because of their inclusion of constituents that are directly protective, or because of the exclusion of constituents commonly found in foods of animal origin.[9] Several other recommendations pertaining to diet and lifestyle are made concerning other known or putative risk factors. There are two major research challenges associated with these recommendations and those arising from other expert reports promoting similar guidelines for a healthy diet.

The first challenge relates to characterising the behaviour of nutrients within complex food systems and the interactions between the constituents of those systems with each other and with human tissues. Evidence linking diet to reduced burden of chronic disease weighs heavily in favour of the protective effects of whole fruits and vegetables, consumed as part of a traditional diet, but this is not reflected in research output. A concerted effort should be made to redress the imbalance between whole-food and high dose, single compound research. It is recognised that the very long-term studies required to determine the impact of any particular intervention on morbidity and mortality rates from chronic disease are difficult to fund and to perform. Furthermore, planning of protocol and interpretation of results from complex food interventions, using early biomarkers of disease risk, is not an easy task. However, science is about meeting such challenges, not avoiding them. The biologically active compounds provided by fruits and vegetables are known to have overlapping effects and probably also have synergistic additive and inhibitory effects on each other. These aspects have barely begun to be addressed in either animal or human experimental studies.

The second challenge relates to public acceptance and action. Three out of four Americans believe that there is too much conflicting information about diet and are confused by the 5-A-Day message. There is no universally accepted convention on which foods should be included in health advice on fruits and vegetables. Are dried fruits included, or fruit and vegetable juices and purees? What is the status of frozen, canned and bottled produce? The lack of more precise guidelines allows complacency about present levels of

consumption. A study of fruit and vegetable intake in Scotland found that among respondents whose intake of fruits and vegetables was low (less than two portions per day), 55% thought that they were getting enough and already eating 'more'. Providing practical, quantified advice on healthy intakes of foods may help to solve this problem.[35] This leads back to the need for research on the public health significance of 'whole' foods, as well as the constituents of those foods, studied within the dietary and cultural environment of specific populations.

Having clear and scientifically supported guidelines, however, does not guarantee compliance. Discussions of approaches to increase fruit and vegetable intake are beyond the scope of this chapter but research on the effectiveness of different strategies is obviously vital if the science and guidelines relating to 'healthy eating' are to be translated into better long-term health.

2.9 Sources of further information and advice

There are a number of relevant European initiatives directly, or indirectly, related to the health benefits of fruits and vegetables and their constituent compounds. For example:

- Concerted Action (FAIR CT 97-3233) 'The role and control of antioxidants in the tomato processing industry' which identified the major antioxidant compounds in tomato and examined processes to maximise their content and bioavailability in tomato products.
- NEODIET (FAIR CT 97-3052) 'Nutritional enhancement of plant-based food in European trade', which sought to understand how best to maximise the bioavailability of selected nutrients and potentially beneficial factors naturally present in plant-based foods, through processing and plant breeding.
- EUROFEDA (QLK-1999-00179) 'Dietary antioxidants in the promotion of health' supports research into defining the factors (antioxidants) that are responsible for ageing and age-related disease and practical ways of reducing their impact.
- MODEM (FAIR CT 97-3100) 'Model systems *in vitro* and *in vivo* for predicting the bioavailability of lipid soluble components of food' which determined the major factors controlling carotenoid bioavailability and developed practical predictive models.
- POLYBIND (QLK1-1999-00505) 'Health implications of natural non-nutrient anti-oxidants (polyphenols): bioavailability and colon carcinogenesis'. This project is studying the effect of polyphenols on health indicators, uptake and metabolism, influence on carcinogen metabolism, effect on cell proliferation and colon carcinogenesis.
- FolateFuncHealth (QLK-1999-00576) 'Folate: From food to functionality and optimal health' which aims to increase folate intakes through an understanding of the absorption from foods and its utilisation *in vivo*.

Further information can be obtained from the CORDIS website (http://www.cordis.lu/en/home.html), and from project coordinators who are identified on website information.

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3

Antioxidants in fruits, berries and vegetables

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3.1 Introduction

Fruits, berries and vegetables contain various phytochemicals with different bioactivities, such as antioxidant activity. This chapter discusses the antioxidant activities reported for fruits, berries and vegetables especially in relation to the compounds that appear to be responsible for the antioxidant activity, their content levels and the compounds' fate during different methods of processing. Antioxidant composition (flavonoids, phenolic acids, tocopherols (vitamin E), ascorbic acid (vitamin C) and carotenoids) of selected commonly consumed fruits, berries and vegetables and their products is presented.

Food processing such as peeling, boiling or juicing may result in no effect, increased inhibition or decreased inhibition of oxidation depending on the changes in the antioxidant components. Transformation of antioxidants into more active compounds improves antioxidant activity, while destruction or loss of antioxidants generally decreases the antioxidant activity, but important exceptions exist. Data on the antioxidant activity of fruits, berries and vegetables and their products therefore vary widely owing to differences in the raw materials as well as a result of different food processing methods that may induce changes in the antioxidant compounds. In addition, data on antioxidant activity of various fruits, berries and vegetables and their products can vary in response to differences in the preparation of samples for antioxidant testing, e.g. preparation of crude homogenates or extracts. When extracts are prepared, the mode of extraction, including solvent type, solvent to sample ratio and extraction time also strongly influence the data. Finally, the use of different oxidation systems and methods to measure antioxidant activity affect the antioxidant results.

While it has not been possible to include all methodological details behind the different antioxidant activity data obtained for various fruits, berries and vegetables and their products, an effort has been made to indicate the test methodologies employed in various investigations. To illustrate particularly how different antioxidant test protocols affect the results, Table 3.2, which compares the antioxidant activities obtained with different antioxidant methods of a number of relevant, pure compounds, is presented.

3.2 Antioxidants from fruits and berries: overview

Fruits and berries are good sources of antioxidants, including carotenoids, ascorbic acid, tocopherols, flavonoids and phenolic acids. It has been known for a long time that the phenolics, as well as some of the other antioxidant components, are closely associated with the sensory attributes of fresh and processed fruits, berries and other plant foods. Especially, the contribution to colour by carotenoids (yellow to orange and red) and anthocyanins (red to purple and blue) is well known. Also the specific involvement of some of the phenolic substances in flavour development and taste sensation is amply documented.[1] Phenolic compounds, including those having potent antioxidant activity, are also substrates for undesirable, oxidative browning reactions occurring during bruising of fruits, when fruits are cut or during their processing.

The possible beneficial biological functions of the traditional antioxidant vitamins, i.e. ascorbic acid, α-tocopherol and to a certain extent beta-carotene (provitamin A) have been intensively studied for at least 50 years and continue to receive high research attention. More recently, the antioxidant functions of flavonoids and other phenolic compounds have received increased attention. The biological roles of these plant phenolics that exert antioxidant activity are yet to be completely clarified, but evidence pointing to the possibility that phenolic phytochemicals also exert various protective effects in humans is accumulating. Because of the possible benefits of phenolic phytochemicals to human health, data on their quantitative occurrence and composition in various fruits and berries are gradually emerging in the literature. Therefore it is now known that flavonoids and other phenolic compounds are particularly abundant in fruits and berries. However, as it is generally recognised in relation to compilation of food compositional data, there are large variations in the levels of the constituents reported, depending on the species investigated, harvest time, fruit maturity stage, geographical origin etc. Differences in the methods employed for extraction and analyses also strongly affect the results and there are thus some inconsistencies in the available data, or very broad ranges for the levels of certain constituents in various fruits.

Some studies have evaluated the phenolic contents in fruits at more than one ripening stage. In the case of plums as well as with red grapes intended for wine making, a marked increase in the content of phenolics of potential antioxidant potency was seen in the fully ripe stage in comparison with the less ripe stage.[2,3] In contrast, no clear differences were observed in other fruits, e.g. peaches and

nectarines,[3] so it appears that there is no general rule correlating phenolic content and antioxidant potency with the fruit ripening stage.

Antioxidant composition (anthocyanins, flavanols and proanthocyanidins, flavonols, hydroxycinnamates, carotenoids, vitamin C and vitamin E) of selected, commonly consumed fruits and berries is presented in Table 3.1. Large amounts of anthocyanins (up to 8100 mg kg^{-1}) are found in the strongly coloured fruits and berries including bilberries (wild clone of blueberries), blackcurrants, cherries, cranberries, red grapes and raspberries. The amount of flavanols is generally below 150 mg kg^{-1} with larger amounts found in blackcurrants, cranberries, red wine grapes, peaches, plums and red raspberries. Apart from a few exceptions such as cranberries and red grapes, fruits and berries are generally also low in flavonols and high in phenolic acids such as hydroxycinnamates. Large amounts of hydroxycinnamates are present in cherries (300–1930 mg kg^{-1}), plums (121–896 mg kg^{-1}) and peaches (81–750 mg kg^{-1}). High molecular weight phenolics, tannins, are also found in fruits and berries with large amounts of ellagitannins in red raspberries (2200 mg kg^{-1}) and cloudberries (1800–2600 mg kg^{-1}) and moderate amounts in strawberries (90–200 mg kg^{-1}).[4] The vitamin C content of fresh fruits and berries is generally high while that of provitamin A carotenoids and vitamin E is low. Blackcurrants (1200–1500 mg kg^{-1}), cloudberries (1000 mg kg^{-1}), strawberries (550–1000 mg kg^{-1}) and orange (510 mg kg^{-1}) are very rich in vitamin C. One exceptional berry is sea buckthorn berry with extremely large amounts of vitamin C (2000 mg kg^{-1}) as well as high amounts of beta-carotene (15 mg kg^{-1}) and vitamin E (32 mg kg^{-1}).

Food processing of fruits and berries into juices and jams, and drying of fruits generally result in lower amounts of antioxidant compounds. For example, losses of anthocyanins in juices and purées of strawberries, strawberry and blackcurrant syrups, cranberry juice, raspberry juice and wine have been reported[5–9] as well as phenolic degradation during processing of apple juice.[10] On the other hand, the manufacturing process had no effect on the qualitative anthocyanin profile of commercial jams made from strawberries, blackberries, raspberries, blueberries, blackcurrants and cherries.[11]

In domestic berry processing practices, a quercetin loss of 15% was observed in strawberry jam, 85% loss in blackcurrant juice, 40% loss in bilberry soup and 85% loss in lingonberry juice in their making procedures.[12] Flavanols are effectively extracted into apple cider, blackcurrant juice and red wine, the amounts being higher than those of the raw materials.[13–17] An increase in ellagic acid in raspberry jams was reported to occur, most likely owing to release of ellagic acid from ellagitannins with the thermal treatment,[18] although according to Häkkinen *et al.*[19] ellagic acid content in strawberry jam was 80% that of unprocessed strawberries.

As for other antioxidant compounds, peeling and juicing result in substantial losses of provitamin A carotenoids, often surpassing those associated with heat treatment.[20] Moreover, the stability of carotenoids differs in different foods even when the same processing conditions are used. Ascorbic acid of fruit juices such as orange, peach, grapefruit, pineapple, apple and mango juice is readily oxidised

Table 3.1 Antioxidant compounds in selected fruits and berries and their products (mg kg fresh weight)

Fruit or berry	Anthocyanins	Flavanols and proanthocyanidins	Flavonols	Hydroxycinnamates	Carotenoids (β-carotene)	Vitamin C	Vitamin E
Apple	4–5[4]	0–15[116]	17–70[4,74]	263–308[4]	0.4[94]	40[117]	2[117]
– juice		0–298[118]	2.5[119]	0.1–162[10,17,120]	0.2[117]	300[117]	0[117]
Bilberry	3450–4635[4]	13–29[4]	41–195[4,12]	170–347[4]	0.5[94]	150[117]	19[117]
– soup			6[12]		0.01[94]	20[117]	5[117]
Blueberry	3970–4840[29]	63–70[29]	115–139[29]	226–315[29]			
Blackcurrant	130–8100[4,62]	205–374[4]	133–157[4,12]	104–167[4]	1[94]	1200[117]	23[117]
– juice	24[17]		36[12]		0.1[117]	380–421[12,117]	11[117]
Cherry, sweet, red	31–4500[22–31]	20–63[28–31]	10–23[29]	100–1900[28–31]	1.2[94]	70[121]	1[121]
Cloudberry	7–15[4]	2–6[4]	34–90[4]	90–128[4]	1.4[94]	1000[117]	31[117]
Cranberry	460–1720[4,6,122,123]	285[4]	139–334[122–124]	191[4]	0.2[94]	120[121]	10[121]
– juice	18–512[124]						
Grapes, table, red	72.5–765[125]	1–160[118]	13–25[125]	5–19[125]	0.3[117]	50[117]	7[117]
– wine, red	0.6–385[13,15]	0–500[118]	10–55[13,15]	4–13[13,15]	tr[121]	0[121]	0[121]
Grapes, table, white	0[125]	0[125]	10–13.5[74,125]	5.5[125]	0.3[117]	50[117]	7[117]
– wine, white	0[15]	0–106[15]		1–34[15]	tr[121]	0[121]	0[121]
Orange			0–5[70]	136–163[126]	0–5[70,74]	510[117]	4[117]
– juice					0.1[94]	300–450[41]	2[117]
Peach	0–17.8[27]	24.5–700[3,27]	0–11.9[27]	54–148[27]	0.9[94]	80[121]	10[121]
– canned	0[127]	tr[127]	tr[127]	11–29[127]	1.0[94]	20[117]	20[117]
Plum	19–76[31,37]	140–600[3]	5.7–27[37]	500–900[23,37]	4.3[121]	54[121]	8.6[121]
– dried (prune)	0[37]	0[37]	42[37]	1800[37]	1.4[94]	0[117]	18[121]
Raspberry, red	200–2200[4,29]	4–480[4,29]	6–39[4,29,128]	3–35[12,29]	0.1[94]	296–380[12,117]	11[117]
Sea buckthorn berry					15[121]	2000[121]	32[121]
Strawberry	202–790[4,29]	9–184[4,29]	7–174[4,29,128]	14–69[4,29]	0.1[94]	420–600[117,128]	6[117]
Strawberry jam	4–22[11]		11.4[12]		0.04[117]	80–236[12,117]	1.0[117]

and lost during staying of the juices with losses ranging from 29 to 41% when stored at room temperature for four months.[21] Kalt *et al*.[22] found a marked difference in the stability of ascorbate in green leafy vegetables when compared with fruits. For example, in spinach more than 90% of the ascorbate was lost within three days after harvest when stored at ambient temperature while losses in ascorbate during storage of blueberries, raspberries and strawberries were minimal.[22]

Antioxidant activity of fruits and berries and their products reported in many studies varies widely and this is partly due to the use of different oxidation systems and methods to analyse antioxidant compounds. For antioxidant testing, either extracts or juices of fruits and berries have been used resulting in different antioxidant compositions owing to choice of extraction solvents (e.g. either water-soluble or lipid-soluble compounds extracted by one method) or use of filtration (e.g. possible losses of antioxidant compounds). The literature has much focused on the antioxidant effects of flavonoids and phenolic acids although ascorbic acid, carotenoids and tocopherols also contribute to the antioxidant activity of fruits and berries. Many of the flavonoids and phenolic acids exert comparable or better radical scavenging activity than vitamin C and E in radical scavenging activity assays.[23]

It is beyond the scope of the present treatise to discuss the problem that arises because the absolute and relative efficiencies of many natural antioxidants vary depending on the test method employed, and especially that the free-radical trapping methods (DPPH, ORAC, TEAC and TRAP assays) may not always mimic the complex multifunctional antioxidant mechanisms of natural antioxidants. It is important to note, however, that relevant antioxidant mechanisms of natural antioxidants and polyphenols such as metal chelation, inhibition of oxidative enzymes etc are overlooked in many of the currently employed rapid free-radical antioxidant test assays. In addition, the possible influences of factors such as antioxidant solubility, partitioning, ionic charge, complexing/interaction with other compounds, type of initiation, pH of the system and so on are not considered in simple radical scavenging tests (discussed in detail by Frankel and Meyer).[24] Glycosides of polyphenols have often been found to be less active as antioxidants compared to the corresponding aglycones in radical scavenging tests.[21] However, this may be an artefact as with more realistic substrates, for example in LDL and phospholecithin liposomes *in vitro* assays, the glycoside/aglycone issue appears to be more complex. Thus, when evaluated at the same micromolar addition level on copper-catalysed LDL *in vitro*, rutin exerted better antioxidant potency than quercetin. Likewise, chlorogenic (5′-caffeoylquinic) acid was better than caffeic acid on human LDL oxidation *in vitro*, when oxidation was induced with AAPH,[25] while no significant differences between antioxidant potency of these two compounds could be established when the LDL oxidation was induced with copper ions.[26] Some of the differences in results obtained in different *in vitro* antioxidant tests with anti-oxidant compounds present in fruits and berries are summarised and exemplified in Table 3.2.

Table 3.2 Radical scavenging and antioxidant activities in different test systems for ascorbic acid and selected phenolic antioxidants purified from fruits, berries and vegetables

Compound	Inhibition (%) of LDL oxidation at 5 μM GAE[29,33,64,129]	Inhibition (%) of lecithin liposome oxidation at 10 μM GAE [64]	ORAC (μM trolox equivalents)[130–132]	TEAC (mM trolox equivalents)[133]
Flavanones				
Naringenin			2.67	0.72
Hesperidin				1.37[23]
Flavonols				
Kaempferol			2.67	1.02
Quercetin	50.6		3.29	2.88
Rutin	67.6		0.56	2.4[23]
Myricetin	68.1		4.3	3.1[23]
Flavan-3-ols				
Catechin	87.8		2.49	2.4[23]
Epicatechin	67.6		2.36	2.5[23]
Procyanidins				
Anthocyanins				
Cyanidin	79.4	pro-oxidant	2.2	2.38
Malvidin	59.3	23.9	2.0	1.80
Pelargonin	39.0	pro-oxidant	1.1	1.30[23]
Delphinidin	71.8	pro-oxidant	1.8	4.80
Hydroxycinnamates				
p-Coumaric	24.5		1.09	1.56
Ferulic	24.3		1.33	1.75
Caffeic	96.7		2.23	0.99
Chlorogenic	90.7			
Other				
Ascorbic acid	45.2 (at 10 μM)	2.5 (at 10 μM)	0.52	1.05
Gallic acid	63.3		1.74	3.01[23]
Ellagic acid	0–36			

3.3 Stone fruits

Stone fruits encompass nectarines (*Prunus persica* var. nucipersica), peaches (*Prunus persica* L.), plums (*Prunus domestica*), sweet cherries (*Prunus avium* L.) and sour cherries (*Prunus cerasus* L.). In general, ascorbic acid is present in highest concentration in the fruit flesh, but the skin fraction contain larger amounts of phenolics than the flesh. Thus, in an investigation of phenolic compounds in nectarines, peaches and plums, the anthocyanins and flavonols (the latter mainly as quercetin glucosides) were found to be almost exclusively located in the peel tissues.[3] However, the flavanols, notably catechin, epicatechin, procyanidin B1 and other procyanidins, were also found in the fruit flesh with mean contents in the flesh of peaches and nectarines in the range 100–700 mg kg^{-1} with

a tendency for contents to be higher in white flesh peach cultivars than in yellow flesh cultivars.[3,27] Plums contain higher levels of epicatechin than catechin, with the total levels of these flavanol diastereoisomers being 5–50 mg kg^{-1} fresh weight of whole plums.[28] More recent values for the total amount of flavanols, which include procyanidins, in the plum flesh alone are in the range 140–600 mg kg^{-1} fresh weight (Table 3.1).[3] The higher values obtained in the more recent investigations[3] may be a result both of a more comprehensive extraction technique as well as use of better analytical methodology.

Cherries, both sweet and sour, appear to be richer in anthocyanins as well as in hydroxycinnamic acid derivatives than peaches, nectarines and plums. Sweet cherries contain up to 3500–4500 mg kg^{-1} of anthocyanins, 100–1900 mg kg^{-1} hydroxycinnamates as caffeic acid and *p*-coumaric acid derivatives, where especially 3′-*p*-coumaryl quinic acid can account for 50–75% and neochlorogenic acid for 15–60% of the hydroxycinnamates, depending on the variety.[28–30] Sour cherries have been shown to harbour higher levels of flavan-3-ols than sweet cherries, mainly epicatechin and catechin: estimates of total contents are in the range 70–170 mg kg^{-1} for sour cherries in contrast to 20–60 mg kg^{-1} in sweet cherries (Table 3.1).[28–31] Individually these compounds exhibit strong antiradical activity, for example in the DPPH *in vitro* assay, when evaluated at different micromolar concentrations.[32] The flavanols are also effective inhibitors of human LDL oxidation *in vitro*.[33] Catechin especially is one of the individual phenolic constituents that are present at high levels in red wines and have high antioxidant activity in inhibiting oxidation of human LDL *in vitro*.[15] A number of flavanone, flavone and flavonol compounds as well as a chlorogenic acid methyl ester and some novel cinnamoyl derivatives – namely the cyclopenta-2,3 and -2,5-diols of caffeic acid – have been purified from sour cherries, varieties 'Balaton' and 'Montmorency'.[34] In an antioxidant assay using phosphatidyl choline liposomes as the oxidising substrate, these novel sour cherry compounds exhibited antioxidant activities that were comparable to the activities of TBHQ, BHT and caffeic acid.[34] Knowledge on their quantitative occurrence in cherries is scarce, however.

The extracts of two different varieties of sweet cherries were superior to various berry extracts (blueberries, raspberries, blackberries and strawberries) in inhibiting lipid oxidation in an *in vitro* phosphatidyl lecitine model system; in contrast, the relative antioxidant activities of the same cherry extracts on human LDL oxidation *in vitro* were lower than that of blackberries and raspberries, but higher than that of blueberries and strawberries when evaluated at the same micromolar concentration of 10 μM total phenols.[29] The antioxidant activities of phenolic extracts of berries against lecithin liposomes were significantly positively correlated to the content of hydroxycinnamates, but the amount of flavanols correlated to the antioxidant potency of extracts of berries in neither the *in vitro* LDL oxidation systems nor in the lecithin liposome assay.[29] Extracts of sweet cherries were found to be the best among a large number of other fruits in inhibiting oxidation *in vitro* of a pool of LDL + VLDL; sweet cherries had an IC_{50} (i.e. the amount required to inhibit oxidation by 50%) of only 0.10 μM total phenols, while red grapes ranked second with IC_{50} = 0.27 μM.[35] Nectarines, peaches and

plums were less potent and were ranked as numbers 14, 15 and 10, respectively, on this antioxidant potency scale.[35]

At a concentration of 10 μM total phenols as gallic acid equivalents, extracts of whole clingstone peach cultivars were demonstrated to inhibit human LDL oxidation *in vitro* by 44–84% depending on the cultivar.[27] Also extracts of the flesh and skins of the peaches exhibited antioxidant activity against LDL oxidation *in vitro*. Extracts of peach peels contained more total phenols ranging from 910–1920 mg kg^{-1} as gallic acid equivalents than the extracts from flesh, where phenols levels were in the range 430–770 mg kg^{-1}: Chang *et al*.[27] found a statistically significant linear correlation between relative antioxidant activity and concentration of total phenols of peach extracts of 0.76. Thus, the relative antioxidant activity of peel extracts was better than the extracts of whole peach and peach flesh extracts, even though the percentage inhibition at 10 μM was in a similar range for all types of peach extracts. The results signified that the antioxidant activity was widely distributed among the extracted peach phenolics. In peaches, the anthocyanins are mainly confined to the peel tissue.[3,27] When the antioxidant activity of clingstone peach extracts was evaluated against LDL oxidation *in vitro*, however, a significant correlation between antioxidant activity and anthocyanins could not be established. In contrast, a strong correlation, $r = 0.96$, was found between the percentage relative inhibitory activity and redness of whole peach extracts when colour was measured on the Hunter scale.[27]

Plums contain high levels of hydroxycinnamic acids (Table 3.1), notably neochlorogenic and chlorogenic acids, with neochlorogenic acid as the dominant compound with content levels in the range 500–770 mg kg^{-1} fresh weight.[28] Individually, these compounds exert potent antioxidant activity on human LDL oxidation *in vitro* and have been shown to inhibit totally the LDL oxidation *in vitro* at addition levels of 10 μM in total phenols.[26] Plum extracts tested *in vitro* were better inhibitors of lipid oxidation in human liver microsomes and phosphatidyl choline than peach, apple, grapefruit and pear extracts.[36] Analyses of methanolic extracts of freshly harvested, unprocessed prune plums, cultivar La petite d'Agen, showed the mean concentration of phenolics to be about 1100 mg kg^{-1} fresh weight, where neochlorogenic acid constituted 73 wt% of the phenols (807 mg kg^{-1}) and chlorogenic acid was 13 wt% (144 mg kg^{-1}); only low amounts of 3′-coumarylquinic acid (10 mg kg^{-1}) were detected.[37] The level of anthocyanins in these plums were 76 mg kg^{-1}, while there was 54 mg kg^{-1} catechin and 27 mg kg^{-1} of other flavonols, mainly rutin.[37] In a study where five Californian plum cultivars were analysed for their phenolic content, high levels of anthocyanins, about 1600 mg kg^{-1} fresh weight, were found in the skin of the blue plum cultivar 'Angeleno'. The 'Angeleno' anthocyanins were dominated by cyanidin 3-glucoside (about 1040 mg kg^{-1}) and cyanidin-3-rutinoside (560 mg kg^{-1}). Other red and blue plum varieties also contained mainly these two anthocyanin glucosides in their skin, but at lower levels, in the range 130–700 mg kg^{-1}. In all the red and blue plum varieties evaluated, only low levels of anthocyanins were detected in the flesh part.[3] In pitted prunes, anthocyanins and catechin were absent, and hydroxycinnamates – dominated by neochlorogenic acid – made up

98% by weight of the phenolic material, where the mean concentrations of phenols were 1840 mg kg^{-1}.[37] Extracts of prunes as well as of prune juice were shown to inhibit the copper catalysed oxidation of lipids in human LDL significantly at 5–10 μM test levels with the prune extract exerting higher antioxidant activity than the prune juice.[37] ORAC measurements evaluated on a per 100 gram weight basis ranked the 'antioxidant power' of dried plums, that is prunes, the highest among a range of other fruits. Thus, by this measurement, the dried plums exerted an antioxidant score of 5770, while fresh plums scored 949;[38] however, part of the increase could be due to the greater dry matter content in dried plums compared to fresh plums.

3.4 Citrus fruits

Citrus fruits are characteristic in containing high levels of ascorbic acid as well as relatively high levels of certain flavonoids. In their peel, citrus fruits also contain the unique glucaric and galactaric acid conjugates of hydroxycinnamic acids, mainly as feruloyl and *p*-coumaroyl conjugates at levels of 170–250 mg kg^{-1} in oranges and 3–10 times less in lemons and grapefruits.[39,40] It appears that the antioxidant potencies of these particular conjugates have not been systematically tested.

Ascorbic acid is considered one of the major nutrients in citrus fruits, owing to its activity as vitamin C, and it seems plausible that the presence of ascorbic acid may influence the antioxidant potency of citrus products. The ascorbic acid levels in various processed citrus juice products manufactured in Florida (orange juices, grape juices) range from ~300 to 450 mg l^{-1}.[41]

Flavonoids in the edible part of citrus fruits are dominated by hesperidin, which is a compound exhibiting only limited antioxidant and antiradical potency in various assay test systems.[23] Hesperidin concentrations in citrus are in the range 5400–5500 mg kg^{-1} dry weight based on analyses of 66 different citrus species.[42] When the ABTS$^{\bullet+}$ radical trapping efficency of orange juice was evaluated in the TEAC assay, the antioxidant activity of orange juice was mainly ascribed to the presence of hesperidin, naringin and narirutin.[17] In contrast, neither orange juice, tangerine juice, grapefruit juice nor hesperidin exerted antioxidant activity on human LDL after *ex vivo* spiking in plasma,[43] and although extracts of grapefruit inhibited ascorbate/iron induced *in vitro* lipid oxidation of human liver microsomes to the same degree as peach extracts, but less efficiently than plum extracts, they only exhibited very weak antioxidant activity when the same liver microsomes were oxidised by NADPH or when the oxidising substrate was phosphatidylcholine.[36]

Citrus essential oils, which contain a large number of volatile components, notably high levels of limonone, exert radical scavenging effects against DPPH, where the essential oil of the Korean lemon variety Ichang lemon, Tahiti lime and Eureka lemon were found to be especially strong radical scavengers on DPPH *in vitro*.[44] Individual volatile components of citrus, notably terpinolene, geraniol

and gamma-terpinene also exhibited pronounced radical scavenging activities on DPPH.[44] However, no clear relationship between specific essential oil constituents or essential oil composition and antiradical scavenging efficiency has been established. Extracts from citrus peel and seeds contain glycosylated flavanones and polymethoxylated flavones, especially of naringin, neohesperidin, hesperidin and narirutin, as well as hydroxycinnamates, with the flavanone content in the peels being higher than in the seeds.[45,46] In a model system using citronellal as the oxidising substrate, seed extracts of various citrus fruits exhibited greater antioxidant activity than the corresponding extracts of peels, but no clear relationship could be established between antioxidant activity and phenolic composition of these peel and seed extracts.[45] Thus, citrus products contain a range of very different types of antioxidant compounds, which are furthermore distributed differently in the separate fruit fractions.

3.5 Grapes

Grapes (*vitis vinifera* and *vitis lubruscana*), especially the dark red varieties, contain generous amounts of flavonoids and relatively high levels also of hydroxycinnamates that all exert potent anti-oxidant activities in various assay systems. The antioxidant activity of wines have received much attention owing to their possible physiological benefits. However, several of the phenolics present in fresh grapes and grape juice are also potent antioxidants in various *in vitro* assays, including several containing biologically relevant lipid substrates, notably human LDL. In fresh grapes and grape juices the polyphenolic compounds are primarily present as glucosides, while the phenolics in wines are principally aglycones. Glycosylation is generally considered to dampen the antioxidant potency of polyphenolics, but the available data on this are conflicting, as the impact of glycosylation and in turn antioxidant solubility and partitioning are very system dependent.

Depending on the variety, red grapes may contain about 100–4000 mg kg^{-1} of anthocyanins, 5–285 mg kg^{-1} flavonols, mainly rutin, 0–25 mg kg^{-1} flavanols, 2–25 mg kg^{-1} hydroxycinnamates, very low levels of hydroxybenzoic acids and hardly any vitamin C, E or carotenoids (Table 3.1).[31] Except for hydroxycinnamates, where the content range is approximately the same in white and red grapes, the levels of phenolics in white grapes are about 20–25 times lower than in dark red grapes, and white grapes do not contain anthocyanins.[2,31] Certain white grape varieties contain flavonols, notably rutin, at the same levels as found in red grapes. Grape hydroxycinnamates are uniquely esterified to tartaric acid, and caffeoyl-, coumaroyl- and feruloyl-tartrates are generally found in grape pulp.

Flavonoids, free hydroxycinnamates and hydroxybenzoic acids are mainly present in grape skins and seeds, but the levels and composition depend very much on the grape variety. Since the seeds and skin of grapes (especially of red grapes) are particularly rich in phenolic compounds, the extraction method employed for extracting phenols from whole grapes strongly influences the yields

of phenolic substances and the antioxidant potency of the extracts. Thus, by using extended solvent contact times (24–165 hours) and crushing the seeds prior to extraction, flavan-3-ols and hydroxybenzoates – i.e. antioxidant compounds of considerable potencies – can be obtained in high levels in extracts of fresh grapes, while they are virtually absent in grape extracts produced during short extraction, for example one minute solvent contact time.[2]

Red wines, extracts of different types of fresh grapes, 'grape skin extract', American Concord grape juice, as well as European red grape juices, strongly inhibit human LDL oxidation *in vitro* and this antioxidant activity is associated with the phenolic compounds.[2,47–50] Thus, not only has the antioxidant activity of similarly diluted grape samples been shown to be proportional to concentration of total phenols, but in certain cases, the antioxidant potency also correlates to the levels of different classes of compounds. Thus, the relative antioxidant potency towards human LDL oxidation *in vitro* is strongly correlated to levels of anthocyanins and flavonols for fresh grape extracts; for Concord grape juice and red European grape juice strongly correlated to the level of anthocyanins, and in white grape juice samples the antioxidant potency on LDL correlates to the levels of hydroxycinnamates and flavan-3-ols.[2,49,50] Extracts of fresh grapes also inhibit both development of lipid hydroperoxides and their degradation to produce hexanal in lecithin in liposomes, and the relative antioxidant potency is statistically correlated with the total phenols.[51] Compared to the data obtained on human LDL oxidation *in vitro*, the grape extracts exhibiting highest antioxidant activity on lecithin liposomes were those of the red table varieties (Red Globe and Emperor) and the white wine grape varieties (Chardonnay and Sauvignon Blanc);[51] these extracts had only low antioxidant potency on human LDL oxidation *in vitro*.[2] The removal of phenolic compounds by polyvinylpolypyrrolidone stripping abolishes the antioxidant activity of grape juices and a mixture of representative carboxylic acids of red wine do not exert antioxidant activity.[16] The presence of ascorbic acid was somewhat surprisingly found not to affect antioxidant activities of Concord grape juices on LDL oxidation *in vitro*.[47]

In contrast, equimolar addition of ascorbic acid (5 μM) to European red grape juice samples significantly increased the antioxidant activities of the red grape juices on human LDL oxidation *in vitro* (Fig. 3.1).[50] The phenolic profile of Concord grape juice is dominated by anthocyanins, levels range from about 300–450 mg L^{-1},[49] where the dominant compound, which is also the major contributor to the dark, purple-bluish colour, is delphinidin-*O*-3-monoglucoside. In the ORAC antioxidant assay employing β-phycoerythrin as the oxidising substrate, Concord grape juice exerted the highest antioxidant activity among commercial fruit juices followed by grapefruit, tomato, orange and apple juice.[52] Phenolic extracts from red grape pomace that remained after red wine production and isolated catechins and procyanidins extracted from grape seeds are all effective inhibitors of human LDL oxidation *in vitro*.[26,33] Grape seed procyanidins also act as free radical oxygen scavengers in aqueous *in vitro* model systems with procyanidin B2 3-*O*-gallate being the most potent compound.[53] The pronounced

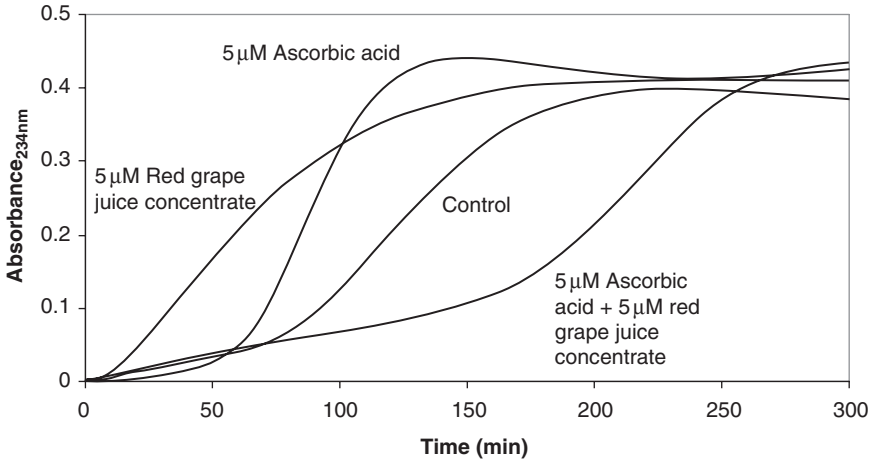

Fig. 3.1 Effect of ascorbic acid addition on antioxidant activity of European red grape juice concentrate on human LDL oxidation *in vitro*.

antioxidant activity of fresh grapes and grape juices is thus attributable to different types of phenolic constituents, but the antioxidant effectiveness in different oxidation systems is correlated to distinct types of phenolics and their relative concentrations in various samples.

3.6 Apple

Apple showed strong antioxidant activity towards oxidation of methyl linoleate, although the apple extracts tested were low in total phenolics as well as ascorbic acid.[4,54] In apple juice, vitamin C activity represented a minor fraction of the total antioxidant activity, with chlorogenic acid and phloretin glycosides as the major identifiable antioxidants.[17,55] Dihydrochalcones such as phloretin glucosides and phloridzin amount to 5–223 mg kg^{-1} in apple juice, this content being greater than that of fresh apples.[55] According to Plumb *et al.*,[36] chlorogenic acid contributes about 27% of the total activity of apple extract in scavenging hydroxyl radicals. Apple polyphenols isolated from gala apple pomace such as epicatechin, its dimer (procyanidin B2), trimer, tetramer and oligomer, guercetin glucosides, chlorogenic acid, phloridzin and 3-hydroxy-phloridzin showed strong antioxidant activities using beta-carotene linoleic acid system and DPPH radical scavenging activities.[36] During conventional apple juice production (straight pressing or pulp enzyming) more than 80% of the quercetin glycosides remained in the press cake and less than 10% was found in the raw juice. It was suggested that quercetin glycosides and antioxidant activity in apple juice could be increased over tenfold by extracting the pulp with an alcoholic solvent such as methanol or ethanol.[56]

3.7 Berries

Berries constitute a significant source of antioxidants, the most significant compounds being flavonoids, phenolic acids and to a minor extent ascorbic acid. Carotenoids may contribute to the antioxidant activity in, for example, carotenoid-rich sea buckthorn berry (*Hippophae rhamnoides* L. cv. Indian-Summer) that had a high antioxidant activity in a beta-carotene bleaching method.[57] The antioxidant activity of berries and antioxidant compounds (mainly phenolics) isolated from berries has been investigated using various antioxidant assays and has resulted in somewhat contradictory findings depending on the choice of methods. For example, high antioxidant capacity is reported for strawberries by using radical model systems,[22,52,58] while in lipid oxidation models (methyl linoleate, LDL) phenolic extracts from strawberries ranked among the least active antioxidants compared to the activities of other berries.[29,54]

The most potent berries were crowberry (*Empetrum nigrum*), cloudberry (*Rubus chamaemorus*), whortleberry (*Vaccinium uligonosum*), cranberry (*Vaccinium oxycoccus*) and rowanberry (*Sorbus aucuparia*), all being wild berries, while the cultivated berries such as strawberry (*Fragaria ananassa*), red currant (*Ribes rubrum*), blackcurrant (*Ribes nigrum*) and red raspberry (*Rubus idaeus*) exerted low antioxidant activity in inhibiting lipid oxidation.[54] Berry extracts inhibited LDL oxidation in the order: blackberries (*Rubus fructicosus*) > red raspberries > blueberries (*Vaccinium corymbosum*) > strawberries.[29] In the same study, blueberries, red raspberries, blackberries and strawberries were active towards inhibition of oxidation of lecithin liposomes. In a more recent study by Kähkönen *et al*.,[4] a statistically significant correlation was observed between the flavonol content and antioxidant activity of berries ($R = 0.78$) and between hydroxycinnamic acid content and antioxidant activity ($R = 0.54$). However, a multiple linear regression analysis revealed that flavonol and hydroxycinnamic acid contents explained only 31% of the variability in the antioxidant response. The level of vitamin C in these berry extracts was low owing to choice of extraction method and sugar removal techniques.

Blueberries and their wild clones, bilberries (*Vaccinium myrtillus*), have been shown to be very efficient antioxidants in many studies.[22,29,52,54,58–62] One of the most potent antioxidant compounds in strongly coloured berries, such as blueberries, are anthocyanins, although blueberries are also rich in hydroxycinnamates such as chlorogenic acid.[4,29] Like several other flavonoids, anthocyanins are powerful free radical scavengers [23,52,63] They also show antioxidant activity in lipid environments such as emulsified methyl linoleate, liposome and human LDL.[62,64] The antioxidant activity of berries for LDL was associated directly with anthocyanins and indirectly with flavonols, and for liposome it correlated with the hydroxycinnamate content.[29]

Fruit extracts (black chokeberry, blackthorn and strawberry) containing large amounts of anthocyanins showed high radical scavenging activity using the DPPH radical assay.[65] Correlation was found between the berry anthocyanin content and ORAC in different cultivars of berries belonging to *Vaccinium*

genus,[60] and in high and low bush blueberries. Also spray-dried elderberry juice (*Sambucus nigra*), containing large amounts of anthocyanin glucosides, inhibited copper-induced oxidation of LDL.[66] In this study, the anthocyanins were able to reduce alpha-tocopheroxyl radical to alpha-tocopherol. According to Smith *et al*.[61] many blueberry fractions had antioxidant activity, especially those rich in anthocyanins and proanthocyanidins.

Kähkönen *et al*.[62] isolated anthocyanins from blackcurrants, bilberries and lingonberries (*Vaccinium vitis-idaea*) resulting in remarkable inhibition of the hydroperoxide formation of methyl linoleate and hexanal formation in LDL. Blackcurrant anthocyanins showed the highest radical scavenging potential against the DPPH radical, followed by bilberry and lingonberry. On the other hand, according to Costantino *et al*.,[59] the activities of black raspberries, blackcurrants, high bush blueberries, blackberries, red currants and red raspberries toward chemically generated superoxide radicals were greater than those expected on the basis of anthocyanins and polyphenols present in the berries.

It is possible that ascorbic acid contributes significantly to the antioxidant activity of berries and berry juices, as Miller and Rice-Evans[17] have reported that blackcurrant juice has an ascorbate sparing effect. The effect of ascorbic acid is not clear, however, since Kalt *et al*.[22] reported that ascorbate made only a small contribution (0.4–9.4%) to the total antioxidant capacity of strawberries and raspberries confirming earlier results on blueberries by Wang *et al*.[52] and Prior *et al*.[60]

Food processing such as juicing, involving juice extraction, heating steps and juice clarification treatment has an impact on the putative antioxidant composition as well as the antioxidant activity of berries. For instance, industrial clarification treatment of blackcurrant juice to remove cloud and sediments, decreases the contents of the four major anthocyanins by 19–29%. Also the level of ascorbic acid and flavonols decreases, but the flavonols apparently relatively less than the other compounds.[67] When tested at equimolar doses of total phenols, the antioxidant activity on human LDL oxidation *in vitro* was improved after clarification treatment. This suggests that the overall composition of putative antioxidants in the blackcurrant juice improved, even though the total level of antioxidants decreased.[67] Thus, to obtain a more comprehensive understanding of the effects of processing, it appears important to accompany antioxidant evaluations with detailed compositional studies of the putative antioxidants.

3.8 Antioxidants from vegetables: overview

The antioxidants present in commonly consumed vegetables include ascorbic acid, tocopherols, carotenoids and phenolic compounds such as flavonols and phenolic acids (Table 3.3). In comparison to fruits and berries, vegetables generally contain much lower amounts of antioxidant compounds. A large amount of vitamin C is found in sweet red pepper (1850 mg kg^{-1}) and significant amounts in Brussels sprouts (up to 900 mg kg^{-1}) and broccoli (750–830 mg kg^{-1}), while

Table 3.3 Antioxidant compounds in selected vegetables and their products, mg kg fresh weight

Vegetable	Flavonols (quercetin)	Hydroxy-cinnamates	Carotenoids (beta-carotene)	Vitamin C	Vitamin E
Broccoli	15–65[71,72,134]	62–148[72]	4–27[70,94]	750–830[68,121]	7[121]
– boiled				640[117]	7[117]
Brussels sprouts	0–6[71]		4.3[94]	900[117]	4[117]
Carrots			11–770[70,94]	60[117]	4[117]
– boiled			101[117]	42[117]	4[117]
Onions	340–420[71,105]		0.1[94]	75[117]	0.4[117]
– blanched	210–290[105]				
– fried	220–370[105]		0.2[117]	57[117]	8[117]
Pea	1.4–1.6[105]		3.6[94]	200[117]	2[117]
– boiled	0.8–1.0[105]		3.6[117]		
– fried	1.3–2.0[105]				
Potatoes		140[76]	0.1[94]	100[117]	1[117]
– boiled				100[117]	1[117]
Spinach	tr[71]		8–240[70]	600[117]	12[117]
Tomatoes	2–14[71]		0.2–623[94]*	140[117]	7[117]
– juice	13[71]			140[117]	7[117]
– ketchup			99[94] *	80[117]	23[117]
Sweet red pepper			1.2–33[70]	1850[117]	22[117]

* Lycopene.

the amounts of vitamin E are generally below 10 mg kg^{-1} in vegetables. According to Hussein *et al.*[68] although there was significant loss in vitamin C during storage of broccoli and green peppers, in most cases there was no difference in loss of vitamin C or beta-carotene between the processed and unprocessed vegetables, and the packaging systems. After storage, artichokes stored at different temperatures showed a decrease of about 40% in the vitamin C content which was most likely to have been associated with the ability of the polyphenol oxidase to catalyse the oxidation of ascorbic acid.[69] Carotenoids contribute to antioxidant activity, with beta-carotene (1–644 mg kg^{-1}) and lutein (up to 203 mg kg^{-1} in spinach[70]) present in all vegetables and lycopene dominating in tomatoes (0.2–623 mg kg^{-1}) and tomato products (Table 3.3). As a result of food processing involving heat treatment carotenoids undergo isomerisation[70] which may decrease their antioxidant activity. On the other hand thermal processing is reported to increase carotenoid concentration, perhaps owing to greater extractability, enzymatic degradation and unaccounted losses of moisture and soluble solids.[20]

In fresh vegetables only glycosylated flavonols and other flavonoids are present but aglycones may be found as a result of food processing.[71] Quercetin levels in vegetables are generally below 10 mg kg^{-1}, except for onions (340–347 mg kg^{-1}), kale (110–120 mg kg^{-1}) and broccoli (30–166 mg kg^{-1}), while

kaempherol has only been detected in kale (210–470 mg kg^{-1}), endive (15–90 mg kg^{-1}), broccoli (60 mg kg^{-1}) and leek (10–60 mg kg^{-1}).[71,72] The content of other flavonoids in vegetables is very low with some exceptions such as flavanones in celery leaves (apigenin, 750 mg kg^{-1})[71] or anthocyanins in purple sweet potatoes.[73] In general, flavonol levels in processed foods are lower than in fresh products.[74] Crozier *et al*.[75] studied the effect of cooking on the quercetin content of onions and tomatoes. With both vegetables, boiling reduced the quercetin content by 80%, microwave cooking by 65% and frying by 30%. All vegetables contain phenolic acids such as hydroxycinnamates where either caffeic acid, ferulic acid, sinapic acid or coumaric acid has been conjugated with quinic acid and/or esterified with for example sugars.[76,77] According to Clifford[76] commercial varieties of American potato may contain up to 1400 mg kg^{-1} dry weight caffeoylquinic acids. In broccoli several hydroxycinnamic acid esters have been isolated in amounts of 62–148 mg kg^{-1}.[78]

Vegetable extracts such as root and tuberous crops (carrots, potatoes, sweet potatoes, red beets etc.), cruciferous vegetables (cabbage, Brussels sprouts, broccoli etc.), green leafy vegetables (lettuce, spinach etc.), onions, tomatoes and other vegetables have been screened for antioxidant activity using different oxidation systems and methods to measure antioxidant activity. Cao *et al*.[79] reported that the antioxidant score of vegetables measured by ORAC assay decreased in the following order: kale > garlic > spinach > Brussels sprouts > alfalfa sprouts > broccoli flowers > beets > red bell pepper > onion > corn > eggplant > cauliflower > potato > sweet potato > cabbage > leaf lettuce > string bean > carrot > yellow squash > iceberg lettuce > celery > cucumber.

Results on spiking plasma with vegetable extracts showed that beans, garlic, onions, asparagus, beet, potato and broccoli ranked highest in inhibiting the oxidation of the LDL and VLDL fractions.[80] On oxidation of pure methyl linoleate at 40°C, the antioxidant activity was the following: pea, legume > cucumber, leaf > pea > onion > carrot.[54] Compared to the poor activity (10–37% inhibition) of these vegetables in inhibiting lipid oxidation, the peel extracts of beetroot, sugar beet and potato showed remarkable antioxidant activity ranging from 86 to 99% inhibition. By measuring the ORAC, Gazzani *et al*.[81] reported that when prepared at 2°C, most vegetable juices showed initial pro-oxidant activity. This pro-oxidant activity was very high for eggplant, tomato and yellow bell pepper. In general the antioxidant activity increased after heat treatment suggesting that the pro-oxidant activity is due to peroxidases which are inactivated at high temperature during food processing.

3.9 Root and tuberous vegetables

Potato tubers (*Solanum tuberosum*), sweet potatoes (*Ipomoea batatas*), carrots (*Daucus carota*) and red beets (*Beta vulgaris* L.) all contain antioxidant substances, but they are very different types of chemicals. Potatoes contain ascorbic acid and are characterised by high levels of conjugated hydroxycinnamates,

present at 500–1200 mg kg^{-1} dry weight, and chlorogenic acid dominates.[82] The phenolics are concentrated in the potato skins; red skinned cultivars harbour up to 7 g kg^{-1} of *p*-coumaryl–anthocyanin conjugates in the peels and around only 25% of this level in the flesh[82] and pelargonidin-3-rutinoside-5-glucoside appears to be the dominant anthocyanin compound in red-fleshed potatoes.[83] Concentrated aqueous extracts of red and brown potato skins, respectively, contained up to 12.5 g kg^{-1} of hydroxycinnamates, and chlorogenic acid accounted for 60–65 wt% of these, followed by caffeic acid (22–24 wt%).[84] Ferulic acid and protocatechuic acid are also among the major phenolic acids in potato peels.[84]

Homogenised potatoes and sweet potatoes only exhibited medium ORAC compared with, for example, kale, garlic, spinach and onions.[79] Ethanolic extracts of whole potatoes have been demonstrated to reduce oxidising DPPH radicals and to inhibit linoleic acid oxidation in suspension.[85] More concentrated extracts of potato peels efficiently retarded carotene bleaching coupled to linoleic acid oxidation,[84] and slowed the oxidation of soybean oil (active oxygen method).[86] By 1964 hot water extracts of potato peels had been demonstrated to exert weak antioxidant activity in retarding development of thiobarbituric acid reactive substances when added to beef slices and in slowing the bleaching time of a carotene–lard solution adsorbed onto filter paper.[87] A large portion of the antioxidant activity of these extracts was ascribed to the presence of quercetin derivatives, caffeic acid and chlorogenic acid.[87,88] This assumption has been corroborated and refined in several later investigations, which attribute most of antiradical scavenging effects and antioxidant activities exerted by potatoes and potato extracts to the presence of chlorogenic, protocatechuic and caffeic acid.[85,86] Anthocyanins extracted from the flesh of coloured potatoes also delay oxidation when tested in an aqueous assay using linoleic acid.[89]

Methanolic extracts of sweet potatoes also exhibit antioxidant activity to retard linoleate oxidation. The phenolics in a methanolic sweet potato extract were identified mainly as caffeoylquinic acids, notably chlorogenic acid, and various 'iso' chlorogenic acids, but the antioxidant activity of this sweet potato extract was not directly related to the phenolic profile, being ascribed as a result of a synergistic action of both phenolic compounds and amino acids.[90] Peonidin glucoside, an anthocyanin purified from purple sweet potatoes, also exhibited antioxidant activity on linoleate oxidation.[73] Recently, a proteinaceous trypsin inhibitor isolated from sweet potatoes was demonstrated to be able to exert antiradical scavenging activity against the DPPH radical and to capture hydroxyl radicals as measured by electron paramagnetic resonance after addition of picomole levels of the inhibitor; the DPPH scavenging efficiency was about one-third that of glutathione.[91] Whether this radical scavenging efficiency may have any relation or quantitative relevance to true antioxidant effectiveness in food or biological systems remains to be investigated.

Carrots are very rich in alpha- and beta-carotenes that range in content from 4000–8700 µg per 100 g (alpha) and 7000–16 000 µg per 100 g (beta) in different orange carrot varieties.[92–95] The major phenolic compound in carrots is chlorogenic acid, but dicaffeoylquinic acids, and several other hydroxycinnamic–quinic

acid conjugates are also present; in total, the level of conjugated hydroxycinnamates is about 1.6 mg kg^{-1} and ascorbic acid contents are 30–50 mg kg^{-1} fresh carrot weight (Table 3.3).[92] Carrot blends and extracts only exert very weak antioxidant activities compared to other vegetables,[54,80] but extracts of carrot peel and leaves have been shown to inhibit formation of diene hydroperoxides in pure methyl linoleate at 40°C, although the inhibitory activities were ≤50% of those of potato peel extracts at the same level of addition.[54] At the time of writing, no clear relationship between the antioxidant activity of carrots and their contents of carotenoids, ascorbic acid or hydroxycinnamates has been recognised. Methanolic extracts of peels of sugar beet and red beetroot contain the same total level of phenolics (about 4.2 mg g^{-1} dry weight of starting material) and exhibited strong antioxidant activities in pure methyl linoleate at 40°C, almost blocking oxidation when 500 ppm dry weight base were added.[54] Betacyanins, the major colour compounds in red beets, were shown to exert potential antioxidant activities in various model systems, including isolated turkey muscle microsomes, human LDL and solubilised linoleate.[96] These compounds contain a phenolic and a cyclic amine group, where the structure of the latter resembles that of ethoxyquin, a strong antioxidant permitted for use in feeds in the USA (but not in Europe).

3.10 Cruciferous vegetables

Broccoli (*Brassica olearacea* L. cv Italica L.), Brussels sprouts (*B. olearacea* L. Gemmifera), red cabbage (*B. olearacea* L. cv Rubra), white cabbage (*B. olearacea* L. cv Alba) and cauliflower (*B. olearacea* L. cv Botrytis) have been reported to show significant antioxidant properties against lipid peroxidation.[97] Phenolic compounds such as flavonols and hydroxycinnamic acids in the cruciferous vegetables may be responsible for the antioxidant activity rather than the main bioactive compounds in cruficers, namely glucosinolates.[98,99] According to Plumb *et al*.[78] purified glucosinolates exhibited only weak antioxidant properties and thus are unlikely to account for the antioxidant effects of extracts from cruciferous vegetables. Compared to other vegetables and cauliflower, kale (*B. olearacea* L. cv Acephala), Brussels sprouts and broccoli were found to exert higher antioxidant activity.[70,80,97,100] White cabbage was reported to show more than 80% inhibition of coupled oxidation of beta-carotene and linoleic acid[81] and it was also an active hydroxyl radical scavenger.[98] However, while measuring lipid peroxidation in microsomes containing specific cytochrome P450s cabbage, cauliflower and Brussels sprouts were found to be pro-oxidants.[72] Food processing involving heat treatment seems to have different effects on various cruciferous vegetables depending on the choice of the antioxidant activity measurement. Boiled (15 min) Brussels sprouts were found to promote peroxidation of human liver microsomes and of phospholipid liposomes,[98] while boiled (5 min) broccoli exhibited 96% inhibition of oxidation of beta-carotene linoleic acid emulsion[100] and boiling for up to 30 min improved the antioxidant activity of white cabbage.[81]

3.11 Other vegetables

Onion (*Allium cepa*) has been studied for antioxidant activity both in lipid oxidation models[54,81,87,88,97,100–102] and in radical scavenging assays.[79,80] Both yellow and red onion were shown to be poor antioxidants towards oxidation of methyl linoleate,[54] moderately active towards coupled oxidation of beta-carotene and linoleic acid[102] and highly active towards oxidation of lower density lipoproteins.[81] Onion had also a poor antioxidant score in the ORAC activity test while garlic (*Allium sativum* L.) expressed a score four times higher.[79] Yin and Chen[103] reported that the presence of garlic bulb, garlic greens, Chinese leek, scallion, onion bulb and shallot bulb significantly delayed lipid oxidation of phosphatidylcholine liposomes. While a thiosulphinate, allicin, is responsible for the antioxidant activity of garlic bulb,[104] compounds other than allicin are involved in determininig the antioxidant effect of other *Allium* members.

Makris and Rossiter[101] assessed the impact of domestic processing, including chopping, maceration and boiling on onion bulbs. While quercetin 3,4′-diglucoside and quercetin-4′-monoglucoside were virtually unaffected by chopping, boiling for 60 minutes caused overall flavonol losses of 20.6% in the onions. In contrast, Ewald *et al.*[105] reported that the greatest loss of quercetin and kaempherol in onion took place during the peeling, trimming and chopping before blanching. Further processing by cooking, frying and warm-holding of blanched onion had only a small effect on flavonoid content. Chopping did not considerably influence the antioxidant capacity of onion bulbs, but boiling did provoke notable changes measured by the coupled oxidation system of beta-carotene and linoleic acid.[101] Boiling of juiced onion for 10 min resulted in pro-oxidant activity that was reversed into antioxidant activity with prolonged heat treatment.[81] On the other hand, incubation of pulped onion at 37°C resulted in improved antioxidant activity partly caused by the enzymatic (endogenous glycosidases and glycosyltransferases) conversion of quercetin diglucosides into the monoglucoside and aglycone forms.[102] After six hours of incubation, 75% of the total quercetin existed in the aglycone form.[106] It was suggested that the increment of antioxidant activity through enzymes naturally present in vegetables could be used to replace food antioxidants.

The antioxidant activity of green leafy vegetables such as spinach (*Spinacia olearacea* L.) has been reported to be low against inhibition of oxidation of LDL[80] and moderate towards oxidation of linoleic acid.[97] On the other hand spinach expressed a very high ORAC activity while that of leaf lettuce and iceberg lettuce was poor.[79] According to Beom *et al.*,[106] blending spinach with other vegetables resulted in increased antioxidant activity in iron-catalysed model systems. Differently processed, that is, minced or enzymatically juiced spinach samples, were found to inhibit formation of lipid hydroperoxides but to act as pro-oxidants in cooked meat.[107] The antioxidant activity of spinach decreased during storage after modified atmosphere packaging (MAP) which could be due to decrease in the ascorbic acid content.[108] The authors also reported a 50% loss of total flavonoids and 60% loss of vitamin C in the cooking water while boiling spinach. However,

the vitamin C content of the cooked tissue was higher than in spinach stored in MAP.

Tomato (*Lycopersicon esculentum*) was reported to exert antioxidant activity in some studies[86,88] while in other experiments it showed no antioxidant activity[109] or acted as pro-oxidant.[85] In beef homogenates, tomato significantly inhibited lipid peroxidation.[81] The antioxidant effect of tomato is most likely to result from synergism between several phytochemical compounds and it is not due to lycopene content alone as pure lycopene and several other carotenoids act as pro-oxidants in the lipid environment.[100,110,111] In a study by Wenli *et al*.,[112] lycopene concentrate extracted from tomato paste containing 50% lycopene and 50% other lipid-soluble substances (probably including tocopherols) was shown to scavenge oxygen radicals effectively and to inhibit lipid peroxidation. Lycopene in tomatoes seems to be more stable compared to other carotenoids to changes during peeling and juicing of vegetables.[113] Among commercial juices tested, tomato juice has a higher oxygen radical absorbance capacity than orange juice and apple juice.[52] According to Anese *et al*.,[114] antioxidant activity of tomato juice decreased after an initial 2–5 hours of heating but was restored after prolonged heating. Gazzani *et al*.[81] report that while boiled vegetable juices were generally found to exert antioxidant activity, tomato juice was pro-oxidant. These contradictory findings may be explained by differences in the amounts of the antioxidant compounds in the tomato juices because Gazzani *et al*.[81] used a filtration method resulting in loss of most of the juice coloration. Apart from lycopene, another interesting antioxidant compound, naringenin chalcone, is present in tomato skin ($64\,mg\,kg^{-1}$) and may be present in juice, paste and ketchup.[55] In tomato processing to ketchup, naringenin chalcone is transformed to naringenin.

3.12 Effect of different processing technologies on antioxidant activity

Food processing involves changes in structural integrity of the plant material and this produces both negative and positive effects. When the negative and positive effects counterbalance each other, no change in the antioxidant activity occurs.[115] The antioxidant activity is diminished owing to inactivation of antioxidant compounds caused by oxidation, for example, by enzymes (polyphenoloxidase and others) or leaching into the cooking water. Both negative changes have a greater impact on the water-soluble antioxidants, vitamin C, flavonoids and phenolic acids, than on the lipid-soluble antioxidants, carotenoids and tocopherols. The positive effects of food processing include transformation of antioxidants into more active compounds, such as the deglycosylation of onion quercetin,[106] as well as an increase in the antioxidant activity owing to inhibition of enzymes.[81] Peeling and juicing result in substantial losses of carotenoids, anthocyanins, hydroxycinnamates and flavanols as the fruit and berry skins and vegetable peels are very rich in these antioxidant compounds. However, the antioxidant activity of fresh fruits and berries is comparable to that of their processed products such as juices

and wine.[4,29,111] Also the antioxidant activity in tomato juice was comparable to that of fresh vegetables in most studies.[52,114]

3.13 Future trends

Dietary antioxidants are gaining a considerable amount of interest as bioactive components with possible health effects. The physiological role of some of these antioxidants, such as vitamin E and vitamin C, is well established. Intervention trials with beta-carotene have proved disappointing with respect to finding a possible positive biological role of dietary carotenoids. On the other hand, flavonoids are very effective antioxidants and it has been proposed that they protect against cardiovascular disease by reducing the oxidation of LDL. There is some epidemiological evidence for this, but as flavonoids are generally absorbed only in very small amounts, their bioactivities *in vivo* remain to be established. A European collaboration has been formed (QLK1-1999-00124, 2000-January 2003) to examine the functional properties, bioavailability and bioactivities of dietary anthocyanins especially towards human cardiovascular health. Thus, before any new information on the identity, bioavailability and bioactivities of dietary antioxidants emerges, the general advice to increase fruit, berry and vegetable consumption both as fresh products and as processed foods remains valid.

3.14 Sources of further information and advice

As interest in functional foods and other products with possible health effects is escalating a large number of industrial enterprises are now producing various 'antioxidant' concentrates. Industrial enterprises range from the traditional juice producers and large companies specialising in natural flavours and colours to new companies specialising in health promoting supplements. There is a sparcity of published knowledge available on the molecular composition and the proven health effects of most of these antioxidant concentrates, but many of them are nevertheless claimed and marketed as having potential physiological benefits, or at least to 'supply high amounts of antioxidants'. Some caution in the evaluation of these advertisements is recommended. At the time of writing, the precise action mechanisms of antioxidants and their individual and combined efficiency have not been elucidated in detail. Thus, despite our rather detailed understanding of the various mechanisms by which natural antioxidants may act, it is currently difficult to predict the activities and efficiencies of various plant extracts of mixed composition without knowing the compositional profiles of the preparations. In addition, certain natural antioxidant phenols may act synergistically or even antagonistically, which further complicates predictions of antioxidant effectiveness of mixed concentrates. Therefore, marketing of most natural antioxidant concentrates is based only on empirical knowledge from tests in model systems. In nutrition, only very limited knowledge is available on the potential long-term

effect of an elevated intake of natural antioxidants when they are consumed in their concentrated form, even when extracted from natural sources of fruits, berries and vegetables. Much more research is therefore needed on the antioxidant effects of natural antioxidant mixtures, on the influence of various types of processing on natural antioxidants and on the possible influence of the natural matrix on the antioxidant and nutritional effects.

For further information, the following review articles and books are recommended:

FRANKEL E N and MEYER A S, 'The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants', *J Sci Food Agric*, 2000 **80** 1925–41.

LINDSAY D and CLIFFORD M (ed), 'Special issue devoted to critical reviews produced within the EU concerted action 'Nutritional enhancement of plant-based food in European trade' (NEODIET)', *J Sci Food Agric*, 2000 **80** 793–1137.

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3.15 Abbreviations

- AAPH: 2,2′-azobis(2-amidinopropane) dihydrochloride
- $ABTS^{\bullet+}$: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonate) radical cation
- BHT: butylated hydroxytoluene
- DPPH: 2,2-diphenyl-1-picrylhydrazyl
- GAE: gallic acid equivalents
- IC_{50}: 50% inhibition
- LDL: low density lipoprotein
- MAP: modified atmosphere packaging
- NADPH: nicotinamide adenine dinucleotide hydrogen phosphate
- ORAC: oxygen radical absorbance capacity
- TBHQ: tertiary butylhydroquinone
- TEAC: trolox equivalent antioxidant activity
- TRAP: total radical trapping parameter
- VLDL: very low density lipoprotein.

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4

Improving the nutritional quality of processed fruits and vegetables: the case of tomatoes

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4.1 Introduction: role of processed fruits and vegetables in the modern diet

A basic idea on which all nutritional scientists can agree is that the increased consumption of diets rich in a variety of fruit and vegetables will improve the health of almost any human population. This diet (of which the Mediterranean diet is the best example) is known to be beneficial for health, especially with regard to the development of chronic degenerative diseases.[1,2] Tomato is one of the most used of the fruits and vegetables in the Mediterranean diet. Therefore, tomatoes appear to be especially important in terms of public health as they are consumed in large quantities and are rich in several compounds believed to provide protection from or reduce the risk of contracting chronic degenerative diseases.

Vegetable products, including tomatoes, contain many substances which may have beneficial effects on health, providing protection from certain pathologies correlated to oxidative processes. These substances have differing functions, such as free radical scavengers, singlet oxygen quenchers, metal chelants and inhibitors of enzymes involved in the formation of the active species of oxygen.[3] Epidemiological studies have demonstrated that tomato consumption provides a protective effect against some types of cancers and ischaemic heart diseases. This protective effect has mainly been ascribed to the antioxidant activity of some tomato components.

Carotenoids are among the first compounds to have attracted the attention of scientists to the effect on health of fruit and vegetables, and tomato is especially

rich in one of them: lycopene. Tomato is the main dietary source of lycopene, the typically red-coloured carotenoid. Other carotenoids, such as β-, γ- and ζ-carotene, lutein, phytoene and phytofluene, are also present, though in much lower concentrations, with vitamin C, and vitamin E in the seeds.[4] Moreover, there is a growing interest in other compounds present in tomatoes, like folates and phenolics, though not enough human studies are available to estimate properly the effect of phenolics particularly on human health. Tomato is an important source of ascorbic acid which exerts a well-known antioxidant and nutritional effect. Like other vegetables, the tomato contains a number of polyphenolic compounds which can exert antioxidant activity.[5] Finally, tocopherols are also found in tomatoes, though in low concentrations.[6] The antioxidant composition of the tomato is complex and rich, and optimisation criteria of processing and storage technologies should take into account the preservation of the whole antioxidant pool and its functional properties.

4.2 Processed tomato products

It is important to note that a large proportion of tomatoes are eaten in the form of industrially processed products. Tomatoes can easily be processed into several products which are consumed in large amounts. During this processing, the main components of tomatoes are preserved and even concentrated. Because of this widespread and large consumption, tomatoes appear as one of the most interesting foods in terms of health.

These findings introduce novel optimisation criteria and goals for processed tomato products. If it is clear that the starting point for the optimisation of tomato nutritional properties is the raw material, great attention must also be paid to avoiding or minimising the detrimental effects induced by technological processing and by the storage of processed products.

Between 25 and 30 million t of tomatoes are processed annually, more than one-third of the overall 70 millions t produced each year. The average per capita consumption is nearly 3.5 kg (on a fresh tomato basis), with variations from zero in some populations, to 14–15 kg in the EU, and to more than 30 kg in Italy and the USA.

The tomato is now the most important vegetable product used in the making of industrial preserves. The 'traditional' tomato-growing nations are the USA, Italy, Greece, Spain, Portugal and France, to which have gradually been added Turkey, the countries of north Africa, Israel, Canada, Mexico, Chile and Brazil and, more recently, China, the southern republics of the former USSR, Australia, Thailand, India and South Africa. Table 4.1 shows the production data for the most recent production campaigns, from which it can be seen that, although this is an industry which produces products of relatively low added value, the USA and the EU alone process 70% of the world's entire tomato production.

Tomatoes can be processed into a number of different products, such as:

Table 4.1 Quantity of processed tomatoes in the more important using countries (in thousands of t) since 1975*

Country	1975	1980	1985	1990	1995	1999
USA	7715	5646	6525	9307	10235	11724
Italy	1575	3083	3785	3850	3535	4900
Turkey	520	600	1100	1500	1920	1800
Spain	827	499	819	1134	916	1480
Greece	979	1500	1180	1150	1178	1200
Brazil	*	*	*	600	930	1100
Portugal	800	454	716	760	831	996
China	*	*	*	420	550	900
Chile	*	275	*	609	822	900
Tunisia	*	*	–	100	435	720
Canada	350	379	476	580	524	480
France	280	416	392	340	281	370
Argentina	*	*	–	267	190	330
Mexico	210	220	230	365	275	310
Israel	163	166	257	300	315	287
Total	*	*	*	21282	22937	27497
World	*	*	*	**22821**	**24959**	**29592**

* Some data have been excluded because of uncertainty.

- *tomato preserves* (such as whole peeled tomatoes, tomato juice, tomato pulp, tomato puree, strained tomatoes, diced tomatoes, tomato paste). Whatever the technological flowsheet, these products are finally packed (tinplate cans, glass jars, multilayer bags) and stabilised by heat treatment;
- *dried tomatoes* (tomato powder, tomato flakes, whole, halved and sliced dried tomatoes). These products are dehydrated by different techniques and low water activity represents the stabilising factor;
- *tomato-based foods* or *tomato-containing foods* in which tomato is one ingredient (such as tomato soup, tomato sauces, chilli sauce, ketchup, bolognaise sauce, etc). In this case many other ingredients can be added to make up the final product, which is canned and stabilised by heat treatment. The variety of tomato-containing foods makes it impossible to identify a general processing flowsheet. Products can be heat treated, refrigerated, frozen or dehydrated, and can be stored in different conditions, depending on their stability.

In addition, many of the above-mentioned products require further home processing before consumption, such as cooking, baking or rehydration.

4.3 Nutritional quality of processed tomato

Processed tomatoes, and in particular tomato paste, have always been considered ‘poor’ products with a low added value destined for use as a basic ingredient in

more elaborate products (sauces, ketchup), both for domestic and manufacturing purposes. These semi-processed products are seen as commodities dominated by price rather than finished products which can command a premium in the market through their intrinsic qualities.

Today, the consumer faces new socio-economic and therefore food factors which tend to favour service (or convenience) quality. A service which, first of all, meets the requirements of new life systems but which also takes into account the renewed attention to hygienic and dietary aspects of food i.e. its nutritional quality, particularly in the light of the supposed antioxidant activities of some microcomponents, particularly lycopene. Tomato products are important foods from a sensory point of view, with good service quality and positive effects towards the prevention of the most important and common diseases of the modern world.

4.4 Macrocomponents

Fresh tomatoes and other processed tomato products make a significant contribution to human nutrition owing to the concentration and availability of several nutrients in these products and to their widespread consumption. Composition tables show that ripe tomato (*Lycopersicon esculentum*, Mill.) contains 93–95% water and low levels of solid matter.

Tomatoes contain usually from 5.5 to 9.5% total solids, of which about 1% is skins and seeds. The percentage of solids in tomatoes varies over wide limits for a number of reasons, such as variety, character of soil and especially the amount of irrigation and rainfall during the growing and harvesting season.[7]

In as much as tomato products, such as pulp and paste, are evaporated to a definite specific percentage of solids, their yield per t of tomatoes varies with the composition of the raw tomatoes used in their manufacture. In tomato juice, the fraction of insoluble solids (cellulose, lignin, pectic substances) varies from 15 to 20% of total solids.[8]

The soluble solids are in main part constituted by free sugars. The free sugars of tomatoes are predominantly reducing sugars.[9] The quantity of sucrose found in tomatoes is so negligible that it may be ignored for all practical purposes.[10] Sucrose rarely exceeds 0.1% on a fresh weight basis. The reducing sugars, which usually make up from 50 to 65% of tomato solids, are mainly glucose and fructose. The total sugar content of fresh tomato is found to vary from 2.19 to 3.55%.[11] Leoni[8] reported that, in general, more fructose than glucose was present (ratio 54/46). The polysaccharides in tomatoes make up about 0.7% of tomato juice. Pectins and arabinogalactans constitute about 50%, xylans and arabinoxylans about 28% and cellulose about 25%.[9]

The acid in tomatoes is generally considered to be almost entirely citric, and free acids are almost always determined as citric monohydrate. Some workers have reported the presence of malic acid in quantities often exceeding those of citric acid, while traces of tartaric, succininc, acetic and oxalic acids have also been reported. Chromatographic analyses reported by Miladi *et al*.[9] have

separated eight organic acids from tomato juice. Malic acid was found to be the second major organic acid in fresh juice whereas pyrrolidone carboxylic acid was found to be the second major organic acid in the processed juice. Processing of tomato juice results in an increase in total acid. It was found that acetic acid is increased by 32.1% apparently owing to oxidation of aldehydes and alcohols during processing and deamination of amino acids, such as the breakdown of alanine via pyruvic acid. An increase in citric and malic acids after processing was also noted. Crean[12] indicated that sugars can decompose on heating in the presence of acids to give acetic, lactic, fumaric and glycolic acids.

There are 19 soluble amino acids in fresh tomato juice. Miladi *et al*.[9] reported that glutamic acid makes up to 48.45% of the total weight of amino acids in fresh tomato juice. Second highest in concentration is aspartic acid. The amino acid with the lowest measurable concentration is proline. Processing of fresh tomato juice at 104°C for 20 min results in a substantial increase in the free amino acids as a result of denaturation and partial hydrolysis of protein. The greatest increase occurs in glutamic and aspartic acids, alanine and threonine. Asparagine and glutamine disappear during processing owing to the loss of amide ammonia (NH_3) to form glutamic and aspartic acids, which partially account for the increase in ammonia in canned juice. It could also be due to glutamine and asparagine deamination and formation of pyrrollidone carboxylic acid.

4.5 Microcomponents of nutritional interest: minerals

Of the minerals present in tomatoes, iron is the most important in terms of providing adequate nutrition. A glass of tomato juice provides about 2.0 mg iron in the reduced ferrous state. This concentration is important both because it is 10–20% of the RDA of iron and because it is consumed in a product that also provides ascorbic acid, which helps retain the iron in its reduced state and is necessary for iron absorption.[13]

4.6 Microcomponents: antioxidants and vitamins

Ripe tomatoes are relatively rich in antioxidants: vitamin C (160–240 mg kg^{-1}), lycopene (30–200 mg kg^{-1}), provitamin A carotenes (6–9 mg kg^{-1}) and phenolic compounds; flavonoids (5–50 mg kg^{-1}); and phenolic acids (10–50 mg kg^{-1}).[14] Also present in small quantities are vitamin E (5–20 mg kg^{-1}) and trace elements such as copper (0.1–0.9 mg kg^{-1}), manganese (1–1.5 mg kg^{-1}) and zinc (1–2.4 mg kg^{-1}) which are present in several antioxidant enzymes. Most often the tomato variety is not indicated and the reported values are a mean concentration of the constituents in tomatoes found in local markets.

Whole red-ripe tomatoes contain nearly all the vitamin C activity in the reduced ascorbic acid form. Dehydroascorbic acid has been reported to consti-

tute 1–5% of the total ascorbic acid in tomatoes.[15,16] The ascorbic acid concentration in fresh ripe tomatoes is about 25 mg per 100 g. Thus, a small tomato supplies about 40% of the adult US recommended daily allowance (RDA) of 60 mg and about two-thirds of the RDA of 40 mg for children. A glass of tomato juice supplies about 35 mg of ascorbic acid or about 60% of the adult RDA and 85% of the RDA for children.

Tomatoes are also a good source of vitamin A, present in the form of carotene. Fresh ripe tomatoes and tomato juice contain 1000 international units (IU) of vitamin A per 100 g. Booker *et al*.[17] gave a figure of 1150 IU per 100 g. On the basis of these figures, a small tomato or glass of juice should supply 20% or more of the adult recommended daily allowance (RDA) of 5000 IU. It is clear, therefore, that in relation to the average consumption, the tomato makes a very important contribution to the vitamin A requirement of the human diet.

Tomatoes also provide small amounts of the B complex vitamins: thiamine, niacin and riboflavin. The content of thiamine, reported in various sources cited by Leoni,[8] varies from 16 to 120 mg per 100 g of ripe fruits and juice. On this basis a small tomato contains only about one-tenth of the RDA for an adult man. The same sources indicated the riboflavin and niacin contents of tomatoes to be rather low (20–50 mg per 100 g for riboflavin and less than 1 mg for niacin). On the basis of these values it is evident that tomatoes make a very small contribution to the 1.7 mg of RDA of riboflavin and 20 mg of niacin required for adults.[11] Cultivars and environmental conditions, such as exposure to light, are also important. From a practical standpoint the stage of ripeness is not an important consideration here because tomatoes are usually canned or consumed only when ripe and because of this the method of ripening seems to have little effect.

4.7 Microcomponents: lycopene and other carotenes

During ripening, tomatoes change in colour from green, typical of chlorophylls, through pink-orange to bright red, owing to the development of carotenoids. These are polyenes, in particular tetraterpenes, which originate from a head-to-tail condensation (with 1,4-bonds) of several isoprenic units; they are divided into xanthophylls, which are oxygen-containing carotenoids and carotenes, consisting solely of hydrogen and carbon atoms. They present a long chain of double bonds, most of which are conjugated. This chain is responsible for their typical absorption of light in the visible region. During ripening, the chlorophylls gradually disappear and become undetectable 7 days after the breaker (turning) stage, tomatoes change in colour from green, typical of chlorophylls, through pink-orange to bright red, due to the development of carotenoids. At the turning stage, lycopene content considerably increases and can reach 80–100 mg kg^{-1} fresh matter at the red stage.[18,19]

Of the carotenoids occurring in ripe tomatoes, lycopene, red in colour, is the last to form and its formation increases especially after the breaker stage (colour

change from green to pink) of the berry. Earlier literature reported that lycopene was found only in the red-coloured strains.[20] So far, little is known about the effect of agricultural practices and soil/climate factors on the oxidant content of tomatoes. It is plain, however, that factors such as water, fertilisation, temperature and light have a bearing on carotenoid level in tomatoes, as have variety, degree of maturity, harvest date, fruit growth and post-harvest storage.[21] Lycopene formation, for example, is inhibited at temperatures above 30–32°C, whereas it is favoured at temperatures from 16 to 21°C.

The other carotenes (hydrosolubles and lyposolubles) occur in concentrations lower than that of lycopene which, in ripe tomatoes, accounts for 85% of total carotenoids.[8] Within the fruit, the lycopene level is higher in the outermost part of the mesocarp's cells; here it builds up in vesicles (aged chloroplasts) which originate from chloroplast transformation–degeneration and which form, with the carotenoid molecules, the so-called LHC (light-harvesting complexes). These complexes consist of sequences of hydrophobic membrane-linked proteins containing several pigment molecules coagulated in the form of elongated needle-shaped crystals. Voet and Voet[22] and Laval-Martin[23] categorised tomato chromoplasts into two types: globulous chromoplasts, containing mainly β-carotene found in the jelly part of the pericarp and other chromoplasts found in the outer part of the pericarp containing voluminous sheets of lycopene. The development and ultrastructure of these sheets of lycopene were studied by Ben-Shaul and Naftali[24] and named crystalloids (or coagula, or clots).

Among the factors that influence the biosynthesis of these compounds are, in addition to temperature (already mentioned), degree of maturation and light; in particular light favours conversion of phytoene to lycopene and β-carotene. Lycopene, like all carotenoids, is an apolar, photosensitive substance. In aqueous suspension, it is in practice stable to high temperatures and oxidation whereas, in solution in organic solvents, it is quite sensitive to both.

In work carried out by Sandei *et al.*,[25] several commercially processed tomato lines have been evaluated in order to compare their lycopene content in fresh material (Table 4.2). The range varied between 2.0 and 3.4 g kg^{-1} of dry matter

Table 4.2 Lycopene content in ripe fruits of several cultivars of processed tomatoes[25]

Cultivar	Seed company	Fruit shape	Lycopene mg per kg of dry matter
DR 10747	De Ruiter	Blocky long	2831
Nun 1365	Nunhems	Square round	2131
To 0426	Peotec	Egg	2395
Forum	Peto ital	Egg	2093
Perfectpeel	Peto ital	Square round	2154
PS 1617	Peto ital	Egg	3457
Nema Crimson	United Genetics	Square round	2043

(100–170 mg kg^{-1} of fresh fruit with a value of 5° Brix). All-*trans* lycopene is the predominant isomer in tomatoes and tomato products (~95%).[26]

4.8 Behaviour of nutrients during processing: vitamins

The vitamin C and vitamin A content of processed tomato products manufactured without fortification is necessarily less than or nearly equal to that of the fresh tomatoes from which the vitamins were produced. The maintenance of high levels of ascorbic acid in products during processing has received considerable attention by food technologists. In the manufacture of tomato juice, ascorbic acid is destroyed, mainly by oxidation (enzymatic or non-enzymatic). The rate of oxidation is dependent on the dissolved oxygen, enzyme content, dissolved copper and temperature of the juice. The longer the tomato juice is held at optimum conditions for oxidation the lower will be the retention of ascorbic acid after processing.

Clifcorn and Peterson reviewed the retention of ascorbic acid during tomato juice manufacture.[27] They reported that an average of 63–70% retention was found during three separate plant surveys and that in some plants retention as high as 94% had been achieved. They emphasised that in plants where retention was high, total elapsed canning time was short (2–3 min) and those conditions that increased the oxidation rate were minimised.

The temperature to which tomato products such as tomato juice are heated in the presence of air is the most important factor in the rate of ascorbic acid destruction; it has been found that the rate of ascorbic acid destruction increases with increased temperature in the presence of air. It is therefore important that juice be brought to the desired temperature as quickly as possible and held for only a short period at high temperature. Guerrant *et al*., cited by Gould,[11] showed that retention of ascorbic acid was greater (92%) after a 15 s preheat before extraction at 57°C. Retention decreased to 54% after a 35 min preheat at 88°C. While cold extraction at 49°C has been suggested, later work has shown that retention is nearly equal for hot- and cold-break processes if the juice is not held at high temperatures while exposed to air for long periods of time prior to extraction.[27] A hot-break process is where juice is extracted after heating to more than 90°C (with inactivation of the pectolytic enzymes). A cold-break process is the same operation carried out at 60–70°C (but without inactivation of the pectolytic enzymes). Any unit operation that incorporates air into the juice will accelerate oxidation of ascorbic acid. Concentrated products present a further problem in retaining vitamin C.

Prolonged heating accompanied by exposure to air resulted in some destruction of vitamin A potency. Prolonged heating also decreased retention of the B vitamins. Cameron[28] reported an average retention of 89% for thiamine, 97% for riboflavin and 98% for niacin.

4.9 Behaviour of nutrients during processing: lycopene

Data and information supplied in the scientific literature on lycopene degradation during common tomato processing, such as heat sterilisation, concentration by

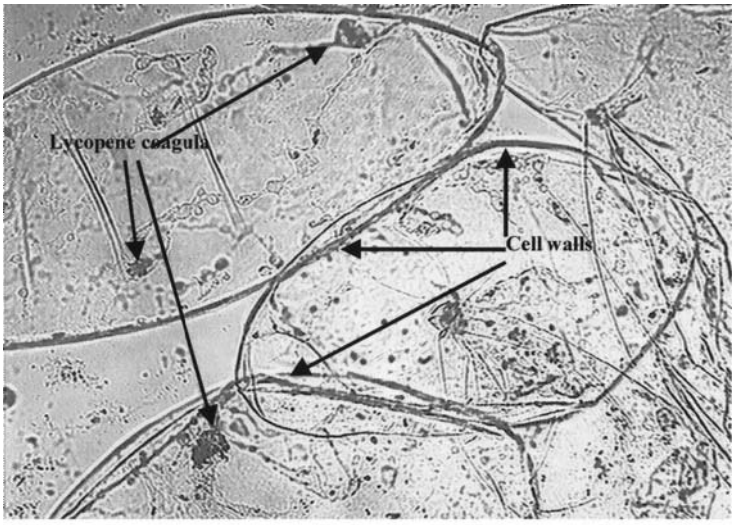
evaporation and dehydration, and also information and data on storage of processed tomato products, though sometimes inconsistent or not completely clear, allow for some general conclusions and comments. Since the operating conditions applied to the tests are either not well defined or do not correspond to those used for industrial treatments, the results should be considered as being often unreliable.

The data seem to suggest that lycopene is stable to heat treatment for tomato concentration and cooking and also during processed tomato storage. The stability is lower for products submitted to treatments which have damaged the cell walls and which have consequently reduced the protective effect with respect to lycopene coagula (Fig. 4.1).[29] Exposure to oxygen, high temperature and low water activity may cause lycopene degradation. Researchers substantially agree that this compound is stable in commercial production processes, in terms of both degradation and isomerisation rate. Even air drying, which is a really severe treatment in terms of oxidative stress, does not cause serious lycopene losses.[30–32] Some of these studies witnessed a relatively high lycopene loss and isomerisation in heat-treated tomato products; a possible reason for these results, which are in contrast to other data, could be the differing analytical methods and procedures that were applied. Various authors have demonstrated that lycopene is much less stable towards isomerisation and oxidation when it is solubilised in organic solvent.

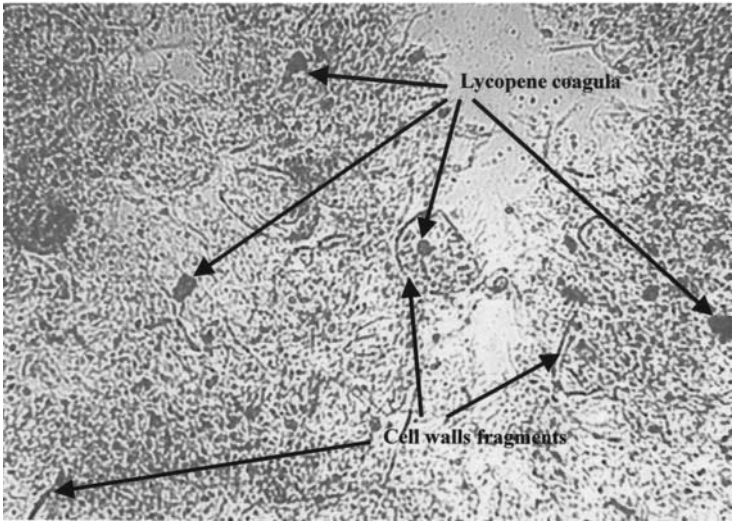
FAIR Concerted Action 97-3233 made an assessment of the available literature which seems to indicate that lycopene is relatively stable during heat treatment, that it possesses a fair stability during storage, with only slight reduction under severe oxidative conditions such as hot-air drying and a light discoloration during deep freezing.[29] However, much research does lead to contradictory conclusions about the supposed marked degradation effect of storage, probably owing to the fact that lycopene was frequently measured in second-stage tomato products, with high oil/fat percentages (sauces) which facilitated partial solubilisation of lycopene and its subsequent higher reactivity and degradation.

There are no data about how the lycopene content is affected by non-traditional heating processes such as microwaves, ohmic treatments and non-thermal pasteurisation processes using high pressure technology.

It therefore seemed interesting to report the results of the effects of storage conditions on the lycopene content of tomato purees obtained by different processing techniques, by summarising the trials conducted by Tamburini *et al.*[33] using a pilot plant. Samples of tomato puree were prepared first by extracting the juice according to a conventional technique, varying the extraction temperature (at ambient temperature, series marked F, at 60°C or cold break, series marked C and at 90°C or hot break, series marked H) and pulper hole size (ø 8/10 mm, series marked 8 and 13/10 mm, series marked 13), then by vacuum-concentrating the juice to 8°Brix and finally by hot filling it into lacquered tinplate cans. The samples obtained were subjected to different storage conditions and the changes in lycopene content were monitored over a 12-month period. In this way, six kinds of tomato puree with different physical characteristics (consistency, colour and granulometry) were obtained.



(a)



(b)

Fig. 4.1 Microscope photographs of a fresh tomato sample (a) and tomato juice sample (b). Reproduced courtesy Volker Böhm – Institute of Nutrition Friedrich Schiller University Jena.

Tables 4.3 and 4.4 summarise all the results; lycopene content values represent the mean of the different storage conditions (temperature) and the different effects of the various juice extraction treatments are highlighted. All in all, the better preservation of lycopene in the hot break-treated product is clearly shown graphically, whereas no granulometry induced protective effect seems to occur, at least under the conditions tested. As the data show, no difference occurs with the different processing techniques applied.

Table 4.3 Physicochemical characteristics of the purees obtained under different extraction conditions

Analyses	Samples					
	F8	C8	H8	F13	C13	H13
°Brix	8.20	8.20	8.33	8.20	8.17	7.98
L	25.67	25.75	26.40	24.73	24.72	25.37
a	31.90	31.94	31.48	30.04	31.37	31.40
b	14.39	14.76	15.13	14.05	14.04	14.36
*a**b*	2.22	2.17	2.08	2.14	2.36	2.18
Lycopene, mg kg^{-1}	201	192	192	196	188	204

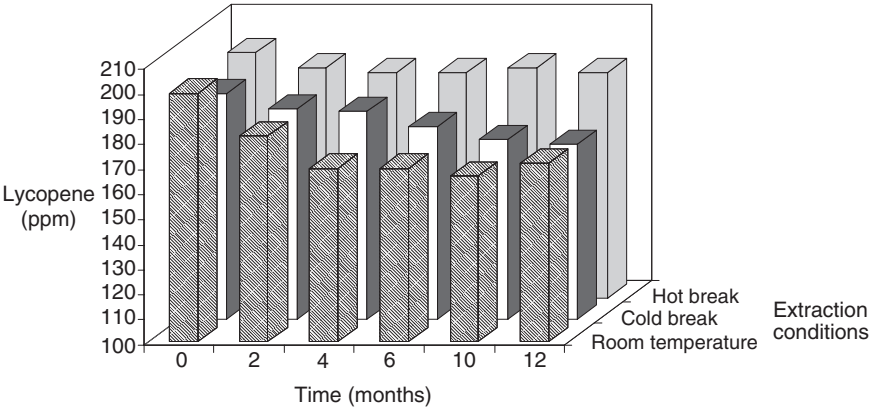


Fig. 4.2 Behaviour of the lycopene during 12 months' storage, as a function of the juice extraction conditions.

During storage, as shown in Fig. 4.2, a slight decrease in lycopene content occurs which seems to be linked to the type of heat treatment applied for juice extraction. An examination of the data obtained seems to reveal that lycopene is substantially stable to heat treatments, which reports the mean values of the variation over time in the lycopene content for all the samples. However, a detailed analysis of the changes occurring over time in samples prepared under different temperature conditions (ambient temperature, cold-break, hot-break) reveals that a hot-break process, although typically involving blanching at high temperature (more than 90°C) for some dozens of seconds, and which could cause a considerable decrease in lycopene content, actually lessens lycopene levels only to a limited extent. It seems to preserve the pigment over time better than either the cold-break process or extraction at room temperature. Lycopene content in the

Table 4.4 Variation over time in lycopene contents (total samples)

Storage time (months)	Lycopene ± s.d. (mg kg^{-1})
0	195.5 ± 1.1
2	188.0 ± 1.1
4	180.7 ± 1.1
6	177.4 ± 1.1
10	176.6 ± 1.1
12	176.9 ± 1.1

samples which have undergone the enzyme-inactivating hot-break process (90°C, samples H8 and H13) remain almost unchanged even after 12 months of storage, whereas the small variations in lycopene content of the cold-break-treated samples (C8 and C13) are similar to those occurring in samples which have received no heat treatment (F8 and F3).

The hot-break juice extraction technique lessens the initial lycopene content because of the severe heat effect, but in allowing the tomato cell structure to remain almost unchanged, preserves lycopene more efficiently over time against reactions that cause its destruction. This is different from what happens with other juice extraction techniques (at room temperature, cold break), where because the protective effect caused by the triggering of pectolytic reactions is absent, a decrease in lycopene content of 8–15% has already occurred after 4–6 months' storage.

The other effects that were analysed (diameter of the pulper holes and storage temperature) cause no significant technologically interesting variations in lycopene content. The results reported so far lead us to the conclusion that while lycopene remains within the original hydrophilic matrix and most of all, within a whole cell, it is quite stable. However, because of the subsequent low reactivity, it probably exhibits lower bioavailability and therefore could in practice be ineffective in exerting its potential antioxidant activity.

The study of the effect of manufacturing processes in the preparation of tomato powders on lycopene content and on colour carried out by Cabassi *et al*.[32] highlighted a moderate loss (5%) of total lycopene content which can be traced back to isomerisation and oxidation phenomena. A comparison of the lycopene content found when different flexible packaging materials were used showed that the greatest preservation of lycopene was obtained by vacuum packaging in Al/polythene pouches. A very good result was also obtained using nitrogen packaging in polymer (polyethylene and polyvinylacetate) trays sealed with a polyethylene terephthalate film. In the packages containing air (and therefore oxygen) lycopene loss was decidedly higher (22–25%). The storage time showed a significant effect which was reflected in an average 13% decrease in total lycopene in the samples during the first month of storage. However, it must be noted that this average

value reflects moderate decreases in vacuum and nitrogen packaging (2%) compared with packaging in the presence of air (24%). The more marked effect found during the first month of storage of the powders suggests that some lycopene, probably that present on the air-exposed surface, is more sensitive to the action of oxidants compared with the lycopene inside the granules themselves.

4.10 Bioavailability of lycopene

Lycopene content must be distinguished from lycopene bioavailability. The above mentioned studies are incomplete because they only measured the lycopene content, not its bioavailability, which is most important for the nutritional quality of the product. Therefore, the most stimulating object of research on lycopene is, perhaps, to evaluate the actual bioavailability for humans in the forms in which lycopene is present in processed tomato products.[34]

Although there are a number of comparative studies on the bioavailability of lycopene in tomato products, there are no proven methods for the quantitative assessment of carotenoid bioavailability, even of β-carotene which has been the most frequently studied. A few studies have been carried out on the bioavailability of lycopene in the human diet. Some of them indicate that the absorption of lycopene is greater from heat-treated tomato juice than from untreated juice, and others indicate that absorption from tomato paste is greater than from fresh tomatoes.

It has been clearly demonstrated that the physical state and processing history of a food item have a very marked effect on the availability of these compounds for absorption. This indicates that disruption of the food matrix and thermal history via the processing technique could be the most important factors affecting bioavailability. It is also known that the bioavailability of carotenoids is markedly affected by the fat content of the general diet, because the presence of lipids is essential for the extraction of carotenoids from the aqueous bulk of the food and for the formation of mixed micelles via which the carotenoids are then absorbed by enterocytes and transferred to the tissues (via plasma lipoproteins). Carotenoids are passively absorbed lipophilic compounds and their bioavailability is therefore affected by those factors that influence their mass transfer from food into the mixed micelles that can be absorbed by the intestine.[35] Interestingly, absorption can be improved by cooking and homogenising the food, thus breaking down the cell structure, as long as the cooking is carried out in the presence of oil or fat.

As long as lycopene remains in the aqueous matrix and more so if it remains inside the undamaged cells it is very stable but has little reactivity. Its bioavailability is therefore small and its efficacy as antioxidant almost zero. In contrast, its high solubility in a lipid medium (for instance in certain products formulated with oil) imparts considerable reactivity as well as complete bioavailability. Its assimilation is decidedly better if foods are cooked and homogenised so as to disrupt the cells and even more so if this occurs in the presence of oils or fats.

However this effect is inevitably counteracted by a more rapid degradation of its antioxidant power.

When lycopene solubilises in a lipophilic matrix, it has considerable reactivity and more availability, thus enabling it to undertake its antioxidant activity. However, this greater reactivity also means that it is more unprotected against the degradation effects of environmental conditions (air, biological matrix components, temperature).

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Part 2

Managing safety and quality in the supply chain

5

Modelling fruit and vegetable production: the case of tomatoes

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5.1 Introduction: the importance of modelling to quality

The tomato is a very popular crop. It can be consumed either fresh or as the main ingredient in a range of processed products. These two major supply chains generate different sets of quality requirements. In short, the fresh market emphasises visual appearance and shelf-life duration,[1] whereas the processing industry gives more value to the dry matter concentration and composition.[2] In both cases, producers have to control their production process to reach the standards defined by their customers.

In this review, a fairly broad definition of quality in tomato production has been adopted, including the fruit sensory properties (appearance and taste), its nutritional and health value (presence of valuable nutrients, absence of chemicals or toxins) and the environmental impact of the cropping system. Many of the physical and biological processes involved in tomato production have been formalised in different ways in order to carry out simulations, make predictions or optimise their management,[3] but much still remains to be done in the simulation and management of quality.

In fact, effort in modelling has been proportional to the ability to control the cultivation system, that is, greater for greenhouse than for field production. In greenhouse production, modelling has focused on yield prediction, optimisation of climate and fertigation (the application of fertilizer through an irrigation system) control and evaluation of strategies of crop management. In field production, it has been dedicated more to the prediction of harvest dates and to the estimation of water and nutrient requirements. In this chapter the processes of tomato production, the various areas of application of models and the future trends in the modelling of tomato production and quality will be reviewed.

5.2 Types of tomato production

The dual use of the tomato has led to two major cultivation systems, one under cover and one in the field. Protected cultivation is specific to production for the fresh market. Its rationale is a gain in productivity. This goal can be achieved through application of transparent cover which reduces the convective and radiative heat losses[4] and increases the crop temperature. Productivity can be increased by extending the production period and by reducing the number of limiting factors through better control of the physical and biological environment of crops.

A greenhouse can contian various types of equipments to control the environment.[5] The temperature can be increased by heating, for example by burning natural gas, oil or coal, or by using thermal screens during the night. The temperature can be reduced by natural (vents) or forced (fans) ventilation, or by absorbing heat through evaporation of water applied via cooling pads or fog systems, or by cooling the cover material using water sprinklers. The light level can be controlled with shading screens, by whitening the cover, by using roof materials that have a greater light transmission and by applying supplementary lighting. Water vapour is released by crop transpiration. The air humidity can be decreased by ventilation, sometimes in combination with heating. It can be increased by evaporation of water using, for example, a fog system. The CO_2 concentration in the air can be increased (or maintained at normal levels when greenhouses are closed and crop photosynthesis is active) by the injection of either industrial CO_2 or flue gases from a boiler.[6] Some of these techniques (such as supplementary light, fog system, injection of industrial CO_2 and so on) are expensive and seldom used for tomatoes. It should be noted that the transpiration of the crop itself effectively reduces the air temperature and increases the air humidity. In this respect, proper management of the development of the canopy is a major contributor in controlling the greenhouse climate.

In soilless culture, the root environment is continuously monitored and controlled (ion concentration, pH, no soil diseases). Roots may develop either in mineral (rock wool) or organic (coco peat) substrates or directly in the nutrient solution (nutrient film technique). As the substrate can be replaced, no soil disinfection is needed. In order to limit environmental pollution, growers are now encouraged to close the fertigation systems; the drained nutrient solution is pumped back, disinfected and brought back to set point by replenishing the water and nutrients.

Finally, protected cultivation facilitates the control of pests and diseases. The use of pesticides can be reduced or suppressed thanks to biological control. For greenhouse tomato crops, the natural enemies of the most damaging pests have been identified. The development of some diseases such as grey mould (*Botrytis cinerea*) can be avoided with proper control of humidity and temperature, thereby limiting condensation on the foliage.

In contrast to greenhouse cultivation systems, the field cultivation system permits much less control of the physical and biological environment. The timing of operations can be adapted to allow the crop to grow under the most favourable

climate conditions. The required nutrients can be provided either in one run before plantation or several times during crop growth. If necessary, water (possibly together with nutrients) is supplied by irrigation. Plasticulture systems equipped with drip irrigation allow the greatest control of water and nutrient availability: a plastic cover spread out on the soil keeps rainfall off and limits soil evaporation. There are, of course, a large number of intermediate cultivation systems between the most sophisticated glasshouse and the most basic field cultivation system. For example, significant areas of tomato crops are cultivated on soil under plastic cover. In this particular case, growers still have some control of the climate but the conditions of water and nutrient supply are close to those encountered in the field.

Different cultivars are used for the two cultivation systems. For long-season production in greenhouses (up to one year), indeterminate (with a vine shape) cultivars are grown with all side shoots removed. New inflorescences continually appear. As a consequence, irrespective of the season, mature fruits can be harvested two to three times per week and delivered to the fresh market. Determinate (with a bushy shape) cultivars are preferred in the field when the growing season is short or when the pest pressure is great. These plants have a grouped flowering and fruiting. This latter characteristic makes such crops suitable for mechanical harvesting.

5.3 Types of modelling

The crop models that are available at the time of writing are based on two different approaches. On one hand, new models appear as a mathematical formalisation of observed processes as the available knowledge increases. Such models are called research models. On the other hand, models can be designed to be part of procedures aimed at solving practical problems; these are called engineering models.[7] Research models are evaluated on their scientific value (realism). They are explanatory or process-oriented models, as the behaviour of a simulated system at a particular hierarchical level is the result of processes described at lower hierarchical levels. The engineering models are evaluated on their operational value (effectiveness). They can be more descriptive, being built from statistical relationships ('black-box' models) or knowledge based (heuristic models).

5.4 Mass and energy balances of tomato crops

5.4.1 Carbon

Basically, the production of biomass by a canopy relies on the net assimilation of atmospheric CO_2, that is the balance between gross photosynthesis and respiration. It depends on the amount of available energy (light) and carbon substrate (CO_2), and on the ability of the canopy to intercept light and assimilate CO_2. In greenhouses, the assimilation of CO_2 is not only important for crop growth, it interacts strongly with the composition of the atmosphere. The daily consump-

tion of carbon by a tomato canopy can be up to 10 times the amount of carbon available in the greenhouse atmosphere.[6] It must be balanced either by ventilation or by CO_2 enrichment.

Longuenesse *et al*.[8] and Gijzen[9] have extensively reviewed models of photosynthesis of horticultural species at leaf and canopy levels. The leaf gross photosynthesis responds to light by a saturation-type curve. Various mathematical formulations have been proposed and tested on tomato data, for example the rectangular hyperbola,[10] the non-rectangular hyperbola[11] and the negative exponential.[12] Despite their slight difference in shape, all these functions include two important parameters: the maximum rate of leaf photosynthesis (P_{max}) and the initial (close to darkness) light use efficiency (α). P_{max} increases with CO_2 concentration and with the conductance of CO_2 transfer from the atmosphere to the chloroplasts. It is limited at low and high temperatures (see examples of parameterisation for tomato in Bertin and Heuvelink[13]). Initial light use efficiency α is positively affected by CO_2 concentration and negatively by temperature. The conductance to CO_2 transfer gets lower at low light intensity, high CO_2 concentration, high vapour pressure deficit (VPD) and under water stress.[14]

Gross photosynthesis has been integrated at canopy scale in different ways. The simplest approach is to multiply the unit leaf activity by the leaf area index or by the projected leaf area ('big leaf' approach). Other models take the transmission of light in the canopy into account using an exponential law of extinction.[15] When the leaf light response curve is a rectangular hyperbola, analytical integration at canopy scale is possible (e.g. in Jones *et al*.[16] for tomato crops). More sophisticated models are based on a detailed description of light distribution and absorption in canopies (see later).

The respiratory efflux of CO_2 is significant: on a daily basis, it can represent a quarter to a half of the gross photosynthesis of a developed greenhouse tomato crop.[6,9] Respiration of plants has functionally been divided in two components: maintenance and growth respiration. Maintenance respiration corresponds to the energy needed to maintain the ionic gradients across biological membranes and pools of macromolecules such as proteins. Growth respiration corresponds to the energy involved in the synthesis of new biomass from assimilates and minerals. Maintenance respiration is calculated as the product of the plant or organ dry weight times a maintenance coefficient. Growth respiration is calculated as the product of the plant or organ growth rate times a CO_2 production factor. In crop models, maintenance and growth respiration are summed to estimate total respiration, generally on a daily basis. Respiration rate increases exponentially with temperature. For tomato, Heuvelink[17] has hypothesised that the maintenance coefficient decreases with ageing of organs. The CO_2 production factor is proportional to the energy cost of biomass synthesis; it varies between organs and with ageing (see Gary *et al*.[18] for tomato).

The crop carbon balance includes carbon exchanges between the atmosphere and the canopy (net photosynthesis), and the partitioning of carbon in the plant between one or several pools of photoassimilates and the growing organs. Gent and Enoch[19] put together simple formulations for gross photosynthesis and res-

piration, and provided a relationship between the availability of photoassimilates and growth. With these simple formulations, the 24-hour dynamics of CO_2 exchanges and of the variations in the assimilate pool of young tomato plants could be simulated.[20,21] Such a simple carbon balance model was reshaped for control purposes by Seginer *et al*.[22]

5.4.2 Water

The water balance in the crop is an important crop property in various respects. Water import contributes to the plant growth, as water status influences cell extension in growing organs and water flow conveys nutrients to growing or storage organs. Water status also partly controls the stomatal conductance and may therefore affect photosynthesis. And last, the evaporation of water during transpiration is connected to the absorption of latent heat: it strongly determines the temperature of the canopy and, therefore, of the air inside a greenhouse.[3]

The modelling of water relations of horticultural crops has been reviewed by Jones and Tardieu,[23] van de Sanden[24] and Jolliet.[25] Research in this domain has been motivated by two main concerns: (1) simulating the water status and its relation with various physiological functions (organ extension, stomatal opening, water flux and so on) and (2) simulating the water flux through the canopy to estimate the water requirements of crops. The basic framework that has generally been adopted is an analogue of Ohm's law: the water volume flux along a certain path is proportional to the gradient of water potential and to the inverse of a flow resistance. For tomato, van Ieperen[26] designed a model describing the pathway of water from the root environment to the atmosphere through one root compartment and three shoot layers within a vegetative plant, and the dynamics of water potential in roots, stems and leaves. Premises for modelling the water fluxes to the tomato fruit through the phloem and xylem vessels can be found in Guichard *et al*.[27] These premises are based on Fishman and Génard's model.[28] The dominating phloem flux depends on the concentration of carbohydrates in the phloem vessels and on the ability of the fruit to unload these carbohydrates.[28] The xylem flux varies with the water potential in the stem, since the fruit water potential remains fairly stable in time and in different environmental conditions.[27] Owing to a high resistance to water flux in its epidermis, the transpiration of the tomato fruit is limited; it was modelled as a function of irradiance and VPD by Leonardi *et al*.[29]

On the canopy scale, the transpiration of tomato crops has been modelled applying the classical Penman–Monteith approach[30] as the sum of a radiative component, proportional to the global radiation absorbed by the canopy, and of a convective component, proportional to the VPD. The canopy resistance to transfer water vapour comprises the aerodynamic resistance that depends on wind speed and air and leaf temperatures, and the stomatal resistance that depends on radiation, leaf air saturation deficit and leaf temperature (e.g. Boulard *et al*.[31] for tomato crops). For operational purposes, the complete analytical model has been simplified to a two-parameter formula, the parameters being either derived from the complex model or identified *in situ*.[32]

5.4.3 Energy

A crop canopy can be compared to a solar collector. The absorbed radiation is the balance between incident, reflected and transmitted global radiation. In their study of light interception by glasshouse crops, Warren Wilson *et al.*[33] measured, for a tomato canopy, an average reflectance of 13% and an average transmittance of 23.5% of the incident light in the photosynthetic active radiation (PAR) waveband. Light absorption was improved by about 10% when the soil was covered with a white plastic sheet. It also increased with the foliage development to almost complete absorption with a leaf area index (LAI) of 4 or above. Light absorption is related to plant density and row spacing as it tends to increase when the plant distribution is more uniform.[34] The distribution of light and its absorption by rows of canopies such as tomato crops have been modelled by using several approaches reviewed by Critten.[15] Among these are the exponential extinction curve, and various models that take light scattering and the distribution of diffuse and direct light[35] and leaf angle distribution into account.[36]

Part of the absorbed radiation is used by photosynthesis for carbon assimilation and biomass production. This proportion is estimated by the radiation use efficiency (RUE), that is the ratio between the energy equivalent of biomass and the absorbed (or incident) global (or PAR) radiation. For a tomato crop, Aikman[37] estimated the absorbed radiation to be about 7% when based on the absorbed PAR or 1.6% when based on the global radiation outside the greenhouse.

A significant part of the absorbed energy is actually dissipated by the crop as latent heat by transpiration. As a consequence, the temperature of a transpiring canopy is lower than the air temperature. This difference generates a flux of sensible heat from the air to the canopy. In a greenhouse, depending on the LAI, 50–70% of the solar energy input is used for evapotranspiration.[3] This justifies the fact that the crop water requirements are estimated from the absorbed or incident global radiation.

5.4.4 Minerals

In the same way as for carbon and water, both mechanistic and black-box models have been designed (see the extensive review of Le Bot *et al.*).[38] The mechanistic models describe specific processes like nutrient uptake, transport and assimilation. Even for nitrogen, the most studied element, the regulation and the integration of these processes on a whole-plant scale are still in discussion. For tomato, two main approaches of mechanistic modelling have been proposed. According to Le Bot *et al.*,[38] the time-course of nitrate uptake is related to the translocation of carbohydrates to the roots to cover the energy cost of nutrient uptake. According to Cardenas-Navarro *et al.*,[39] nitrate uptake is related to the maintenance of a steady internal ion concentration.

More general (black-box) models link the demand of nutrients directly to the growth rate. It has been established for several elements (nitrogen, potassium, phosphorus) that a critical concentration in plant tissues should be maintained to

approach the potential growth based on total intercepted radiation. For nitrogen, this critical concentration gradually declines with the accumulation of biomass during the vegetative phase.[40] Le Bot *et al*.[41] parameterised this relation for tomato plants. To explain this decline in nitrogen content, Caloin and Yu[42] suggested two compartments in the biomass, one mostly active for growth and having a high nitrogen content and another dedicated to structures and storage having a lower nitrogen content. With crop development, the second compartment tends to dominate. This model was calibrated for a greenhouse tomato crop by Bellert *et al*.[43] A comparable approach to the nitrogen demand by processing tomatoes has been implemented in the EPIC model to evaluate different fertilisation policies in terms of crop growth and nitrogen dynamics in the soil.[44]

Few models are available at the time of writing for other nutrients.[45] A first model simulating the flux of calcium in pepper fruit and its relation to the occurrence of blossom-end-rot (a quality defect also observed on tomato) was reported.[46]

5.5 Yield formation

Tomato has been a pioneer species for crop modelling. The formation of yield (organ appearance, dry matter production and partitioning) has been thoroughly studied and formalised by various approaches, again either mechanistic or empirical. The approach of fruit growth has been based on models of dry matter production. Water fluxes towards the fleshy tomato fruits (around 95% water) have only recently been studied and modelled.

5.5.1 Production of biomass

Different approaches of modelling biomass production have been developed for different crop species including tomato. In the 'photosynthesis-driven' models, integration of net photosynthesis and conversion of the resulting photoassimilates into biomass are used to compute the accumulation of dry matter. Challa and Bakker[47] estimated the potential production of greenhouse crops in various regions of the world using this approach. It is also the first step in most of the tomato crop models.[16,17,48] Bertin and Heuvelink[13] compared the dry matter production estimated by the models of Jones *et al*.[16] and Heuvelink.[17]

In the RUE approach, the production of biomass is considered to be a sequence of energy conversions from the incident radiation to the energy content of biomass. Interception of radiation is linked to the leaf area index by a saturation-type curve; the coefficient of conversion of intercepted light into biomass is higher for C_4 (e.g. maize) than for C_3 (e.g. tomato) species and it increases at high CO_2 concentration. This approach was validated at different conditions for greenhouse tomato crops.[49–51] A similar approach has been used for different species including tomato in the STICS modelling platform.[52]

5.5.2 Timing of development

Development processes include the formation of new organs and their ageing and phase transitions on the whole plant (e.g. vegetative versus generative periods) and organ (e.g. fruit setting) scales. Formation and ageing of organs mainly depend on temperature,[53] following a bell-shaped curve that can be partly described by the Arrhenius equation.[54] Such a response curve has been calibrated for the formation of new leaves and trusses and for the fruit development from flowering to maturity, and introduced in most tomato crop models (e.g. De Koning).[55] Under the hypothesis that the response of development rate to temperature can be considered to be linear in a limited range of temperature, daily temperatures can be summed to calculate a 'thermal time' expressed in degree-days that is, by definition, independent of the temperature regime.[56] (Tijskens and Verdenius[57] revisited the modelling of biological processes that depend on temperature.)

In tomato plants, fruit setting is the phase transition from flowering to fruit growth. It has been observed that the higher the source-sink ratio (i.e. the fraction of the plant potential growth rate that can be met by the current production of photoassimilates), the more successful is fruit setting.[58] This relation was formalised in the TOMGRO model.[59] In this model, the dynamics of flowering, fruit setting and fruit ageing determine the age structure of the populations of vegetative and generative organs at any time during production.

5.5.3 Dry matter partitioning

The dry weight of harvested organs depends on the fraction of dry matter that is allocated to them. In the case of fruit species such as tomato, the vegetative–generative dry weight balance is a key component of crop models. This ratio can change with the plant development stage, and dynamically with the strength of vegetative and generative sinks. The sink strength of an organ or a group of organs is their ability to attract photoassimilates; it is the potential growth rate when no competition for carbon resources exists among organs.[60] It varies with the stage of development of the organ, increases with temperature and is not affected by the availability of assimilates themselves. Heuvelink[61] demonstrated that, in tomato, all the organs of a tomato plant have the same access to the carbon resources. Consequently, (1) the vegetative–generative dry weight allocation ratio depends on the number and age structure of leaves, stem internodes and fruits, and (2) when the source activity (net photosynthesis) is lower than the sink demand, the actual growth rate of all organs is limited in the same proportions. These concepts have been implemented in the tomato crop models designed for indeterminate cultivars.[62] Until now, only a few attempts[63] have been made to verify and validate this theory for determinate cultivars.

5.5.4 Dry matter content of fruit

The high water content of mature tomato fruits results from xylem and phloem influxes and transpiration efflux during fruit growth. As mentioned earlier, mod-

elling of lateral fluxes within the plant (from stems to fruits) and of the fruit transpiration has only been studied quite recently. These processes will be introduced in a tomato crop model provided carbon and water fluxes can be coupled. To this end, the dynamics of water potential in the stem and of carbohydrate content in the phloem and the possible variations in water transport resistance in the fruit peduncle and epidermis have to be determined.

At the time of writing, tomato crop models are based on the assimilation and partitioning of carbon only. The dry weight of harvested fruits is calculated and converted into fresh weight by applying a coefficient of dry matter content that is either fixed[16] or variable with the season.[55] In the latter case, the fruit dry matter content is higher in summer than in winter as environmental conditions in summer tend to favour water stress (when radiation, VPD or salt concentration in the nutrient solution is high). The dry matter content of mature fruits is also genetically determined: it is generally higher in small fruits (cherry, cocktail) than in large fruits.

5.6 Formation of product quality

The quality of tomato fruits covers a number of different characteristics among which more attention has been paid to fruit grade. The average fruit fresh weight can be modelled based on the weight and number of harvested fruits. The fruit potential growth rate is a genetic parameter. In tomato, it increases from cherry over cocktail to round and beefsteak cultivars. Within the range of genetically determined fruit grades, the actual fruit size can be controlled in greenhouses by climate and crop management. Larger fruits can be obtained by increasing net assimilation with, for example, CO_2 enrichment or by decreasing competition for assimilates by, for example, fruit pruning. These behaviours are simulated by the TOMGRO model: in the SIMULSERRE simulator, different strategies of climate and crop management can be evaluated in terms of the time course of weekly yield and average fruit grade.[64]

At the time of writing, the modelling approach to fruit colour, flavour and texture has been less mechanistic as the underlying processes are complex. Colour change during ripening involves the conversion of chloroplasts to chromoplasts with the degradation of chlorophyll, the synthesis of carotenoids and the accumulation of lycopene, resulting in red-coloured fruits. These processes respond differently to temperature and, consequently, fruits become yellow rather than red below 12°C and above 30°C.[65] Such physiological changes can be simply characterised by colorimetry by the *L*, *a* and *b* values of the three-dimensional Hunter system[66] or by *a* and *b* converted into *Hue* and *Chroma* to provide, together with *L*, an assessment of colour that correlates well with the consumer's perception.[67] Thai and Shewfelt[68] showed that the *Hue* value changed sigmoidally with time and responded to temperature, and that the *Chroma* and *L* values were variables dependent on *Hue*. A simple statistical model resulted, simulating tomato colour changes under constant and changing temperature regimes. Tijskens and Evelo[65] carried out a comparable

analysis using the *a* value and *a/b* ratio and introduced a correction for the biological age of the fruit at harvest. Yet the prediction accuracy of coloration decreased when harvest shifted from the pink to the mature green stage.

Together with size and colour, the occurrence of defects and abnormalities participate in the visual appearance of tomato fruits. Jahns *et al*.[69] characterised size, colour, shape (eccentricity), defects (brown spots), cracks (reduced reflectance) and uniformity (green-red distribution) by image analysis. They designed a fuzzy model of quality rating by consumers based on these optical properties.

The pleasantness of tomato fruit is explained mostly by aroma, sweetness and mealiness (characterised by a loose and granular structure of the flesh). As aroma is correlated to sweetness, Verkerke *et al.*[70] used multiple linear regression to link sweetness and mealiness assessed by a trained panel to a set of texture measurements and chemical analyses. Similarly, Schotte *et al.*[71] found a logarithmic relation between firmness measured by an acoustic impulse–response technique and estimated by experts. Firmness decreased exponentially with time and this dynamic was affected by the maturity at harvest (with an interaction with the cultivar), by temperature and by the season (the deterioration constant being higher in spring than in other seasons).

Keeping quality during storage and distribution is an integrative variable. It quantifies the time of product acceptability which depends on both the product properties and consumer behaviour. The keeping quality of tomato fruits could be correlated to firmness at harvest.[72] Then a general modelling framework was formulated by Tijskens and Polderdijk[73] in which the decay kinetics of one or several quality attributes depend on temperature, initial value and limit of acceptance by the consumer.

Noticeably, most of the models of fruit quality are not explicative and few of them (colour, firmness, keeping quality) are dynamic. Ongoing research is conducted on the physiology of the formation of the tomato fruit quality in terms of chemical composition (sugar, acid, aroma contents and so on), appearance (colour, cracking, blossom-end-rot and so on) and health promoting compounds (antioxidants). If models are still unavailable for most of these quality variables, some can be related to the carbon, water or mineral fluxes of the fruit[74] for which modelling frameworks exist (see previous sections of this chapter and Fig. 5.1). For example, the sugar content could be linked to the carbon availability (but acid or aroma contents could not),[75] the frequency of cracking of the fruit epidermis has been linked to the crop water status[75] and the occurrence of blossom-end-rot has been related to the calcium flux transported by the xylem network.[76]

5.7 Interactions with pests and diseases

Few simulation models of pests and diseases are available although their effects are of major importance in tomato cropping systems, in relation with environmental and health concerns. Seghi *et al*.[77] reviewed some empirical models that forecast diseases from climatic data in processing tomato crops. In the 1970s, the

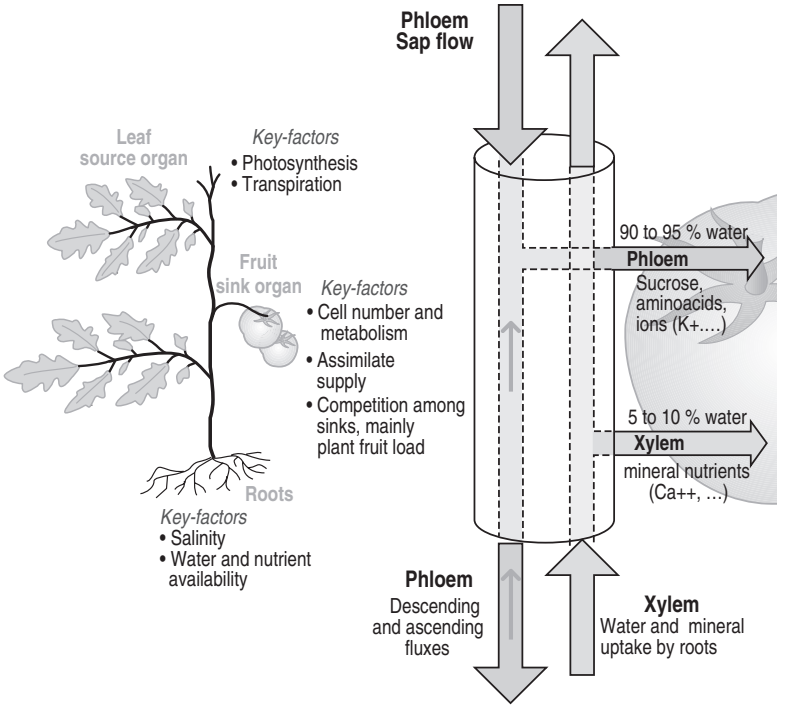

Fig. 5.1 Main factors affecting the water, carbon and nutrient fluxes from source organs to fruits in tomato plants. Reproduced from Guichard *et al*., (2001)[74] with kind permission of EDP Sciences.

FAST model was designed to predict the severity of risk of early blight (*Alternaria solani*) outbreaks.[78] The risk is evaluated from models predicting dew (favourable to spore formation) and rain (favourable to spore formation and infection). The two models are based on sets of weather data: duration of wet periods and air temperature during these wet periods for the dew model, and rainfall, temperature and duration of periods of high humidity during the past week for the rain model. Another disease-warning model, BLITECAST, was coupled to FAST. Based on air temperature and time of high humidity, it predicts outbreaks of late blight (*Phytophtpora infestans*). In the 1990s, CU-FAST predicted both early blight and anthracnose (*Colletotrichum coccodes*) episodes.[78] It estimates the duration of wetness based on rainfall, duration of periods of high humidity and minimum daily humidity. A step further was TOM-CAST that was developed to control early blight, anthracnose and Septoria leaf spot (*Septoria lycopersici*). This modified FAST programme needs only the dew model to be run.[78] The outputs of all these models are variables cumulating the daily rating values of the severity of risk. Patterson and Nokes[79] incorporated to TOM-CAST a model of fungicide (chlorothalonil) persistence on tomato foliage.

In their review on the control of grey mould (*Botrytis cinerea*) in greenhouse tomato, Nicot and Baille[80] identified only a small number of models for greenhouse vegetables, one designed to forecast the fungus epidemics in cucumber in an unheated greenhouse and another to simulate spore germination on tomato leaves. The crop–pathogen interactions have seldom be modelled. For example, the effect on tomato yield of disease induced by *Septoria lycopersici* was estimated: a good linear relation was observed between tomato yield and healthy leaf area duration.[81] Pest models are also rare: an example of a research model simulating the parasitoid–host relation between *Encarsia formosa* and the greenhouse whitefly (*Trialeurodes vaporariorum*) on tomato crops was published by van Roermund *et al.*[82]

Plant protection has been a field of particular development of knowledge bases, often designed for diagnosis purposes. Bouto[83] and HYPP[84] are examples of databases for the identification of pathogens, pests and weeds for a range of crop species, including tomatoes.

5.8 Areas of application: yield prediction and crop management

Management can be defined as the sequence of three operations: planning, implementation and control. The planning operation sets up the strategy which encompasses the goals assigned to the cropping system and the means to achieve these goals. Implementation performs the translation from the strategy into actions, while control ensures the proper applications of these actions by constantly monitoring the process and revising the mode of application of the action. The decision process leading to the determination of the actions to be taken is complex. It depends on uncontrolled external factors, on complex interactions between the crop and its environment and on the knowledge of the crop state.

In view of this, the first application of crop models is to provide information that is otherwise not readily accessible to the grower, either because no measurement system is available or because the cost of obtaining the information would be prohibitive. The second application is to represent crop processes in optimisation routines. In the following subsections an overview is presented of current works using models as information providers (crop management and protection) and as process representations (climate and fertigation control).

The demand for yield prediction varies with the tomato cultivation system. In field production, determinate cultivars are selected to obtain fruits ripe for a single harvest. The expected time of harvest and expected amount of product are predicted to enable an integrated planning of production and processing. For example, Wolf *et al.*[85] estimated the times of emergence, flowering, turning stage and harvesting of tomatoes for processing based on the heat sums. McNeal *et al.*[86] went a step further and predicted the mass of fruits at harvest using a greenhouse tomato crop model (TOMGRO) adapted to field conditions. In greenhouse production, yield is planned for a long period of time. In negotiations with

the product buyers, growers must be able to announce their weekly production for the next couple of months. For this purpose, a simple tomato crop model named TOMPOUSSE was developed to predict the weekly yield and average fruit grade from information available on the farm.[49] The same model can be used as a simulator to evaluate different strategies of crop management (truss pruning, CO_2 enrichment, changes in stem density). De Koning[55] used a similar approach in a model of dry matter partitioning to optimise shoot density and number of fruits per plant.

These crop models, used to evaluate the biological consequences of policies of crop management, are still far from real decision support systems (DSS). For this purpose, the models should describe not only the dynamics of the crop and of its physical environment (greenhouse climate and/or soil), but also the decision-making process itself and its interactions with the biophysical system. For example, the GX/Sim system[87] is a greenhouse simulation platform that can specify the decision rules the grower uses to adapt the climate settings to the current climate and crop conditions.

In the CONSERTO project,[88] a dynamic model of the greenhouse production system has been designed with three components: the decision system, the instructions-to-actions system and the biophysical system. The decision system describes the management strategy (climate, manual operations such as fruit and leaf pruning, training and harvesting) applied over a cultivation period to realise production objectives. The instructions-to-actions system converts these decisions into actions via automatons (the climate and fertigation control system) and workers. The biophysical system comprises a greenhouse climate and a tomato crop model (TOMGRO)[16,59] implemented in an object-oriented framework.[89] The outputs provide not only information on physical and biological performances of the system under a set of actions but also indicators (e.g. the plant vigour or predictions of important events such as flowering or fruit maturity) useful for the decision system.

5.9 Areas of application: climate control

Our understanding of model-based climate control encompasses all the approaches where new climate set-points are determined using either information output from the model or the knowledge contained in the model itself. Optimal control is probably the most widely used method to exploit available models and determine 'optimal' crop environmental conditions.[90–92] Climate control application of crop models within the framework of optimal control also requires a model of the greenhouse climate because the control variables directly modify the climate. The plant behaviour is driven indirectly through its responses to modifications of the environment.

In one of its simplest forms, the climate optimisation problem is defined as follows: using a crop dry matter accumulation model and an algebraic expression of the greenhouse climate model, find the day- and night-time temperatures that

maximise a cost function, balancing the relative growth rate and the heating costs (CO_2 enrichment can also be included). Gal *et al*.,[93] Seginer,[94,95] Seginer *et al*.[96] and Critten[97] showed that the optimal solution can be expressed as a direct function of the external climate conditions for each time instant independently. In practice this allows for the offline computation of lookup tables that indicate what actions should be taken under current conditions. Seginer *et al*.[22] have studied the temperature optimisation problem, only based on plant need. They used a dynamic model of the carbon balance of the crop with a temporary carbohydrate pool to derive the day and night temperatures that maximise the relative growth rate, for a given daily radiative flux. The results are that young crops need higher temperatures than old ones where the maintenance respiration rate is higher and that for a given situation, several couples of day and night temperature are optimal. Tchamitchian *et al*.[98] and Tap *et al*.[99] have used a dynamical greenhouse model instead of an algebraic one to introduce the damping of temperature caused by the structures in the greenhouse. Solving the climate problem, either for tomato or for lettuce, respectively, proved to be a rather difficult numerical problem.

Coupling a dynamical model of the greenhouse climate to a lettuce growth model, van Henten[100] used the singular perturbation approach[101] to tackle the problem of models with different magnitudes of time constants. A new development in this area (Tap, personal communication) applies the same method to a simplified tomato crop model. Daily optimisation of the climate (so-called fast processes) under the constraint of long-term optimisation of the crop production (so-called slow processes) can then be solved.

Although many theoretical applications of models to climate control have been studied, none or very few have been put to test in practice. A technical reason is that, at the time of writing, optimal control produces time-varying set-points which cannot be implemented on commercial greenhouse climate computers.

5.10 Areas of application: irrigation and fertilisation

In both field and greenhouse production, there is an increasing pressure to improve the policies of irrigation and fertilisation that should both satisfy the objectives of production and quality and avoid losses of nutrients in the environment. At the time of writing, empirical methods are used; they should be improved with mechanistic models that are being developed.

The supply of water to the crop must fit its water requirements. In soil-less culture, irrigation is usually calculated based on radiation measurements. Several relationships have been established between the crop water uptake and the incident radiation for tomato and as well as for other vegetable crops (formulae reviewed by Jolliet[25]). The VPD should also be taken into account when radiation and VPD are uncoupled, for example in changing climatic conditions and when using systems of climate control such as thermal screens or fog systems.[32] The water demand depends on a crop coefficient that increases with the leaf

area development. In soil culture, the availability of water in the soil compartment must be considered: it depends on the hydraulic properties of the soil and on the root development. In the field, the rain flux must enter into the water balance.

In greenhouses, computers are used to monitor radiation and to control the quantity of water that is provided for open systems (on soil or soil-less), that is, the calculated evapotranspiration plus about 25% run-off to avoid salt concentration in the root substrate. In closed soil-less systems, the water input must fit the crop demand to maintain the total volume of circulating nutrient solution. In the field, new DSS are designed to calculate the proper water supply. For example, the IRRIGERE software, designed for field tomatoes, estimates the daily evapotranspiration from climate and crop development and the soil water reserve from the soil characteristics and the root depth.[102] Irrigation will not meet crop demand when water stress is needed to increase the quality (dry matter content) of fruits. In that case, the objective is to exhaust the water available in the root zone at fruit harvest. With these constraints, irrigation is proposed when the watering dose gets higher than a threshold value of 3 mm.

Few attempts have been made to build fertilisation strategies using models of crop requirement, even in soil-less culture. In this cultivation system, nutrients are usually supplied in excess together with water. Therefore there is no way to control the crop growth or product quality through the regulation of fertigation. Marcelis *et al*.[103] proposed the combination of models and sensors to optimise the nutrient supply in closed systems.

5.11 Areas of application: plant protection

The epidemiological models presented earlier in this chapter were explicitly designed to build disease-warning systems. For example, TOM-CAST has been implemented in eastern North America in networks grouping tomato growers, the processing industry, extension services and universities. Weather sensing can be automatic or manual, data are centralised and disease severity values or advice of fungicide spray are disseminated to growers by phone or fax.[78] For pest control, the model designed by van Roermund *et al*.[82] can be used to evaluate strategies of parasitoid release for biological control under various climate conditions.

From these strategies of plant protection, actions can be implemented: fungicide spray, insect release or climate control. In this last field, the use of knowledge engineering was suggested by Kozai.[104] It was then realised in the SERRISTE expert system,[105] in which prevention of grey mould is a major constraint for the selection of the proper time-course of temperature and humidity in greenhouses.

Lastly, the control of the system state has been made possible by knowledge bases used for diagnosis. Blancard *et al.*[106] and Guay and Gauthier[107] developed expert systems for identifying tomato diseases.

5.12 Current and future developments in modelling

From this overview of the various processes of tomato production that have been modelled, it appears that a large range of methods have been mobilised to design research or engineering models. In the fields of carbon and, to a lesser extent, water and nutrient uptake, mechanistic approaches have often been preferred. Much effort has been dedicated to the formation of yield, mostly based on the space and time integration of net photosynthesis. Practical outputs of this research can be found in the controls of greenhouse climate, of irrigation and fertilisation, and of crop management. Yet, before models could be used for designing strategies or producing decisions, they often had to be simplified.

In the fields of fruit quality and crop protection, the complexity of the processes involved led more to the design of statistical or heuristic models. Interestingly, the lack of mechanistic knowledge has not been a limitation to the development of applications. Priority has been given to engineering models for the control of post-harvest conditions and of integrated crop protection. For this reason, the level of complexity of these models has been kept in adequation with the demand for information for building management strategies. However, poor understanding of some processes is still a limitation. For example, the relationship between pre-harvest crop management and the quality of the mature fruit has not clearly been described and formalised. Consequently, there are still only minor connections between the control of climate under cover and of fertigation, and the formation of fruit quality at and after harvest.

The present challenge of vegetable horticulture is to improve its sustainability and, to this end, satisfy a set of constraints that have been grouped in the framework of *integrated production*. The concept of quality should be global by integrating external product quality (the only one considered by the market in most cases[1,2]), internal product quality, ecological quality of production and processing, ethical and socioeconomic quality of production, and processing and working conditions of the people involved.[108] Therefore, models and DSS should more and more combine various dimensions of the vegetable cropping systems.

Some modelling approaches seem to be well adapted to this goal. For example, the SERRISTE project[109,110] has opted to use artificial intelligence techniques to represent the knowledge involved in both the greenhouse climate management task and the crop response. Agronomical knowhow, obtained from experts, is represented through a set of variables and a set of constraints relating these variables. For example, the target daily mean temperature domain is obtained by:

- computing an optimal temperature from the forecasted available radiation;
- making adjustments for the tomato cultivar;
- positioning a 1°C window around this value according to the vigour status of the crop.

A constraint is expressed as a linear combination of variables, the result of which is forced to a fuzzy domain. As an example, the temperature difference between

day and night (a linear combination) must belong to a domain extending from 2 to 5°C, values which may be changed depending on the current conditions (e.g. switch from 5 to 3 if grey mould has been observed). A constraint satisfaction algorithm determines the sets of variable values that satisfy all the constraints. Declared knowledge and numerical models are mixed in what is called a knowledge base. Two years of experiments in experimental stations in three different regions of France have proven the feasibility and the real agronomic success of this approach.[105]

As proven by Guerrin and Dumas,[111] the combination of declared and numerical models allows the scope of the system that can be represented to be broadly widened and thus may be a way to connect the various types of models that have been identified in this review. Moreover, building a declarative model may on many occasions be faster and cheaper than the experimental and theoretical work that would be needed to obtain a numerical model of the same processes. However, designs of hybrid models mixing declarative and numerical models and use of artificial intelligence techniques for crop management support are still limited.

5.13 Sources of further information and advice

For more information, readers can refer to some general books on the tomato crop[112] and on greenhouse cultivation.[5,113] *Scientia Horticulturae* published a special issue on 'crop modelling in horticulture' (1998 **74**, issue 1–2) and *Agronomie* on 'greenhouses, environment and product quality' (2001 **21**, issue 4).

Various sections, commissions and working groups of the International Society of Horticultural Science (ISHS) organise scientific meetings and publish issues of *Acta Horticulturae* on topics related to the present review (see www.ishs.org).

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6

Use of HACCP in fruit and vegetable production and post-harvest pretreatment

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6.1 Introduction: food safety and quality

This chapter discusses use of the hazard analysis critical control point (HACCP) system of food safety management with particular reference to the growing and post-harvest pretreatment of fruit and vegetables. It is not the intention here to review the processes involved in the growing and pretreatment of named fruit and vegetables in the context of food safety, or to examine specific food safety issues relating to fruit and vegetables. The purpose of the chapter is to bring a broad understanding of HACCP sufficient to guide the reader in the development of food safety management systems, in relation to products of their choosing, designed to protect consumers from foodborne harms arising from the growing and basic processing of fruit and vegetables.

Today, food businesses of all kinds recognize that food quality and safety are critical to continued consumer satisfaction, competitive advantage and profit. In this, the food businesses that constitute the fruit and vegetable sectors of the food supply system are no different from any other. Like all other food businesses they need their own particular understanding of food quality and safety and they have had to come to terms with consumers' changing perceptions of food quality and increasing awareness of food safety issues.

Among the factors that affect the way consumers understand concepts of food quality and safety, the multiple food retailers, or supermarkets, are significant. As more food produce is moved through a reducing number of supermarket chains the power of supermarkets over food supply businesses grows. What consumers

believe to stand for quality has become more closely aligned to the supermarkets' own definitions of quality, of which food safety is a part. Consequently, as greater numbers of consumers shop in supermarkets, suppliers are forced to adjust their own understanding of quality in line with that of supermarkets if they are to stay in business. At one time variation in the size and shape of a given fruit or vegetable, or the presence of a scar or blemish, did not denote poor quality. The apples placed in a bag by a greengrocer were all allowed to be a little different. Potatoes, carrots and parsnips came in different sizes and with residues of earth that indicated their origins as products of the land. In the modern consumer food marketplace the supermarkets have taken control of the education of consumers in matters of food. Now fruit and vegetables of the same kind have to appear all the same size, colour and shape, and with no obvious defects, if consumer expectations are to be met and continuous satisfaction is to be achieved. But although the supermarkets may influence consumers' understanding of food quality, consumers' awareness of food safety issues has, in recent years, been influenced significantly by the media reporting of food safety problems and food scares. Consumers now perceive quality, and consistency of quality, to be an important factor in guiding food choices and in demonstrating value for money in their purchases. They also know that whatever the standard of quality in the products they buy, the foods must be safe to eat, and they expect both growers and retailers to ensure this.

The term 'quality' can encompass many aspects of a food product and, indeed, the services that a food business provides in association with that product. Many definitions of the word quality exist. Crosby (1984) states that quality 'has to be defined as conformance to requirements'. The International Organization for Standardization (ISO, 2000a) defines quality as 'The degree to which a set of inherent characteristics fulfills requirements'. Clearly, consumers are able to appreciate the quality of food products but their assessment of quality tends to be subjective. They usually judge the quality of, for example, an orange or a lettuce in subjective terms, as bad, poor, good or excellent. A food producer, on the other hand, must understand quality in objective terms and this normally leads to identifying and quantifying quality parameters in order to measure and describe quality. If quality cannot be measured it cannot be controlled and quality parameters must be established in order to achieve the control needed to ensure that quality conforms to or fulfils requirements. Food safety is bound to be a quality requirement of any food product. The fact that a food product is unfit to eat because it has spoiled means that it is not of the right quality, but this does not necessarily make the food unsafe to eat. In contrast, if a food product is not safe to eat, for whatever reason, the food is then not of the right quality. Logically, it follows that food safety is a subset of quality.

There are sound commercial reasons why food businesses should manage aspects of food quality other than food safety in ways that will ensure customer and consumer requirements are met at all times. In matters of food safety commercial perspectives apply, but, undeniably, all food businesses have moral and legal duties to provide consumers with foods that will cause no harm.

Indeed, though laws may state requirements for, and set limits on, certain kinds of conduct by food businesses for the safety of consumers, what is considered to be ethically right ought not to be defined entirely by the law. Though maximum residue levels (MRLs) may be set by law to limit agrochemical residues associated with fruit and vegetables (as well as other food crops), it can be argued that working to the limits specified in law is an abdication of moral duty to the consumer in favour of the grower's interests as protected by the law. Legislation can be subject to bad judgement, political bias and lobbying by parties intent on protecting their own interests. Laws can be wrong and fail to protect those who ought to be protected. In the application of agrochemicals, conduct by the grower ought to reflect what is right, in the broad interests of consumers, and not just what the law requires. The minimum use of approved chemicals to achieve the required degree of crop protection, and not just use up to legally permitted levels, is considered the right thing to do by enlightened agriculturalists. Such thinking is now being reflected in the developing philosophy of integrated farm management.

In matters of food safety, recognition of both the moral and legal duties of food producers is important. In the United Kingdom (UK) the Food Safety Act 1990 offers the concept of the 'due diligence defence' as the acceptable defence in the event of a food business being prosecuted under the Act. Section 21 of the Act states that 'it shall be a defence for the person charged to prove that he took all reasonable precautions and exercised all due diligence to avoid the commission of the offence by himself or by a person under his control'. The term 'reasonable precautions' is interpreted to mean the implementation and maintenance of a system for food safety management, while 'due diligence' means that the system should be operated effectively to ensure the production of safe food. While UK law proposes, in effect, that the use of an effective food safety management system can provide demonstration that the law has been complied with, this may not be so in all countries. But all food businesses in all countries ought to operate such a system in recognition of their moral duty to consumers, irrespective of whether or not this requirement is framed in national law. Sadly though, without the encouragement of legislation, some food businesses will give insufficient weight to their moral duty to protect consumers and deny adequate expenditure on formal food safety management systems.

The UK's Food Safety Act 1990 does not advocate a specific approach to food safety management. The European Union (EU) Directive 93/43 on the hygiene of foodstuffs states *the* approach to food safety management required to be taken by all food businesses in the EU. It requires the implementation of five of the seven principles defining the HACCP system for food safety management. Because it is stated in an EU directive this requirement is interpreted in the UK's Food Safety (General Food Hygiene) Regulations 1995, though the regulation is sometimes erroneously understood by food safety practitioners to state only a requirement for hazard analysis, without recognition of the need to identify and maintain critical control points. Although EU and UK laws require that food safety management systems embody only the first five HACCP principles, many

food businesses utilize all seven. Indeed, food businesses supplying major food manufacturers and supermarkets are almost certainly bound to be required to operate complete HACCP systems. Also, this will be a clear requirement for any business accredited to standards such as the British Retail Consortium (BRC) *Technical Standard for Companies Supplying Retailer Branded Food Products*, or the European Food Safety Inspection Service (EFSIS) *Standard for Companies Supplying Food Products* which are revised periodically (see section 6.9.1 for contact details).

6.2 Food safety and the grower

Conventionally, agricultural produce has not been thought of as food until the point of harvest, or, in the case of animals, the point of slaughter. Food safety issues such as BSE (bovine spongiform encephalopathy) in cattle, and concern about *Salmonella enteritidis* PT4 and *Campylobacter* spp. in poultry, *E. coli* O157:H7 in beef, the contamination of fruit and vegetables with *Listeria monocytogenes*, pathogenic *E. coli*, *Salmonella* spp. and *Shigella* spp., and agrochemical residues in fresh produce have all brought considerations of food safety down to the level of farmers and growers as the second link in the food supply chain. The problem of BSE has emphasized the fact that farm inputs businesses, such as animal feed producers and agrochemical manufacturers, constitute the first link in the food supply chain. This perspective is reinforced when the role of biotechnology companies as suppliers of genetically modified (GM) seed to farmers and growers is taken into account and the safety of GM crops as novel sources of (novel) food is addressed by regulatory processes.

Many of the activities undertaken by growers in the production of crops bear upon matters of food safety and some can be critical. The sequence of events involved in crop production, from the treatment of seeds, to propagation, planting, crop management and treatments during growing, harvesting, post-harvest storage and handling, and immediate post-harvest processing, or pretreatment, can all influence the safety of the final food product. Where little, or no, further processing is involved in the preparation of fruits and vegetables for the consumer food market, the responsibility of the grower to assure food safety is most clearly defined. This is particularly so when products are destined for immediate consumption without preparation or cooking by the consumer. In the case of minimally processed products, such as salad materials intended for use in retail pillow-packs or in the production of fresh sandwiches, the grower's responsibility for food safety is also clear. Even in instances where produce receives various degrees of further processing by other agents, the grower may still bear some responsibility for preventing foodborne hazards from moving up the food chain. For growers, HACCP provides the best way to manage the safety of produce and many 'assured produce schemes' now incorporate food safety management requirements relating directly to, or based on, HACCP principles.

6.3 The hazard analysis critical control point (HACCP) system

Originally developed for the US space programme as a method of making safe foods for astronauts, HACCP is now recommended by the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) of the United Nations (UN) as a means of food control with specific application to enhancing the safety of food. In accordance with the Codex Alimentarius Commission, HACCP is recommended as the method of choice for food safety management by governments and by professional and trade bodies associated with the food industry. The HACCP system is entirely compatible with the implementation of quality management systems such as those developed against the International Organization for Standardization's publication ISO 9001: 2000 (ISO 2000b) which provides a model for the development, implementation and maintenance of quality systems.

HACCP itself is not a quality management system or quality assurance (QA) system in the proper sense of these terms. The express purpose of HACCP is the management of food safety. The concepts applied in the use of HACCP to identify and control hazards can be translated to issues of quality and, for instance, QA systems based on the concepts can be developed to control factors affecting aspects of food quality not related to food safety. HACCP itself should be reserved specifically for the management of food safety and not confused in its application by liberal reinterpretation as a dual method of food safety management and QA. This is not always so. At times, food businesses are tempted, or recommended, to use HACCP principles for both food safety and quality purposes. Consequently, management systems are established which combine requirements critical to food safety with those important to the control of quality factors unrelated to food safety. This can be a recipe for confusion and, sometimes, disaster. Though food safety is logically an element of quality, to ensure clarity of purpose food safety management systems should be developed and operated discretely, and in parallel with the systems used for controlling other aspects of food quality.

6.4 Good agricultural practice

When HACCP is used in food manufacturing it is sound practice to establish good manufacturing practice (GMP) as the foundation on which to build HACCP systems. The Institute of Food Science and Technology (IFST, 1998) states that GMP consists of two components: effective manufacturing operations and effective food control. These components interrelate and interact, and when working in concert will lead to the manufacture of food products which meet specifications and customer requirements. The concept of GMP is that all that is required to make food products, and control the quality of food products in line with specifications, is adequately defined and documented, and that manufacturing

operations and food control procedures are carried out according to the documented requirements which, essentially, form a quality plan. The relevance to HACCP is that GMP causes elements of the food manufacturing operations and food control procedures that have bearing on matters of food safety to be properly defined, documented and controlled. For example, basic issues of food hygiene, such as staff training and hygienic conduct, and the cleanliness of the manufacturing environment, may not be part of a HACCP system, but can influence the ability to prevent the occurrence of hazards, for instance, the contamination of food products with bacterial pathogens. GMP is synergistic with HACCP and working together the two will lead to increased confidence in the power to make safe food products.

Though GMP has been developed for the food manufacturing industry, the principles on which it is based are readily transferable to the growing of fruit and vegetables. In this instance the appropriate term is good agricultural practice or GAP. Growers undertake many activities that have the potential to generate hazards that may be associated with produce given the right circumstances, but which might not be included as requirements for control within the scope of HACCP systems. General site organization and cleanliness will lead to the minimization of sources of contamination that might compromise food safety. The handling, storage and methods of use of agrochemicals ought to be carried out in defined ways that are unlikely to lead to the creation of food safety hazards. Equipment used to apply agrochemicals, for example crop sprayers, should be calibrated to ensure correct levels of chemical application and should be cleaned between uses to prevent the possibility of cross-contamination between chemical compounds. Such requirements are good practice, though not necessarily a part of a HACCP system. Other examples include the treatment and use of animal wastes and farmyard manure as fertilizer. In recent years the use of animal faeces in certain agricultural practices has become a point of concern because of the potential for contamination with bacterial pathogens, and particularly because of the possible presence of *E. coli* O157:H7 which has a very low infective dose level. While it might be construed that the management of animal wastes to eliminate bacterial pathogens constitutes part of a HACCP system, the use of animal wastes in growing is analogous to the use of an ingredient in the manufacture of a food product. In food manufacturing, ingredients are selected partly on the basis of their safety. If a hazard is potentially associated with an ingredient but it cannot be controlled in the manufacturing process, the likely course of action will be to eliminate use of the ingredient. Animal wastes which have been properly composted to ensure the destruction of bacterial pathogens can be considered as safe 'ingredients' in the growing of fruit and vegetables. The treatment and control of animal wastes should be governed by GAP (Chambers, 1999) and not become part of HACCP systems. Similarly, water used for irrigation should be free of contamination by animal wastes and any other materials that might lead to the presence of bacterial pathogens (or other harmful substances, such as heavy metals). The specification and control of irrigation water is a matter for GAP and not HACCP. Controlled by GAP, manures and irrigation waters, as inputs to the

processes of growing fruit and vegetables, and water itself in pretreatment processes, should be specified in terms of their freedom from pathogens (and other contaminants). Confirmation of compliance with specifications is then likely to be part of the HACCP system. Confirmation may be achieved through, for example, microbiological analysis, but this is an unlikely and probably impractical method. Access to records demonstrating that manures have been adequately composted, and that irrigation waters have been appropriately treated and handled, should be sufficient.

6.5 Applying the HACCP concept

Food safety is defined as the 'Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use' (CCFH, 1997). The HACCP system provides a systematic method of food safety management and is based on the seven Principles of HACCP (CCFH, 1997):

Principle 1: Conduct a hazard analysis.
Principle 2: Determine the critical control points (CCPs).
Principle 3: Establish critical limit(s).
Principle 4: Establish a system to monitor control of the CCP(s).
Principle 5: Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
Principle 6: Establish procedures for verification to confirm the HACCP system is working effectively.
Principle 7: Establish documentation concerning all procedures and records appropriate to these principles and their application.

The HACCP concept is implemented through a logical sequence of activities, known as the HACCP study (described in section 6.6). The HACCP study should yield a HACCP plan which is implemented as the HACCP system. By definition (CCFH, 1997) a HACCP plan is 'A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration'. A HACCP system is 'A system which identifies, evaluates and controls hazards which are significant for food safety' (CCFH, 1997). As the term implies, hazard analysis is concerned with understanding the hazards associated with a food product. A hazard is defined (CCFH, 1997) as 'A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect'. Biological hazards include:

- poisonous plants and plant materials, e.g. deadly nightshade berries
- poisonous fungi
- infective pathogenic bacteria, e.g. *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*

- intoxicating pathogenic bacteria, e.g. *Staphylococcus aureus*, *Clostridium botulinum*
- toxigenic fungi, e.g. *Aspergillus flavus*, *Aspergillus clavatus*
- viruses, e.g. Norwalk virus, hepatitis A
- protozoan parasites, e.g. *Cryptosporidium parvum*, *Toxoplasma gondii*
- allergenic materials.

A number of microbial pathogens are responsible for a variety of foodborne illness. Some pathogens cause temporary inconvenience with symptoms such as vomiting and diarrhoea. Others cause serious and immediate effects, such as spontaneous abortion, or long-lasting conditions, for instance haemolytic uraemia syndrome (HUS) causing kidney failure and death, often in children, reactive arthritis, and Guillain–Barré syndrome. From the perspective of the grower, faecal materials contaminating fruits and vegetables which do not receive any kind of processing designed to kill vegetative pathogens associated with faeces can be problematic. *E. coli* O157:H7 is linked to HUS, as well as a number of other serious conditions. *Campylobacter* spp. have been associated with Guillain–Barré syndrome (a cause of paralysis in adults and children). *Listeria monocytogenes* can cause abortion as well as meningitis. All three organisms can be found in faecal matter, thus, for instance, manure can represent a significant source of microbial hazard unless managed adequately through GAP.

Chemical hazards can cause short-term illness from which a full recovery is usual, as well as long-term illnesses and death, for example brain damage and death resulting from long-term intoxication by heavy metals. While the health effects of some chemical contaminants are well documented, the effects (or freedom from effects) of some synthetic agrochemicals may be a matter of conjecture rather than proven science. For instance, though an agrochemical may be approved for use on the basis of toxicity research concerning the single substance, little may be known of the so-called 'cocktail effect' when residues of the substance exist in combination with others. Growers must, therefore, be cautious in the use of agrochemicals and ensure that records are kept of their correct use as advised by manufacturers, and that records allow traceability to manufacturers in the event that questions are asked about product safety and issues of legal liability arise. One of the benefits of using HACCP to manage food safety is that the system permits identification of sources of foodborne hazard within the food chain and enables businesses to avoid becoming accountable for food safety problems generated in other parts of the chain. Chemical contaminants that should be considered in developing HACCP plans include:

- naturally occurring environmental contaminants, e.g. heavy metals
- industrial contaminants, e.g. dioxins, polychlorinated biphenyls (PCBs)
- contaminants arising from agricultural practices, e.g. pesticides (insecticides, herbicides and fungicides, etc.)
- contaminants arising from the handling, storage and processing of foodstuffs,

e.g. seed treatment compounds, machine lubricants, cleaning agents, pest control poisons
- contaminants arising from food packaging, e.g. plasticizers and other packaging material additives, adhesives, inks, metals leached from cans.

Physical hazards can be problematic in the production of some fruits and vegetables. They can represent a source of harm to consumers in that they may damage tissue (externally and internally) through laceration, damage teeth and block airways. Physical hazards include:

- slicing hazards – sharp glass fragments, sharp plastic fragments, wood splinters, sharp metal filings and swarf
- dental hazards – glass particles, pieces of wood, pieces of hard plastic, stones, metal fragments and parts, e.g. nuts, washers
- choking hazards – wood, stones, metal fragments, string, nuts, e.g. peanuts.

During the preparation of a HACCP plan a variety of sources may be consulted to establish the identity and nature of hazards associated with food materials, products and processes. These include text books, scientific journals, food research organizations, consultants and academics, national and local government organizations with responsibility for food safety, and sources on the Internet.

6.6 The HACCP study

As a preventative approach to ensuring consumer safety, the HACCP concept of food safety management fits with QA philosophy generally and the operation of HACCP systems should fall within the scope of a grower's QA strategy. The resources needed to develop, implement and maintain HACCP systems should be furnished as part of a grower's overall QA provision. Though the employees of a business can directly influence the safety of products through their actions, the business as a whole carries a corporate responsibility for assuring no harm comes to consumers through eating their products. The ultimate responsibility for food safety and consumer protection in any food business should rest with top management (the chief executive or someone of equivalent status) who should also be responsible for ensuring that the resources for HACCP are adequate. This is necessary even though top management may have little direct contact with the mechanics of QA and food safety management. Inadequate resources in terms of qualified personnel, physical resources and time can be one of the key problems faced by food businesses in carrying out HACCP studies and in implementing, operating and maintaining HACCP systems. Without the commitment to providing adequate resources there is little point in embarking on a HACCP study. Ensuring this commitment is a primary function of top management.

The HACCP study defines the basis for the systems of consumer protection against foodborne hazards established by food businesses, such as those operated

by growers. It consists of a 12-stage process which aims to (a) analyse hazards, that is, identify the hazards most likely to be associated with a given product and the process by which it is produced, and (b) establish critical control points, that is, places in the production process where methods for the control of hazards can be applied.

6.6.1 Stage 1: Assemble the HACCP team (and define the scope and terms of reference of the study)

A HACCP team should be assembled and the scope and terms of reference of the study should be defined. The team should be multidisciplinary, containing members with the expertise required to deal with the range of issues that will arise during the study. A microbiologist (or someone with an adequate knowledge of microbiology) is usually an essential member of the team as microbial hazards must be appraised. It is also important to include members who have expertise in the crop production and pretreatment operations to be considered in the study. If the appropriate expertise is not available within an organization it may be necessary to use, for instance, the services of a consultant. The HACCP study should relate to a given crop product, its associated production process and any subsequent pretreatment processes, as relevant. The hazards arising in the production of a crop and the way they arise may be almost unique to that crop in the place where it is grown. It cannot be assumed that the factors affecting the production of a crop in one place will be identical to those affecting production of the same crop in another place. The use of generic HACCP plans is sometimes advocated as a short cut to the development of HACCP systems. Generic HACCP plans can be useful as sources of ideas and information, but the adoption of these plans can be dangerous as local factors may not be considered and hazards may be overlooked. It is better and safer to develop HACCP plans and systems locally, and avoid importing errors and misconceptions through the use of generic plans.

The scope of the study should be defined, stating the crop product to be considered by the study and identifying the production processes involved. The scope of the study sets a limit on the HACCP plan to be developed, defining where it starts and finishes. This is important, especially where complex production processes are concerned. It can be simpler (and safer) to break down processes into component operations and carry out a series of HACCP studies on 'bite-sized chunks', which link together later to form the overall HACCP system. For instance, seed preparation and propagation may form one HACCP study, with crop production, harvesting and post-harvest pretreatments forming others. When carrying out a HACCP study it can be tempting to consider the three categories of hazard – microbiological, chemical and physical – at the same time. This can lead to complications and by defining the terms of reference of the study an agreement is made to limit the study to, for example, microbial hazards, with the intention of considering chemical and physical hazards later. Knowledge gained during an initial study can be transferred to later studies, thereby reducing subsequent workloads.

6.6.2 Stage 2: Describe the product

A complete description of the product should be developed. The aim is to provide information that will enable the identification of hazards associated with intrinsic characteristics of the product itself, or from conditions concerning, for example, its packaging, storage, transport and distribution. In the manufacture of formulated foods, intrinsic preservation factors such as pH, salt-in-moisture content and water activity (a_w) are important to consider as they relate to the survival and growth of bacterial pathogens. In the case of fruit and vegetables such factors may be of lesser value, though, for instance, characteristics of the product resulting from harvesting, post-harvest handling and storage, and so on, may be relevant to the presence or development of hazards and should be considered. Similarly, the packaging of a crop may be defined as a part of the final product and should be assessed for its potential to be hazardous, for example, plastic bags can represent a suffocation risk for young children, or give rise to hazards, and the use of wooden boxes and pallets should be assessed in this respect. Also of importance may be the use of specific storage conditions, such as modified atmosphere storage, which might give rise to the development of hazards, for example anaerobic bacterial pathogens.

In describing the product, reference should be made to product specifications, for example specifications agreed with customers defining the quality and food safety parameters to which the product should conform. Crop production methods may result in products carrying pesticide residues, or potentially being contaminated with bacterial pathogens, and limits for both should be set in specifications. Other factors which may be specified are, for instance, physical hazards e.g. stones or fragments of wood resulting from harvesting operations and the use of harvested product management chemicals such as sprout suppressant compounds used on potatoes.

6.6.3 Stage 3: Identify the intended use of the product

The intended use of the product should be identified. The primary purpose is to identify whether the way the product is used (by a processor or consumer) could give rise to a hazard and to identify any sensitive groups for whom the product might be intrinsically harmful. It is also important to identify whether specific market requirements need to be observed. For example, only organic products or products which have not been genetically engineered may be supplied to certain markets, and some markets require certain varieties of produce, like potatoes suitable for crisp and snack food production (though these are not necessarily food safety issues). Fruits eaten without washing before sale and which might not be washed before consumption may be harmful if contaminated by pathogenic bacteria, or by high levels of pesticides or other toxic agrochemical compounds. For example, peaches and nectarines consumed whole, without removal of the skin, may have the potential to poison consumers in the event that the surface of the product is contaminated with harmful substances. Similarly, salad crops such as lettuce may not be adequately washed by consumers and may, therefore, present

the risk of food poisoning if the crops have been contaminated by enteropathogenic bacteria through the use of improperly composted manure or faecally contaminated irrigation water. In the case of crops destined for processing, the customer should be aware of the possible presence of contaminants, probably through agreement of the product specification. The customer then should be responsible for ensuring that hazards in the form of known contaminants are controlled. For example, some manufacturers of minimally processed prepared salads use chlorinated water to wash salad crop materials to reduce levels of contamination by one or two log cycles, and exercise controls that prevent cross-contamination (Carlin and Nguyen, 1999).

When fruit and vegetables are sold to consumers for use in the preparation of meals or for direct consumption the responsibility lies with the producer, and also retailers, to ensure that the products are free from hazards or that consumers are advised accordingly. Crop production methods should ensure that, for instance, pesticide residues do not exceed MRLs, but for additional confidence in the safety of the product, and the ability of the producer and retailer to demonstrate due diligence, it may be that some products are washed to reduce chemical residue levels, for example in the case of some apples, oranges and potatoes. Where controls are not implemented the responsibility for control falls to consumers who should be warned of the possible existence of a hazard so they can take the appropriate course of action. This is the principle seen in the example of food products which contain, or may contain, nuts and which are labelled to warn consumers suffering from nut allergy, who fall into the category of 'sensitive groups'. It is necessary to identify sensitive consumers who may be harmed by characteristics of the product which do not normally represent a hazard to most consumers. Such consumers include children, pregnant women, old people and people whose immune systems are depressed. They can be more susceptible to infection by certain pathogenic bacteria, such as *E. coli* O157:H7 and *Listeria monocytogenes*. Because babies constitute a sensitive group, fruits and vegetables produced for the manufacture of baby foods may be more tightly specified than for other purposes.

6.6.4 Stage 4: Construct a flow diagram

Describing a food product and identifying its intended use generates information that serves in the identification of hazards associated with the materials used to make the product and with characteristics of the product. Many aspects of the production process may also have the potential to give rise to hazards and it should, therefore, be analysed to ensure the identification of possible hazards.

A flow diagram of the production process (Fig. 6.1) should be prepared which identifies the inputs to, and the outputs from, the process, as well as the operating conditions and parameters required to produce the product. Inputs include seed, seed treatment agents, irrigation water, manure, fertilizers and pesticides, as well as water used in post-harvest, pretreatment operations such as hydrocooling to remove field heat and washing to remove soil and contami-

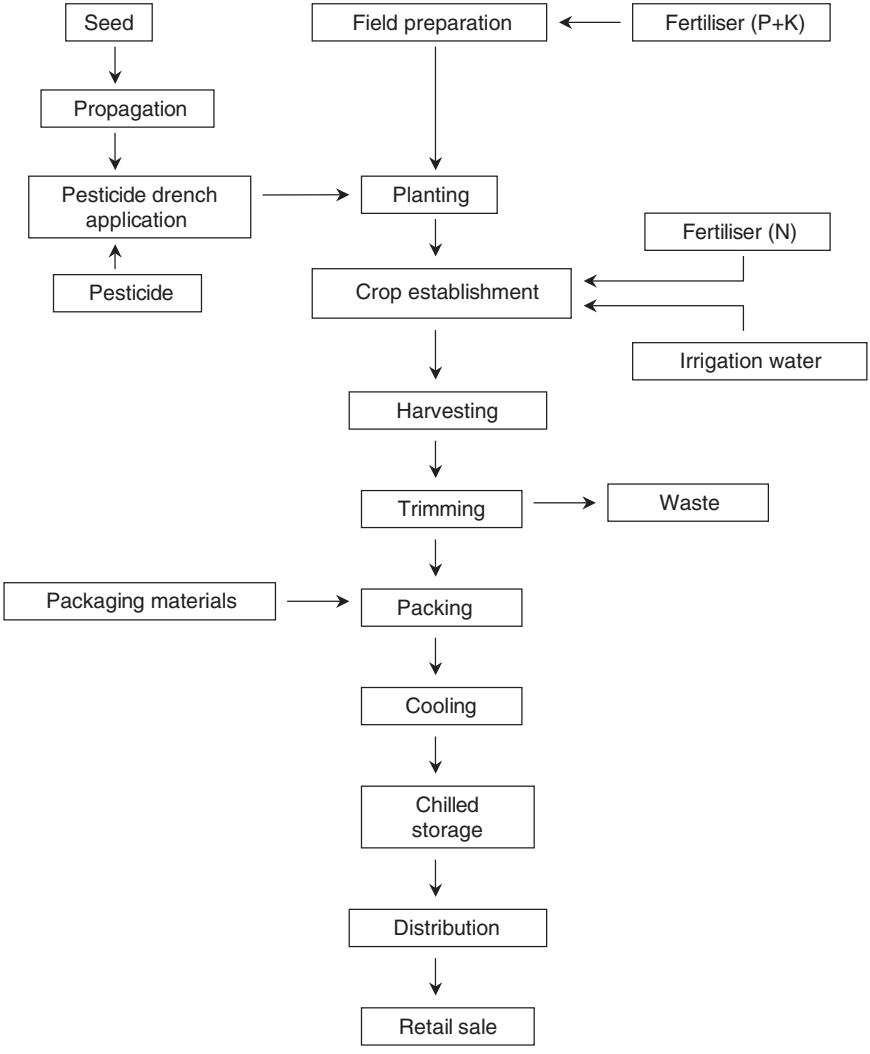

Fig. 6.1 Example flow diagram for the production of a field salad crop.

nants, and so on. Clearly the product itself is the principal output, but others may be product that has been rejected in grading owing to damage or deterioration, or waste botanical material (e.g. from trimming and other preparation processes, soil from washing operations, etc.), all of which, given the right circumstances, could give rise to hazards. The process itself will comprise a sequence of various operations including, for example, seed propagation, field or site preparation, fertilizer applications, planting, growing, irrigation, pesticide applications, harvesting, post-harvest handling and post-harvest pretreatments, like cleaning and trimming, as well as storage and transport, any of

which may present certain hazards. The scope of the flow diagram should be constrained by the scope of the HACCP study. It should be logically and systematically structured, and provide sufficient detail to allow the identification of hazards associated with the process without constant reference to additional information.

6.6.5 Stage 5: Confirm the flow diagram

The flow diagram may be prepared by referring to information and data concerning the crop production process and the various operations carried out as part of the process. Sources of information may include specifications or data sheets for seed, fertilizers, pesticides and so on, procedures for site preparation and crop production, procedures for harvesting, post-harvest crop management and post-harvest processing, specifications for packaging and storage. Whatever the sources used in its preparation, the completed flow diagram should be confirmed as a true representation of the production process, not a reflection of a theoretical process which differs from the true process because changes have been made which are undocumented and, possibly, unapproved. Ideally, confirmation should be made by 'walking the process', whereby the flow diagram is compared with what actually happens, as it happens. It may not be practical, however, to observe a complete growing cycle to confirm the diagram, so reference must be made to procedures, records and the experience of personnel to confirm accuracy and veracity.

6.6.6 Stage 6: Identify and analyse all potential hazards, assess the risks and identify the preventive measures (HACCP Principle 1)

The information gathered about the product and its intended use and the information contained in the process flow diagram form the basis of the hazard analysis stage of the HACCP study, along with any other relevant information, for example literature on the foodborne hazards associated with given fruit and vegetable products. All of the potential hazards associated with inputs to the crop production process, the process itself, outputs from the process and the product should be listed. Each hazard should then be analysed in turn with regard to its nature and the risk associated with it. Risk is the combination of the severity of the adverse effects of the hazard on the health of the consumer and the likelihood of the hazard occurring. It is not an easy thing to quantify. Attention should be given to establishing the risk associated with hazards, because the results will help to focus the HACCP plan on the hazards most important for ensuring the safety of consumers.

Preventive measures should be identified for every hazard that, by its nature, demands control for reasons of consumer safety and compliance with the law. If a hazard is considered to be such a low risk that it is unlikely ever to occur, it may be justifiable to exclude it from further consideration. A preventive measure is an activity, procedure or feature of a process that either prevents the occur-

rence of a hazard, or eliminates it, or reduces it to an acceptable level. The sources of hazards in crop production and pretreatment are diverse and cannot be comprehensively considered here. They are dependent on the nature of the crop and specific production processes involved. As already stated, they may be introduced with inputs to the production process, or arise as part of the process, or they may be a consequence of some aspect of an output of the process. Equally, they may arise from the product itself, possibly owing to a condition or characteristic of the product. They may be introduced by people, or be derived from the environment, and they may occur as a consequence of a failure in the general management of the crop production processes and associated processes. Table 6.1 gives a form suitable for keeping a record of the evaluation of hazards.

6.6.7 Stage 7: Determine the critical control points (CCPs) (HACCP Principle 2)

A critical control point (CCP) is 'A step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level' (CCFH, 1997). Preventive measures are applied at CCPs. For every hazard identified in the hazard analysis stage (Stage 6) each step in the process must be assessed to determine if the step constitutes a CCP. Experience and judgement can be used to decide whether a process step is a CCP, but reference is often made to the CCP decision tree (Fig. 6.2). Answering each of the four questions in the CCP decision tree provides an invaluable aid to the identification and clarification of CCPs. For instance, the moisture in fruit is not a hazard, as moisture represents no harm to consumers. The presence of moisture can allow mould growth and the development of mycotoxins which are potentially hazardous, for example patulin resulting from the growth of *Aspergillus clavatus*, sometimes found in apple juice. Selecting good quality apples, which are not bruised or damaged, for processing into fruit juice and maintaining them in the right condition under appropriate storage, is necessary to prevent mould growth and the formation of mycotoxins. Product selection (a YES response to question 2) and product storage (a NO response to question 4) each represent CCPs in this instance.

Care must be taken not to create more CCPs than are needed to ensure food safety as the complexity and costs of maintaining the HACCP system will increase unnecessarily. It can be argued that the possible hazard of excessive pesticide residues on fruit and vegetables may need control through different preventive measures concerning, for example, operator training, use of the correct pesticide, application of pesticide at the advised concentration and rate, and the maintenance and calibration of spraying equipment. Of these different areas of apparent control, application at the advised concentration and rate can be defined as a CCP. Training operators, using the correct pesticide and maintaining and calibrating spraying equipment are all activities that should be dealt with under GAP. The identification of CCPs can be recorded with details of hazards in Table 6.1.

Table 6.1 Worksheet for hazard analysis and CCP identification

Process step	Hazard	Control measures	Q1*	Q2	Q3	Q4	CCP Yes/No	Justification for being a CCP

*See Fig. 6.2

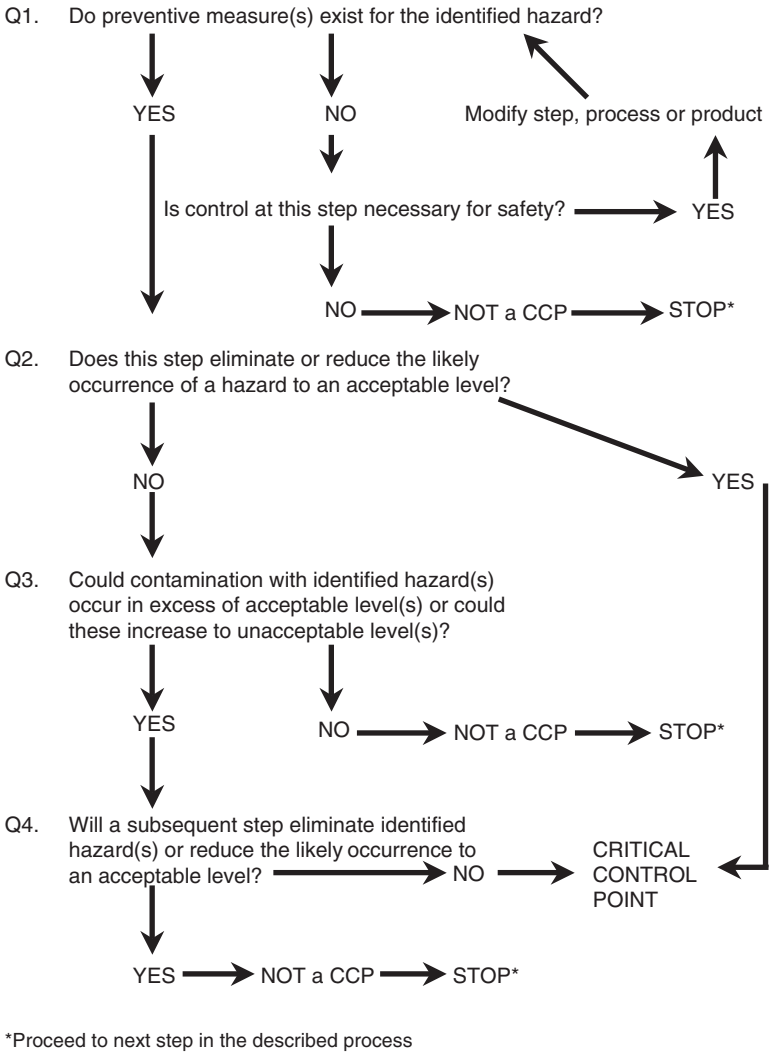

Fig. 6.2 CCP decision tree. Note that for each hazard identified, each question should be answered in relation to each step of the production process. Source: *FLAIR*, undated. *HACCP User Guide*, Concerted Action no 7, Food Linked Agro Industrial Research, 191, Rue de Vaugirard – 75015, Paris.

6.6.8 Stage 8: Establish critical limits for each CCP (HACCP Principle 3)

A critical limit is 'A criterion which separates acceptability from unacceptability' (CCFH, 1997). Critical limits establish parameters for the operation of preventive measures at CCPs and often concern quantitative values such as time, temperature, pH, a_w, concentration, application rates and so on. Critical limits may be set by regulations (e.g. pesticide MRLs defined by law), they may be recommended

by industry codes of practice, or, for example, be established by manufacturers of proprietary agents and growers or customers, by reference to scientifically accepted values. Provided the critical limits established for a CCP are being adhered to, product safety with respect to the CCP should be assured. In some instances operational limits for some elements of crop production processes may be set with reduced tolerances compared to the critical limits of CCPs to provide a safety margin for the management of CCPs. Records of the control activities at CCPs should be maintained for HACCP system verification and maintenance, as well as to provide evidence of due diligence in the production of safe food.

6.6.9 Stage 9: Establish a monitoring system for each CCP (HACCP Principle 4)

Monitoring activities are carried out to confirm that the controls exercised at CCPs remain effective to ensure food safety. CCPs are monitored and the monitoring activities or procedures must be capable of detecting that control has been lost. Monitoring generally consists of a planned sequence of observations or measurements and the results of monitoring are essential to show that the HACCP system is operating effectively. Monitoring methods should be as simple as possible and may concern various forms of measurement, such as temperature, time, concentrations, pH and so on, or they may be based on the observation of procedures and the checking of records. The HACCP plan should identify how each CCP is to be monitored, the frequency of monitoring and who is responsible for ensuring that monitoring is carried out. Records of monitoring activities should be maintained for HACCP system verification and maintenance, as well as for due diligence purposes. Although conventionally, monitoring activities show that control has been lost at CCPs, it can be beneficial to use monitoring, when possible, to indicate that a CCP is going out of control. Rather than allow control to be lost and then have to take corrective action to remedy the problem, it makes sense to adopt a preventive position to ensure control is maintained, as far as possible, at all times.

6.6.10 Stage 10: Establish corrective action procedures (HACCP Principle 5)

In the event that monitoring shows that a CCP is no longer in control, corrective action must be taken, (a) to return the CCP to a state of control and (b) to identify and manage any potentially non-conforming (unsafe) product. A corrective action procedure should be established for each CCP identifying a specific course of action for the return of control. It should also define requirements for identifying, segregating and testing implicated product, as appropriate, to prevent non-conforming product being inadvertently despatched to customers. Each corrective action procedure should identify the personnel responsible for taking action and controlling product, as well as the personnel responsible for verifying that control has been returned. The corrective action procedures constitute the corrective action plan for the HACCP system.

6.6.11 Stage 11: Establish verification procedures (HACCP Principle 6)

When the HACCP plan is complete it can be implemented (see section 6.7, below) as the HACCP system, and both validation and verification activities should be carried out. Put simply (ILSI, 1999), validation concerns answering the question: Will the system work when we put it into practice? whereas, verification should answer the question: Are we doing what we planned to do? Validation is defined (CCFH, 1997) as 'Obtaining evidence that the HACCP plan is [likely to be] effective'. Validation amounts to an assessment of the scientific and technical content of the HACCP plan. Verification is defined as (CCFH, 1997) 'The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan'. In effect, verification is a check that what the HACCP plan says will be done is, in fact, done. As validation concerns checking that the elements of the HACCP plan are complete, that decisions and assumptions made during the HACCP study are sound, and that the plan is adequate to create a workable and effective food safety management system, it must be carried out before HACCP system implementation and verification. ILSI (1999) recommends a series of validation activities intended to collect objective evidence which confirms the adequacy of the plan in relation to the seven principles of HACCP:

Principle 1 (Hazard analysis): Confirm that the skills of the HACCP team members were correct for the task, that the flow diagram was suitable for the purposes of the study and that all significant hazards and appropriate preventive measures have been identified.

Principle 2 (Identify CCPs): Confirm that CCPs suitable to effect control have been identified for all significant hazards and that the CCPs are at appropriate stages of the process.

Principle 3 (Critical limits): Confirm that adequate critical limits have been identified for each hazard in relation to relevant CCPs.

Principle 4 (Monitoring): Confirm that monitoring methods and systems are capable of demonstrating the effectiveness of control measures at CCPs, and that procedures exist for the calibration of monitoring methods and systems, as appropriate.

Principle 5 (Corrective action): Confirm that corrective action procedures exist for each hazard and the relevant CCPs are sufficient to return the CCPs to control and to prevent non-conforming product from reaching customers. Also, confirm that responsibility for taking corrective action and the authority for verifying corrective action and approving the disposition of non-conforming product have been identified.

Principle 6 (Verification): Confirm that procedures and a plan for the verification of the HACCP system have been established.

Principle 7 (Documentation): Confirm that documentation describing the entire

HACCP system exists and that records required to support the system have been established.

Fundamentally, validation should seek to confirm that the HACCP plan is comprehensive and will be effective as a means of protecting consumers, through the control of identified foodborne hazards, when implemented as the HACCP system. Auditing techniques, such as those employed in quality systems auditing, should be used for validation.

Verification activities should confirm that the HACCP system has been implemented in compliance with the HACCP plan, and procedures (and any other methods or tests deemed necessary) should be established for this purpose. Verification should confirm that:

- Hazard analysis and the identification of preventive measures was carried out properly (also a check on validation).
- The identification of CCPs and setting of critical limits was carried out properly (also a check on validation).
- The control of hazards at CCPs is effective and records of CCP control are kept.
- Monitoring methods are effective and monitoring records are kept.
- Corrective action procedures work effectively, that customers are protected from receiving non-conforming product and records of corrective action are kept.
- Verification procedures themselves are carried out properly.
- Documentation covering the entire HACCP system has been established and records to support the system are completed properly and are retained for an appropriate period of time.

Because of the length of crop production cycles it may be necessary to stagger verification activities throughout the year to cover different parts of the process as they come into play. During an extended period of verification matters such as deviations from intended production processes, for example owing to unforeseen circumstances such as the weather, pests and so on, will be of particular interest, as will the implementation of preventive and corrective actions concerning such deviations. Once completed, the results of verification and any information gained from the experience of deviations may be used to modify and improve the HACCP plan.

6.6.12 Stage 12: Establish documentation and record keeping requirements (HACCP Principle 7)

A variety of documents and records are needed to develop the HACCP plan and to support the HACCP system, and a variety will result from the HACCP study. The study should yield a HACCP control chart (Table 6.2) which is central to the HACCP plan and substantially defines the operation and control of the HACCP

Table 6.2 Model of HACCP control plan for field crop production*

Process step	CCP no	Hazard	Control measure	Critical limit(s)	Monitoring**		Corrective action**
					Procedure	Frequency	Procedure
Seed delivery	1	Unacceptable pesticide residues in seed	Seed meets spec.	As stated in spec.	Check certificate of analysis on delivery	Each delivery	Reject delivery Review supplier
Pesticide drench	2	Microbial pathogens in drench water	Use potable water	Absence of pathogens	Confirm water quality with supplier	Annually	Agree measures for improvement with supplier
Site preparation	3	Contamination with pathogens from unrotted manure	Check history of manure use on site	No unrotted manure used in past 2 years	Confirm site history	Prior to site preparation	Use site only if free from manure deposits or choose another site
Irrigation	4	Microbial pathogens in water	Use clean water	Absence of pathogens	Confirm water quality with supplier	Prior to use	Agree measures for improvement with supplier or use another source
Harvesting (glass control)	5	Contamination with glass from machinery	Glass policy – only use glass when needed and care taken when glass involved	No glass contamination of product	Check all glass for damage	Daily	Segregate and check implicated product before approving for use
Harvesting (wood control)	6	Contamination with wood from packaging	Care taken when wood is involved	No wood contamination of product	Check packaging materials for damage	Daily	Segregate and check implicated product before approving for use
Harvesting (staff control)	7	Contamination with microbial pathogens from staff	Good personal hygiene practised by staff	Staff adhering to personal hygiene policy	Observation and supervision of staff	Continuous	Appropriate management of staff breaking the rules
Storage	8	Growth of microbial pathogens	Select temperature and humidity suitable to prevent growth	Adequate temperature and humidity to maintain product quality, but unsuitable for microbial growth	Check storage temperature and humidity	Daily	Segregate and check implicated product before approving for use. Rectify temperature and humidity
Transport	9	Contamination with microbial pathogens from transport vehicles	Use only approved vehicles and hauliers Check vehicles before use	Vehicles clean, hygienic and fit for use	Check records of vehicle inspection	Daily	Agree measures for improvement with haulier or use another approved haulier
	10	Growth of microbial pathogens	Check temperature and humidity suitable to prevent growth prior to despatch	Temperature/humidity suitable to maintain product quality, but unsuitable for microbial growth	Check records of vehicle temperature and humidity assessment	Daily	Review control procedures Agree measures for improvement with haulier or use another approved haulier

* A plan for soft fruit production would be similar, but with, e.g., field pesticide applications included.
** The responsibility for monitoring and corrective action would normally be given.

system. Other documents that will become part of, or will be referenced in, the plan include product specifications, purchased product specifications (covering products such a seed, agrochemicals, etc.), the process flow diagram, crop production procedures, procedures concerning preventive measures and the control of CCPs, monitoring procedures, corrective action procedures and verification procedures. Records will include CCP control records, monitoring records, corrective action records and verification records. Also, records should be kept of HACCP plan amendments and HACCP system modifications arising from validation and verification activities.

6.7 Implementing and maintaining HACCP systems

Implementation and maintenance are not HACCP principles, though they might well be. The thorough implementation of the HACCP plan and the effective maintenance of the HACCP system are as critical to ensuring food safety as the development of a good plan in the first place. Mortimore and Wallace (2001) define an eight-step approach to implementation. Adaptation of this approach to emphasize the implementation of preventive measures, or confirmation of their adequacy if they already exist, leads to a ten-step process, as follows:

1. *Determine the approach to implementation* – this requires the HACCP system to be implemented as a complete system, in one go, or to be broken down into more manageable and practical units.
2. *Agree the activities to be undertaken and the timetable* – this requires the various implementation activities needed to bring the HACCP system into being to be identified and responsibility for completion of the activities to be assigned to named people. Various techniques can be used to establish a timetable for implementation, e.g. the use of Gantt charts.
3. *Confirm the existence of adequate preventive measures, or implement preventive measures, as necessary* – in some instances preventive measures may already exist as part of an operational process, but in others the HACCP study may have demonstrated the need for additional preventive measures which must be implemented.
4. *Conduct training in the operation of preventive measures or confirm that adequate operation exists* – confirmation must be made that the preventive measures are operating satisfactorily and, in some instances, additional staff training in their operation may be necessary, e.g. in the case of new preventive measures.
5. *Set up CCP monitoring methods* – methods for monitoring the control of CCPs must be established.
6. *Conduct training in CCP monitoring* – the staff responsible for carrying out CCP monitoring activities and those responsible for taking corrective action when monitoring shows the loss of control must be trained appropriately for their duties.

7. *Complete 'once-only' activities* – this requires the completion of activities needed to put everything in place to complete the HACCP system, such as procedure writing, the creation of records, establishing document and record control systems, process engineering and modification, and staff training.
8. *Confirm the monitoring systems are in place* – when the CCP monitoring methods are in place, staff have been trained in their use and supporting once-only activities have been completed, confirmation must be made that monitoring systems are in place and operating adequately.
9. *Confirm implementation is complete and operate the HACCP system* – when confirmation is made that implementation activities have been completed, the HACCP system can become fully operational.
10. *Audit to confirm adequate implementation* – adequate implementation of the HACCP system should be achieved through audit using standard QA auditing techniques. It will be necessary to run the system for an agreed period of time to generate records that can be examined to determine the state of implementation and operational effectiveness, and one (or more) complete growing cycle(s) may be needed to have complete confidence in the system.

Although the HACCP plan may be operational in the form of the HACCP system, both the plan and the system will need to be maintained. The HACCP plan should be reviewed annually to confirm that it still addresses all food safety requirements. However, if changes to the product or process are made which impact on food safety during the year, these should be incorporated within the plan, at the appropriate time, and implemented as modifications to the HACCP system. HACCP system audits should be carried out to confirm that the system still complies with the requirements of the plan and corrective actions should be taken to rectify non-compliances. Other activities will also demonstrate the continuing suitability and effectiveness of the HACCP system, for example, review of CCP control and monitoring records, review of HACCP corrective action records, customer complaints and so on, and action may be taken to revise the HACCP plan and system based on the results of these activities. Additional factors that may give rise to the need to revise the HACCP plan and system are changes to the product specification, for example customer or legal requirements causing the revision of critical limits, and the emergence of new hazards, for example the recognition of new bacterial pathogens of concern.

6.8 Future trends

Many developments concerning the nature and use of HACCP and in relation to the application of HACCP can be expected over the next decade. Mayes (2001) recognizes the continued globalization of the world's food industry as one of the driving forces for the increased use of HACCP in the food supply chain. He suggests that HACCP will become the benchmark method for food safety

management, as advocated by Codex Alimentarius, and that World Trade Organization (WTO) member countries that adopt Codex standards will not have to justify their sanitary measures under the WTO's Sanitary and Phytosanitary (SPS) agreement. There would, then, seem to be advantages in working to Codex standards, but as Mayes indicates, the global acceptance of HACCP as the standard for food safety management raises issues about standardized methods of application, and assessment of the effectiveness of implementation and the ability to control foodborne hazards.

With the development of global food supply chains the potential for the movement of foodborne pathogens (foodborne disease organisms and food poisoning organisms) from one country to another is increased, with the possibility of increasing the risk of harm from 'alien' pathogens for sensitive groups in populations and, possibly, populations themselves. Owing to the speed at which the globalization of food supply is being encouraged by Western governments, international food production and manufacturing businesses and major supermarketing companies, some urgency would seem to be justified in establishing a standardized, water-tight approach to food safety management at all levels of the international food supply system. The circumstances and history of the UK's BSE disaster demonstrate how a major threat to public health can appear almost from nowhere, with consumer protection lagging behind the occurrence of the hazard. In this respect, the disaster illustrates a severe limitation of HACCP, especially when applied to food safety in the context of the global food supply chain.

The effectiveness of HACCP systems is contingent on the identification of known hazards, yet, by definition, the unpredicted hazard is the hazard that we do not expect and will not plan to control. In recent years we have become acutely aware of the problem of 'emergent pathogens' and that, in some instances, we need to accumulate a body of scientific knowledge and experience concerning such organisms before we can properly establish food safety controls. The global movement of foodstuffs, particularly unprocessed and minimally processed foods, such as fresh vegetables and meat sold through supermarkets, has the potential to expose consumers to new and possibly virulent foodborne pathogens which will not always be accounted for in HACCP plans. How the world's public health experts deal with such threats remains to be seen.

The development and implementation of HACCP systems relies on the correct interpretation of the seven principles of HACCP. Different businesses and different HACCP teams can make very different interpretations and create systems which fail to control hazards as comprehensively and as effectively as they might. One only has to look at the way the requirements of the EU Directive 93/43 on the hygiene of foodstuffs are dealt with in the UK's Food Safety (General Food Hygiene) Regulations 1995, and then interpreted by food businesses and local food law enforcement officers, to see what kind of variation is possible in the management of food safety based on standard guidelines. The development of a standardized interpretation of the principles of HACCP and their application in different food sectors is needed (avoiding the inherent problems and limitations of generic HACCP plans), as are standardized approaches to conducting HACCP

studies, implementing and maintaining HACCP systems, documenting HACCP plans and HACCP training. The Codex Alimentarius Commission is tackling the issue of standardized approaches to the use of HACCP and many of the Codex publications on HACCP, for example the document on training in food hygiene and HACCP (FAO, 1998), serve as valuable guides in this.

Alongside the issue of standardizing the use of HACCP is that of standardizing the assessment of HACCP systems. Businesses that implement HACCP systems are generally responsible for assessing the suitability and effectiveness of their own systems. Questions inevitably arise about the consistency of approach to auditing HACCP systems and this is a matter that is likely to receive attention in the future. A factor that impinges on this issue is that of third party audit. In some instances customers, for example supermarkets, require their suppliers' HACCP systems (and quality management systems) to be audited independently. A number of organizations whose business is the assessment of quality management systems (e.g. Lloyds Register Quality Assurance), also provide third party HACCP system audit services, and many food businesses have their system audited as part of the overall assessment of quality and food safety management against, for example, the BRC and EFSIS standards. The growth in organizations offering third party HACCP system audit services would seem to demand the development of nationally, and internationally, accepted methods of HACCP system approval and audit.

During the 30 years or more that it has taken for HACCP to become widely used by the food industry, the organizations that have been most active in adopting this approach to food safety management have tended to be larger food businesses. Small and medium sized enterprises (SMEs) have found HACCP a difficult concept to grasp (often because of a lack of scientific and technical ability within the businesses) and the requirements of HACCP difficult to implement (often because of a lack of human and physical resources). Developments in HACCP are likely to take into account the variable nature of food businesses and their varying ability to utilize the methodology.

As the concept of a global, integrated food supply system generating an integrated food supply chain – 'from field to fork' – develops, the use of HACCP by all food businesses in the supply chain will be advocated by public health authorities and by certain businesses within the food supply chain. For instance, supermarkets will require their suppliers to use HACCP for consumer protection, and to enable them to demonstrate that they have acted diligently when sourcing products from suppliers. Such developments will, of course, take HACCP into businesses which, traditionally, have not been overly concerned with matters of human food safety, such as farms and agricultural inputs businesses, because food safety was formerly seen as mainly a province of food processors and manufacturers. Incidents such as the BSE disaster have emphasized the fact that actions and occurrences in one part of the food supply chain (e.g. in animal feed production) can have dramatic and disastrous consequences for other parts of the chain (e.g. consumers and farmers). The assurance of food safety throughout the whole food chain must, therefore, be addressed by the businesses that constitute

the food supply system. This will, of course, result in HACCP being used in many kinds of food business where it has not previously found application and where interpretation will raise difficulties. For instance, following a serious outbreak of *E. coli* O157:H7 poisoning in Lanarkshire in 1996/97, UK abattoirs and raw meat processors are now required to implement HACCP systems. As a result abattoirs and meat cutting plants have been advised (MLC, 1999) that a number of CCPs for bacterial pathogen control exist at points in the process involving hide removal, evisceration and carcass dressing. By definition, hazards are either prevented, eliminated or reduced to acceptable levels at CCPs. Given the nature of abattoir operations some contamination of carcasses is inevitable. Patently, the kinds of controls operated in abattoirs will not prevent, eliminate or reduce pathogens (such as *E. coli* O157:H7) to acceptable levels. Indeed, abattoirs do not normally have appropriate, rapid methods even to monitor levels of pathogen control. Logically, cooking meat properly is the way to control meat-borne pathogens, such as *E. coli* O157:H7. It would seem that the UK's meat industry has been poorly advised, through a misinterpretation of HACCP, to implement control measures which, rightly, belong to GMP and not HACCP. Growers of fruit and vegetables can learn from the experiences of other food businesses, such as abattoirs and meat cutting plants, and save themselves from the creation of over-complicated HACCP systems, with all the operating costs entailed.

As stated earlier in this section there are likely to be developments concerned with standardizing the interpretation of HACCP and such developments should yield benefits in the clearer understanding of HACCP for businesses where this approach to food safety management has not normally been used. Alongside developments in standardized approaches, we are also likely to see work which stresses the importance of GMP (or GAP, in the case of farmers and growers) for establishing sound management practices and prerequisites for the development of HACCP plans. Clearly, GMP (or GAP) can provide a firm foundation for HACCP systems, but its importance in creating sensible systems which are cost effective to operate should not be overlooked, and, possibly, needs stressing through work which emphasizes the integration of GMP (or GAP) with HACCP. In relation to such developments we can also expect to see developments which further the food industry's understanding of how to integrate beneficially HACCP with quality management systems developed against the ISO 9001: 2000 quality system standard. Finally, it is important to raise the issue of risk assessment. This is, perhaps, one of the most difficult issues to contend with during the development of HACCP plans. When faced with the possibility of a hazard occurring in the production of a food, stating the likelihood of its occurrence and deciding, therefore, whether it is a hazard that must be controlled can be a difficult and, at times, worrying task. Consequently, HACCP plans are often written that take into account every hazard conceivable, irrespective of whether or not they are likely to occur. Establishing preventive measures, CCPs and monitoring systems for hazards that are highly unlikely to occur only adds to the costs and complexity of implementing and maintaining HACCP systems. Improvements are needed in

how to understand risks and carry out risk assessments that lead to effective, practical HACCP systems which do not suffer from over-design and unnecessary complication.

6.9 Sources of further information and advice

6.9.1 Organisations

The British Retail Consortium, 5 Grafton Street, London, W1S 4EG, UK. http://www.brc.org.uk/

Campden and Chorleywood Food Research Association (CCFRA), Chipping Campden, Gloucestershire, GL55 6LD, UK. http://www.campden.co.uk

The European Food Safety Inspection Service: EFSIS Limited, PO Box 44, Winterhill House, Snowdon Drive, Milton Keynes MK6 1AX, UK. http://www.efsis.com/index.htm

U.S. Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857–0001, USA. http://www.fda.gov/default.htm. See also: the USFDA Foodborne Pathogenic Microorganisms and Natural Toxins Handbook (Bad Bug Book) at http://vm.cfsan.fda.gov/~mow/intro.html

ILSI (International Life Sciences Institute) USA, One Thomas Circle, 9th Floor, Washington DC, 20005, USA. http://www.ilsi.org/

ILSI (International Life Sciences Institute) Europe, Avenue E. Mounier 83, Box 6, B-1200 Brussels, Belgium. http://europe.ilsi.org/

6.9.2 Books

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MORTIMORE S and WALLACE C (1998) *HACCP: a Practical Approach*, 2nd edition, Gaithersburg, Aspen Publishers.

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FAO (1998) *Food Quality and Safety Systems*, Rome, Food and Agriculture Organization of the United Nations.

IFST (1998) *Food and Drink: Good Manufacturing Practice*, 4th edition, London, Institute of Food Science and Technology (UK).

ILSI (1999) *Validation and Verification of HACCP*, Brussels, International Life Sciences Institute.

ISO (2000a) ISO 9000: 2000 *Quality Management System – Fundamentals and Vocabulary*, Geneva, International Organization for Standardization.

ISO (2000b) ISO 9001: 2000 *Quality Management System – Requirements*, Geneva, International Organization for Standardization.

MAYES T (2001) 'The future of HACCP,' in *Making the Most of HACCP*, eds Mays T and Mortimore S, Abington, Woodhead Publishing.

MLC (1999) *HACCP Systems in Abattoirs and Meat Cutting Plants: Guide to Implementation*, Milton Keynes, Meat and Livestock Commission.

MORTIMORE S and WALLACE C (2001) *HACCP*, Oxford, Blackwell Science.

7

Maintaining the post-harvest quality of fruits and vegetables

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7.1 Introduction

To achieve a high quality processed product, it is important that the raw materials used in the product are also of high quality. This chapter focuses on maintaining fresh produce quality prior to its processing. It assumes that the produce has been selected on the grounds of suitability for end-use and presents an overview of how the harvest quality may be maintained up to the point of processing or consumption.

In section 7.2 the author identifies appearance, texture and flavour as the qualities which are most likely to be key to acceptability of produce whether consumed fresh or processed. The factors that influence quality deterioration in fresh produce are then explored in sections 7.3–7.6. The tissues of fruits and vegetables remain alive after harvest. They eventually die through natural senescence, rotting or when they are consumed, cooked or similarly processed. All living tissues respire and the consequences of this are quite profound for the maintenance of quality and maximisation of shelf-life of these products. Factors that slow respiration can slow senescence and maintain quality; however, some respiration must continue or the products will rapidly senesce and die. Cooling the produce can slow many undesirable changes in fruits and vegetables but many fresh commodities are intolerant of low temperatures. Thus understanding the physiology of fresh produce is fundamental to understanding their stability and likely shelf-life.

It would be a great advantage to managing fresh produce quality if the shelf-life of a product could be accurately predicted. In practice the variability inherent in fresh fruits and vegetables makes this very difficult. In section 7.7 the commercial application of shelf-life testing for fruits and vegetables and its rationale is discussed. The methods in common use for measuring fresh produce quality are reviewed.

The demand for all-year-round supplies at ever-higher quality standards by both the processing industry and retail sector is driving the development of new technical and managerial strategies. Although refrigeration throughout the cool-chain is likely to remain the most important technology for maintaining product quality, a broader range of approaches are increasingly in use, such as modified atmospheres during transport, storage and in individual produce packages. In sections 7.8–7.12 the broad range of post-harvest technologies used to maintain quality and extend shelf-life of fresh fruits and vegetables are briefly reviewed. Finally in section 7.13 some technologies that are likely to become available or of increasing importance to the fresh produce industry in the near future are suggested. One clear trend is that more fresh produce will be consumed in a minimally processed form, that is partially or fully prepared for consumption. The shelf-life of these products is often much reduced compared to that of the intact product. Non-destructive, on-line quality testing, the expansion of non-chemical control of fresh produce diseases and disorders and the availability of genetically modified crops are predicted by the author to have the most influence in quality management in the coming years.

7.2 Quality criteria for fresh produce: appearance, texture, flavour and aroma

7.2.1 Introduction

The specific qualities required in fruits and vegetables will depend on their end-use and the selection of appropriate cultivars for particular products is of paramount importance. The quality of an individual product is also affected by its specific preharvest 'experience'. So, for example, the position of a fruit on the tree will determine its nutrient and water status and its exposure to environmental factors such as sunlight or pests and diseases. All these factors may ultimately influence post-harvest shelf-life (Hofman and Smith, 1994; Sharples, 1984). Experience may enable those who regularly handle certain produce types to predict variations in shelf-life of produce from different sources, for example, based on soil type or weather factors before and during harvest.

Fresh fruits and vegetables are not considered to be high-risk products with respect to food safety as they normally become completely undesirable for consumption long before any hazardous microorganisms or toxins might develop. There is, however, evidence that sealing fresh vegetables in modified atmosphere packaging may extend shelf-life, while still allowing the growth of pathogenic bacteria, in particular *Listeria* spp and *Escherichia coli* O157 (Phillips, 1996). For most fresh produce, shelf-life is best defined as the period within which the product retains acceptable quality for sale to the processor or consumer. It is necessary, therefore, to identify what 'acceptable quality' means before it can be decided at what point the product no longer satisfies those expectations.

For the fresh produce market, specific minimum quality standards exist in many countries; however, owing to the international nature of the fresh produce

market, there is a trend towards international standardisation of quality grades. The European Commission was one of the first organisations to develop international standards for fresh fruits and vegetables (MAFF, 1996a–c). Many of these standards have been adopted by the Organisation for Economic Cooperation and Development (OECD). Usually, standards required for multiple retail outlets are considerably more stringent than these minimum standards and will be defined for the supplier by the retailer. Providing the quality standards have been met, the factors which limit storage and shelf-life fall into the following categories: appearance, texture and flavour/aroma. With respect to the processing industry, each company will have its own carefully defined quality criteria based on the nature of the processing undertaken. These criteria will be agreed in advance with the supplier.

7.2.2 Appearance

Appearance is the key factor for consumers in making purchases of fresh produce. As the multiple retail sector has come to dominate food retailing in many countries, consumers have come to expect fresh produce to have near perfect visual appearance. Displays of fruits and vegetables are characterised by uniformity of size, shape and colour. Vital components of visual quality include colour and colour uniformity, glossiness, and absence of defects in shape or skin finish and freedom from disease.

The importance of appearance in the processing industry will depend on which part of the produce is used in the product and whether the appearance can readily be enhanced during processing, for example by the use of natural colouring additives. In most products, the peel will be removed from the produce, so purely surface blemishes will be of little consequence. Internal flesh colour is usually more important than peel colour. Size and shape may be highly important where processing is automated rather than manual; however, for some products these attributes are less important, for example for juice extraction.

Many fruits and vegetables undergo colour changes as part of the ripening process. Unripe fruit is usually green (the so-called ‘ground colour’) and in many types of fruit, the green colour becomes lighter during ripening and maturation owing to breakdown of chlorophyll, for example in apples, grapes, papaya. This may reveal underlying yellow or red pigments (Tucker, 1993). Peel and pulp often undergo different colour changes, as in apples and bananas. In some cases, fruit colour is a strong indicator of eating quality and shelf-life, for example, tomatoes and bananas, whereas in others it is not. Many pre-harvest factors can affect fruit colour independently of other ripeness characteristics. So, for example, the peel of oranges grown in tropical regions may remain green despite having attained acceptable eating quality. Yellowing of green vegetables such as broccoli and spinach will reduce their quality as may browning of cut tissues, for example butt-ends of Brussels sprouts. Other aspects of appearance which reduce quality include the loss of freshness, like the wilting of leafy crops, loss of surface gloss or skin wrinkling and the development of external and internal defects

caused either by natural senescence, physiological disorders or the growth of disease organisms.

7.2.3 Texture

Eating quality includes a complex of textural properties which are not readily defined or measured. Crisp firm tissues are generally desired in vegetable crops; however, the development of tough fibres during storage in stem crops such as asparagus is not at all acceptable. Some aspects of texture can be judged visually as described above, for example, where produce has begun to wilt or shrivel. Although some degree of softening is required for optimal quality in fruit, over-softening is undesirable and is a sign of senescence or internal decay. The maintenance of textural quality is often critical in certain types of processing, for example in canning and freezing.

7.2.4 Flavour and aroma

Flavour is a complex of taste and aromatic components. Total flavour can rarely be assessed by the consumer prior to purchase but it is critical in the repeat purchase of a particular product or product cultivar. Key taste components in fresh produce are sweetness, acidity, astringency and bitterness. Sweetness of some fruits may increase dramatically during ripening owing to starch to sugar conversions, for example in apples, bananas, mangoes and pears. At the same time, astringent factors (tannins) will disappear (Tucker, 1993). Sugar levels of fruits are often measured to determine whether produce has reached the required ripeness for marketing. Sugar levels do not usually fall significantly during storage; however, maintaining the sugar to acid balance can be important to the fruit flavour balance, for example, in citrus species and grapes. Acid levels generally decrease during storage. If the acid/sugar ratio falls too low, the product can become bland and lose acceptable eating quality. This will also be of importance in processed products in which extra sugars or acids are not added. Bitter components can develop in various fruits and vegetables under certain storage conditions (see physiological disorders in section 7.6.1) or when infected with certain pathogens.

Aroma can be determined to some extent before purchase by the consumer but it tends to be important as a positive factor only in highly aromatic products such as certain cultivars of melons or mangoes. With the emphasis on visual quality which has dominated retailing, it has been claimed that flavour and aroma have been lost from many fresh products as breeding has concentrated on cultivars which will survive the rigours of post-harvest handling without loss of visual and textural quality. Refrigeration also tends to limit the development of aroma volatiles in ripening fruits. The aroma profile can change dramatically during the post-harvest life of fresh produce, particularly in climacteric fruits in which the dominant volatile may be quite different in the unripe fruit, the ripe fruit and the over-ripe or senescing fruit (Morton and Macleod, 1990). Unpleasant aromas

may develop from a number of causes described in later sections (7.3.2 and 7.5). An unexpected or unpleasant aroma may make a product unmarketable even if all other quality factors are quite acceptable. Therefore aroma can be an important factor in the storage and shelf-life of fresh produce.

7.3 Quality deterioration of fresh produce: respiration, ethylene, senescence and breaking of dormancy

7.3.1 Introduction

Many factors can lead to loss of quality in fresh produce, hence the common description of these products as 'perishable'. Some of these factors are part of the life cycle of living produce, that is, over-ripening of fruits or sprouting in root and bulb crops. Others are a consequence of the act of harvesting. Once severed from the mother plant, the plant organ is deprived of its source of water, nutrients and antisenescent hormones. As a consequence normal factors such as transpiration and respiration lead ultimately to water loss and senescence of the product. The growth of pathogens or physical damage will cause direct loss of product quality through their visual impact but both also stimulate senescence. Furthermore, the storage environment will play a highly significant role in determining the speed of all quality changes.

7.3.2 Respiration

Fruits and vegetables are living commodities and their rate of respiration is of key importance to maintenance of quality. It has been commonly observed that the greater the respiration rate of a product, the shorter the shelf-life. Immature products such as peas and beans tend to have much higher respiration rates and short shelf-lives caused by natural senescence whereas the opposite is true for mature storage organs such as potatoes and onions.

Respiration is the metabolic process by which cells convert energy from one type of chemical structure into another form more useful to the cell for driving metabolic reactions. Under normal circumstances, fresh produce undergoes aerobic respiration, during which oxygen and glucose is consumed while carbon dioxide, water and heat are produced (Kays, 1991). In non-storage tissues, for example in leafy crops such as lettuce or spinach or immature flower crops such as broccoli, there is little by way of energy reserves and hence excessive respiration will eventually lead to metabolic collapse. Cell membranes will break down and allow the contents to leak out. Saprophytic bacteria may grow in these tissues and give rise to off-odours. Visible symptoms of tissue collapse and yellowing caused by senescence breakdown of chlorophyll in the chloroplasts may appear. Without adequate cooling, respiratory heat will further stimulate respiration leading to even more rapid deterioration.

Certain types of fruits (known as climacteric) can be harvested unripe and ripened artificially at a later stage (e.g. avocados, bananas, mangoes, tomatoes).

During ripening, the respiration of these fruits increases dramatically over a short period of time (Biale, 1960). Without careful temperature control, the fruit will rapidly over-ripen and senesce leading to internal tissue breakdown and the production of volatiles characteristic of the over-ripe fruit. Failure to control respiratory heat also can increase water loss from the produce. Furthermore, the increased warmth and moisture levels, which can develop in storage, are highly conducive for the development of bacterial and fungal infections.

7.3.3 Ethylene

Ethylene is a plant hormone that plays a key role in the ripening and senescence of fruits and vegetables (Reid, 1992). All plant cells produce low levels of ethylene; however, anything that causes stress to the plant tissues will stimulate ethylene synthesis. Stressors may include excessive water loss, physical damage or pathogenic attack. Climacteric fruits produce high levels of ethylene during initiation of ripening and the hormone is believed to stimulate and coordinate the physiological and biochemical changes which occur during ripening. Exposure to exogenous ethylene can lead to an acceleration of maturation and senescence, for example, green vegetables lose their chlorophyll more rapidly, thickened fibres can develop in asparagus, premature ripening can occur in unripe fruits and cabbages and cauliflowers can lose their leaves through accelerated leaf abscission.

7.3.4 Senescence

Senescence is the natural ageing of the plant tissues and is stimulated by the presence of ethylene and anything else that speeds up respiration rates as described above. Senescence ultimately affects all aspects of quality, ending in the death of the product. Some senescence changes can specifically affect certain types of fresh produce processing, for example, changes to the chemical and physical structure of the cell wall (Jimenez *et al*., 1997). Although in fresh produce, texture is highly dependent on cell turgor (see section 7.4 below), the integrity of the cell wall is important to the texture of some processed products (Femenia *et al*., 1998). In some fruits and vegetables (e.g. apples and tomatoes), the breakdown of intercellular adhesion between cells leads to a condition known as mealiness which is generally perceived as a loss in textural quality (Van der Valk and Donkers, 1994). In potatoes, so-called senescence sweetening is where, over time, storage starch is gradually converted to sugars. Concentrations of reducing sugars of greater than 0.1% in potato tissues being processed into chips and crisps can lead to browning or blackening of the product during the cooking process (Van der Plas, 1987).

7.3.5 Breaking of dormancy

Root, tuber and bulb crops have a natural dormancy period that can be considerably extended under suitable storage conditions. Storage and shelf-life is often

limited by the breaking of dormancy. Most commonly this is seen as the growth of sprouts, for example, in onions or potatoes. Under high moisture conditions, the development of roots may also occur. Neither sprouts nor roots are acceptable in marketed produce (Schouten, 1987). Although roots and shoots can be trimmed off during processing, the internal quality of the produce generally deteriorates during the breaking of dormancy owing, for example, to the conversion of stored starch into sugars that are transported to the growing points.

7.4 Quality deterioration of fresh produce: water loss

Plant tissues are covered with protective tissues, which serve to protect the plant from insect and pathogen attack, physical injury and excessive water loss. The primary protective layer is the epidermis but if the plant organ undergoes secondary growth, a multilayered periderm may develop, for example, on apples or potatoes. The epidermis is covered with a waxy cuticle of cutin while the cell walls of periderm tissues generally become impregnated with suberin. Both cutin and suberin can reduce water losses from plant surfaces; however some water loss is inevitable. Water vapour can permeate the cuticle and is also lost through lenticels, which are gaps in the periderm which form to enable gas exchange for respiration. If the epidermis or periderm is damaged, water loss can be massively exacerbated.

Mature plant organs such as stems, roots and some fruits develop strengthening tissues such as collenchyma or lignified schlerenchyma to maintain their structure. The presence of tough fibrous components is not, however, desirable in fresh produce, so many vegetable crops are harvested immature. Structure and thus textural properties of fresh produce are almost entirely dependent on the maintenance of adequate cell turgor pressure, that is, the force generated when the solute filled vacuole presses against the relatively inelastic cell wall. If too much water is lost from the tissues, turgor pressure will fall, leading to wilting or shrivelling of the product.

The speed of post-harvest water loss is dependent primarily on the external vapour pressure deficit; however, other factors will influence the situation. Products with a large surface to volume ratio such as leaf crops will lose a greater percentage of their water far quicker than large spherical fruits. The specific structure of the cuticle and the extent of suberisation in the periderm appear to be more important than thickness in improving resistance to the movement of water vapour. Produce varies in the percentage of water which can be lost before quality is markedly reduced. Fruits with thick peels can lose a considerable amount of moisture from the skin without compromising edible quality, for example citrus species, bananas. The appearance of the fruit will, however, deteriorate steadily with increasing water loss. Other thin-skinned fruits are more susceptible to water loss, for example, table grapes (Ben Yehoshua, 1987). Furthermore, dehydration of all products can stimulate the production of ethylene (as described above).

7.5 Quality deterioration of fresh produce: fungal and bacterial pathogens

The most important microorganisms causing post-harvest wastage of fresh produce are fungi. This is particularly true for fruits, where the relatively acid conditions tend to suppress bacterial growth. Vegetables with a higher pH can, however, suffer high losses from bacterial infections. The most important pathogens of fruits and vegetables are described by a number of authors (Beattie *et al*., 1989; Coates *et al*., 1995; Dennis, 1983; Snowdon, 1990; 1991). The majority of pathogens rely on damaged tissues to obtain entry into fresh produce (wounds or sites of physiological injury). For example, the *Penicillium* species which cause blue and green mould infections of citrus and other fruit crops are classic wound pathogens, incapable of invading an undamaged fruit. An intact, fresh commodity is resistant to the majority of potential pathogens. The physical barrier of the skin and the presence of antimicrobial compounds in the skin and flesh are sufficient protection.

Some pathogens can gain entry through natural openings such as stomata and lenticels. Bacteria may use this penetration route. The most common group of bacteria causing significant reductions in shelf-life is the soft rotting species of the genus *Erwinia.* Under suitable conditions of warmth and the presence of free water, the bacteria can readily colonise produce such as potatoes through the lenticels. They produce large quantities of extracellular enzymes which rapidly macerate the tissues. Sometimes, soft rots are accompanied by the growth of saprophytic bacteria which give rise to highly unpleasant off-odours (Lund, 1983).

Only a small number of fungal pathogens are capable of direct penetration of the undamaged skin of the produce. On the whole, these latter pathogens are particularly problematic owing to the fact that they may infect produce before harvest but remain quiescent in the tissues until conditions become favourable for growth. This phenomenon is largely seen in fruits, where initial pathogen development and subsequent quiescence occurs in the unripe fruit. As the fruit ripens, quiescence is broken and the pathogen colonises the fruit tissues (Swinburne, 1983). *Colletotrichum gloeosporioides* is a common pathogen showing this behaviour on a number of tropical fruits such as mango and papaya. Typical symptoms on ripe fruits are sunken, lens-shaped lesions, which may develop salmon-coloured sporing structures. *Colletotrichum musae* causes similar symptoms on bananas. *Botrytis cinerea* may also show quiescent behaviour on certain fruits, for example, in strawberries, fungal spores contaminate the flowers, germinate and the hyphae grow into the developing fruit where they remain symptomless until the fruit is fully ripe. The subsequent disease development can be extremely rapid and the whole fruit is completely colonised and covered with a grey, sporulating mycelium within a few days at 20°C.

Skin diseases may remain superficial but cause large market losses owing to the blemished appearance of the produce. The potato industry has a major problem with a number of skin diseases, such as black scurf (*Rhizoctonia solani*),

black dot (*Colletotrichum coccodes*), silver scurf (*Helminthosporium solani*) and common scab (*Streptomyces scabies*) which can spread rapidly on the tubers after the temperature rises in retail outlets (Snowdon, 1991).

On the whole, fungal and bacterial infections are stimulated under high humidity conditions and in particular in the presence of free water. Pathogens of fruits and vegetables are variable with respect to their ability to grow and reproduce at different temperatures; however, most will grow between 6 and 35°C. Some will survive and even grow slowly at temperatures as low as 1°C, for example, *B. cinerea*. The incidence of particular pathogen species is thus affected by both pre-harvest and post-harvest conditions. So, for example, *B. cinerea* is particularly important on produce grown in cool temperate climates, whereas infections caused by *Botryodiplodia theobromae* or *Aspergillus niger* tend to cause serious losses in warm regions.

Certain pathogens can impact heavily on the fresh produce processing industry: for example, the presence of just a few citrus fruits infected with *Alternaria* rot in a consignment can result in off-flavoured juice (Patrick and Hill, 1959). The presence of certain cell wall degrading enzymes from infecting pathogens, for example *Rhizopus* spp., can cause continuing softening of canned products even after the fungus has been killed during the sterilisation process (Harper *et al*., 1972).

7.6 Quality deterioration of fresh produce: physiological disorders and physical injury

7.6.1 Physiological disorders

Physiological disorders are adverse quality changes that occur in fresh produce because of metabolic disturbances. These disturbances can be caused by internal factors such as mineral imbalances or may be due to non-optimal environmental factors such as inappropriate storage temperatures or atmosphere composition. The symptoms may be unique to a particular condition on a specific produce type; however, in many cases the symptoms are similar in a range of conditions with differing underlying causes. Mild symptoms are often confined to superficial tissues which may not be too significant if the produce is to be processed, but can strongly decrease marketability of the fresh product owing to visual disfigurement. Furthermore, physiological disorders can increase the susceptibility of the commodity to invasion by pathogens. The onset of disorders may be determined by pre-harvest conditions, the cultivar, maturity or stage of ripeness.

Poor nutrition will generally give rise to poor field growth and field symptoms. There are, however, a number of nutritional imbalances, which have no obvious pre-harvest significance but which give rise to symptoms during post-harvest storage. One of the most important nutrients in this respect is calcium which plays an important role in maintaining cell wall stability. A classic example is ‘bitter pit’ in apples in which hard, sunken brown pits develop both on the skin and internally. Affected tissues have a slightly bitter taste.

There is a wide range of disorders related to exposure of produce to temperatures which are too high or too low. High temperatures caused, for example, by excessive exposure to the sun or inappropriate post-harvest heat treatments, may cause skin damage and uneven fruit ripening. Only a few commodities destined for fresh consumption can survive mild freezing, for example parsnip and onions, however, the majority of fruits and vegetables destined for fresh consumption cannot tolerate any freezing at all. Ice crystals form inside the cells leading to membrane rupture, and the tissue collapses upon defrosting leading to unacceptable textural changes. These changes are less obvious to the consumer in produce with a relatively low water content and/or which will be cooked before consumption, for example, peas, sweet corn, parsnips, potatoes, carrots, broccoli and spinach.

Chilling injury is quite distinct from freezing injury and may occur at temperatures well above freezing point (Saltveit and Morris, 1990). Tropical and subtropical commodities are particularly susceptible although there may be considerable differences in chilling sensitivity between cultivars and between immature and mature or unripe and ripe produce. Symptoms include water soaking, surface pitting, internal discoloration, failure to ripen, accelerated senescence and increased susceptibility to decay. Symptoms may not become obvious until the produce temperature has been raised to non-chilling levels. At temperatures below 8–10°C and maximal at about 2°C, Irish potatoes are susceptible to reversible low temperature sweetening (Burton, 1989). The reducing sugars produced cause problems to the processing sector (see section 7.3.4 above).

If produce is stored in an atmosphere with insufficient oxygen or excessive carbon dioxide, for example in poorly ventilated stores, respiratory disorders can develop. At higher temperatures, the produce respires more quickly so that an unsuitable atmosphere can develop more rapidly. Symptoms depend on the product in question, so for example, potatoes may develop a black centre whereas lettuces may have pale midribs. Some apple cultivars suffer external injury and others develop internal browning owing to excessive carbon dioxide in the tissues. Very low oxygen levels can lead to alcoholic fermentation with accompanying off-odours. Tolerance levels are variable, for example, some apple cultivars tolerate levels less than 1% O_2, whereas sweet potatoes are highly sensitive and fermentation may set in if O_2 levels fall below 8%. Anaerobic conditions will also encourage the growth of soft-rotting bacteria in potatoes.

A range of specific symptoms in stored fruits and vegetables have been attributed to exposure to ethylene (Kader, 1985). Some examples include russet spotting of lettuce (at concentrations >0.1 ppm) which is associated with increased activity of phenylalanine ammonium lyase (PAL) and phenolic content, formation of the toxin pisatin in peas, and production of phenolics in sweet potatoes and in carrots. In carrots, the phenolic isocoumarin gives a bitter flavour and bitter flavours have also been noted in beetroot.

There are also a number of well-defined miscellaneous disorders of certain fresh produce which are beyond the scope of this book. Further information can be found in books by Snowdon (Snowdon, 1990; 1991).

7.6.2 Physical injury

Physical injury is possibly the most important cause of loss in fresh produce. This is not due to the direct losses, although these can be significant in some crops but rather to the indirect effect of creating a wound in the surface of the produce. This wound is an ideal entry point for many post-harvest pathogens as described above. Injury also allows water loss which compromises the quality of the produce. Furthermore, physical injury stimulates ethylene production in plant tissues, which can lead to premature yellowing or ripening of commodities.

Physical injury can arise at any stage of the life of the crop, from insect injuries in the field to poor post-harvest handling. Many fungi invade through the stem end where the produce was severed from the mother plant. Poor packaging can create problems from cuts caused by sharp edges or hard parts of adjacent produce, for example pineapple crowns, to grazes caused by lack of padding or underfilling of cartons allowing movement of produce within the pack during transport and handling. Bruising can occur from dropping or compression bruising can occur if produce is stacked too high or packs are overfilled. Significant levels of wastage occur in the potato industry owing to internal bruising of potato tubers during storage and handling (Balls *et al*., 1982). The shelf-life of many fresh products is considerably reduced by physical damage caused by rough handling at the retail level, particularly where the produce is loose and can be 'picked over' by the potential customer.

7.7 How quality of fruits and vegetables is measured: appearance, texture and flavour

7.7.1 Introduction

To ensure optimal quality of the produce sold for fresh consumption or for processing, it is essential to be able to monitor quality changes during storage. Ideally those who manage the fresh produce chain would also like to be able to predict the likely shelf-life of the produce. Some types of produce may need rapid transport, for example, out of season, highly perishable produce may need to be air freighted rather than carried by ship from overseas. Other products with a longer shelf-life can be stored and released as the market requires.

The commercial measurement of shelf-life of fresh produce is usually carried out by the quality control staff of retail supply companies (importers and distribution centres). It is considered to be part of the due diligence procedure expected by the customer. Samples of product are removed from the packing line and placed in shelf-life rooms at a temperature that roughly reflects the likely retail conditions. The produce will be assessed for quality changes over a period of time which covers the shelf-life period expected by the retailer for a particular product plus a couple of extra days. Commodity specific evaluation sheets will be filled in and archived. Shelf-life tests are used to forewarn of potential quality problems and will enable action to be taken promptly to identify and limit the problem. They provide some comeback to retailers if there is a problem which may have occurred since the

produce left the supplier. For larger organisations who provide particular products all year round, shelf-life testing may reveal temporal patterns in quality, which can be used in decisions such as when to change the supply source.

At the time of writing, accurate prediction of shelf-life is not really feasible for fresh produce. Efforts to try to develop predictive models for produce shelf-life based on both internal quality factors and environmental factors experienced by the produce have been described in the scientific literature (Polderdijk *et al*., 1993); however, success in this area remains elusive. The difficulty is primarily due to the inherent variability in all the quality factors of fruits and vegetables that might be used to determine shelf-life. Even if the measurement of certain qualities were able to predict shelf-life accurately, individual differences in produce means that, ideally, each individual item would need to be assessed and tests would need to be extremely rapid. At the time of writing, many of the tests in use cause damage to the produce and therefore can only be used on a small sample of the produce.

In many processed products, for example juices, purees and chopped canned or frozen produce, the impact of raw product variability problems can be reduced when the produce is mixed or blended together. However, it is worth emphasising again that top quality products can only be made from top quality raw ingredients, so the ability to measure raw product quality is no less important in the processing industry than the fresh produce sector.

7.7.2 Appearance

Colour

Measurement of colour in horticultural crops is reviewed by Francis (1980). The fresh produce industry uses produce-specific colour matching charts to assist in the grading and shelf-life assessment of many fruits. These charts are cheap and easy to use for training personnel. In larger pack houses, photoelectric techniques may be installed to sort strongly coloured products into at least three grades. For research purposes, colour is generally measured using a surface colour-difference meter (e.g. those manufactured by Minolta or Hunter). This type of instrument measures the characteristics of light reflected from the product surface. The output is processed to give a standard data based on a tristimulus system, for example, numbers for hue, chroma and lightness, which together accurately describe the colour of the object (Minolta Co. Ltd., 1994). The main limitation of this kind of spot colour measurement is the lack of uniformity in the produce itself, for example an apple or mango may be a completely different colour on one side compared to the other.

External and internal defects

The assessment of visual defects such as skin blemishes or greening in root crops is largely carried out by manual operators. Produce may be removed if it has greater than a certain percentage of its surface covered with the blemish in accordance with set quality standards. Some commercial applications of video imaging

techniques (machine vision) exist, for example, some factories use machine vision-based sorting to pick out green, black or unpeeled tubers from potatoes that are due for processing (Clarke, 1996). At the time of writing, the only method in use commercially for determining the presence of internal defects is to cut open samples of produce from each consignment of produce or removed at regular intervals from the pack line, and score the incidence of any discoloration, cavitation or other defects.

7.7.3 Texture

Firmness

The firmness of produce is, in many instances, a fairly good indicator of textural properties and is relatively easy to measure mechanically. Firmness can be assessed visually to some degree, for example whether a product appears shrivelled or flaccid. Resistance to light manual pressure is still a common means of evaluating firmness, although clearly this is highly subjective, with considerable experience required for accurate assessment. The most common method of assessing firmness is with a penetrometer such as the Magness-Taylor firmness tester or the Effegi penetrometer. These measure the total force required to puncture through a given portion of the fruit or vegetable to a standard depth using a standard diameter probe. The test may be carried out through the peel or a portion of the peel may be removed and the flesh firmness only determined. Non-destructive compression testers are also available on the market and can be created simply from penetrometer devices (Macnish *et al*., 1997). Shear instruments are used to measure the tenderness of peas and broadbeans destined for processing, for example, the 'Tenderometer', which uses two sets of hinged grids which simulate the action of chewing jaws (Salunkhe *et al*., 1991).

Firmness can also be assessed using vibration tests. If produce is tapped sharply, sound waves are propagated through its tissues and can be picked up with a microphone or piezoelectric sensor. The characteristics of these sound waves vary depending on the stiffness of the tissues (amongst other factors) and have shown good correlations with fruit firmness. Although the underlying physical principles of these tests have long been understood, it is only relatively recently that the tests are being applied commercially. An Israeli company (Eshet Eilon) is producing a non-destructive bench top firmness tester 'Firmalon' based on acoustic resonance for use with various fruits like apples and pears. (The 'Peleg Firmness Tester' is also available from Technion in Israel.) An on-line acoustic resonance firmness tester 'AvoScan' has been developed by a UK-based machinery company (Sinclair International, Norwich) based on research by Peleg *et al*. (1990). This is being used commercially to categorise fruits such as avocados into separate retail categories (for example 'ready to eat' with an expected short shelf-life).

Other textural factors

In the laboratory, universal testing machines (e.g. those made by Instron) are in common use for evaluating various components of the strength of plant tissues,

which change with storage. For example, mealiness is a textural defect common in some apple and potato varieties as they age. The development of artificial jaws attached to force gauges can simulate bite action and better evaluate textural qualities such as mealiness which limit shelf-life with respect to eating quality. These kinds of measurements are only used for research as suitable commercial applications have not yet been developed.

7.7.4 Flavour

Taste components

Sweetness is an important component of fresh fruit quality and will give a good indication of the state of fruit ripeness and hence potential shelf-life. In the fresh produce sector, sweetness is normally measured in terms of total soluble solids (TSS) content in °Brix. In most fruits and vegetables, sugar makes up the main component of TSS which is thus a reasonable indicator of percentage sugar levels. TSS is measured using a refractometer or a hydrometer. The former instrument operates on the basis of the refraction of light by juice samples and the latter on the basis of the density of the juice. Light reflectance in the near infrared has been correlated successfully with TSS in a number of commodities. This property is being developed as a non-destructive method of measuring sugar levels in crops such as melons.

Acidity is generally measured by titration with a suitable alkaline solution such as sodium hydroxide. Maturity standards for citrus species are based on Brix-to-acid ratios and both TSS and acidity are important measures of table grape quality. There is no rapid objective method for measuring bitterness or other undesirable flavours in fruits and vegetables. Sensory evaluation is the only commercial test used in the fresh produce sector. In the laboratory, bitter or astringent components (generally caused by phenolic compounds) can be extracted and measured by various analytical procedures, for example, high performance liquid chromatography.

Aroma components

The measurement of aroma is currently assessed by the industry on an informal basis, relying on off-odours in shelf-life samples being noted by produce quality managers. Laboratory measurements have traditionally been conducted by headspace analysis using gas chromatography (Wehner and Kohler, 1992). Separated components can be identified objectively (chemically) by various means or subjectively using 'odourmeters'.

Sensory evaluation

There are relatively few instrumental tests which give results which correlate well with consumer assessment of quality in fresh produce. Colour measurement is one of the few exceptions. The most comprehensive way of assessing overall quality is to use panels to conduct sensory evaluation of the products. People on

the panel may be trained to assess certain quality components in a statistically quantitative fashion (Lawless and Heymann, 1998). Alternatively a consumer panel may be used. In this case the assessment is hedonic, that is, made in terms of personal preferences. In the fresh produce sector, the use of sensory tests may simply involve the quality controller acting as a single 'expert' taster. Alternatively, informal taste panels may be run, say, once a month, using up to 15 members of staff, who may or may not be regular members of the panel. Recent initiatives by retailers, particularly in the UK, are encouraging the industry to standardise the use of trained sensory panels for the measurement of quality attributes.

7.8 Maintaining the quality of fresh produce: precooling

7.8.1 Introduction

Table 7.1 provides some examples of the variation in commercial storage conditions and expected shelf-life of some representative fruits and vegetables. The prevalence of physical damage or the presence of pathogens can, however, confound shelf-life predictions. The main factors causing deterioration in fresh produce were described in sections 7.3–7.6. Maintaining quality thus requires action to be taken to limit these factors. In some cases these are preventative measures, for example, providing suitable packaging to prevent physical injury. However, a wide range of proactive technologies must be applied to maximise the shelf-life of perishable commodities. Of primary importance are methods to reduce produce respiration, water loss and the growth of pathogens. Of these, refrigeration dominates as the most fundamental of all post-harvest technologies.

7.8.2 Precooling

Precooling to remove field heat as quickly as possible after harvest is essential for slowing down the rate of deterioration of highly perishable products. The method chosen is largely determined by the type of product in question and the cost to benefit ratio (Kasmire and Thompson, 1992; Mitchell, 1992).

Room and forced air cooling

In room precooling, harvested produce is placed in a refrigerated area. Typically refrigerated air is blown horizontally just below the ceiling, sweeping over and down through the containers of produce below. Upon reaching the floor, it moves horizontally to the return vent to be recycled. More rapid cooling is effected with forced air or pressure precooling. In this case, refrigerated air is forced along a pressure gradient through each package. This is achieved by lining up stacks of containers (pallet loads or individual cartons) on either side of an exhaust fan to give an air plenum chamber. Air is prevented from moving down between pallet

Table 7.1 Range of storage periods for selected fruits and vegetables under typical storage conditions of temperature and relative humidity

Commodity	Temperature (°C)	Humidity (%)	Storage period
Apples	–1–4	90–95	1–8 months
Aubergines (eggplants)	8–12	90–95	1–2 weeks
Avocadoes (unripe)	4.5–13	85–90	2–5 weeks
(ripe)	2–5	85–90	1–2 weeks
Bananas (green)	13–15	85–90	10–30 days
(ripe)	13–16	85–90	5–10 days
Beans (French)	7–8	95–100	1–2 weeks
Broccoli	0–1	95–100	1–2 weeks
Cabbage (green)	0–1	95–100	3 months
(white)	0–1	95–100	6–7 months
Carrots (immature)	0–1	95–100	4–6 weeks
(mature)	0–1	95–100	4–8 months
Cauliflower	0–1	95–100	2–4 weeks
Celery	0–1	95–100	1–3 months
Citrus (easy peel)	4–8	90	3–8 weeks
Courgettes (zucchini)	8–10	90–95	1–2 weeks
Cucumbers	8–11	90–95	1–2 weeks
Garlic	0	70	6–8 months
Grapefruits	10–15	90	4–16 weeks
Grapes	–1–0	90–95	1–6 months
Kiwifruits	–0.5–0	90–95	2–3 months
Leeks	0–1	95–100	1–3 months
Lemons	10–14	90	2–6 months
Lettuce	0–1	95–100	1–4 weeks
Mangoes	5.5–14	90	2–7 weeks
Melons	4–15	85–90	1–3 weeks
Mushrooms	0	90–95	5–7 days
Onions	–1–0	70–80	6–8 months
Oranges	2–7	90	1–4 months
Pears	–1–0	90–95	1–6 months
Peas	0–1	95–100	1–3 weeks
Potatoes (immature)	4–5	90–95	3–8 weeks
(mature)	4–5	90–95	4–9 months
Soft fruits	–1–0	90–95	2 days–3 weeks
Spinach	0–1	95–100	1–2 weeks
Stone fruits	–1–1	90–95	1–7 weeks
Sweet peppers (capsicum)	7–10	90–95	1–3 weeks
Tomatoes (green)	12–15	90	1–2 weeks
(ripe)	8–10	90	1 week

Note: storage conditions and storage life may differ from cultivar to cultivar. The data were adapted from the more comprehensive tables provided by Snowdon and Ahmed (1981).

loads or the sides of cartons by sealing these gaps with flexible baffles. The cold air from the room thus has to pass through the holes in the packaging and around the produce inside. This greatly speeds up the cooling time from one-quarter to one-tenth of that of conventional room cooling.

Hydrocooling
Water is better than air at transmitting heat. Many produce types can be cooled by bringing them into contact with flowing cold water (hydrocooling). Packaging restricts water movement and greatly reduces cooling efficiency. Produce is therefore usually hydrocooled in bulk bins and is rarely used after packaging. This method is commonly used for stem vegetables, many leafy vegetables and some fruits like tomatoes and melons. Some crops cannot be cooled in this way, for example strawberry, because free water on the surface greatly increases the risk of disease. Proper sanitation (usually by chlorination) of the water is required to prevent the build up of bacteria in the water and subsequent contamination of the produce.

Icing
Application of crushed ice may be appropriate for a few crops. This is generally used for temporary cooling during transport from the field, for example leafy greens, for package icing during shipment to retail outlets and in displays of produce at the retail level, for example root and stem vegetables, Brussels sprouts and some flower-type vegetables like broccoli. The primary disadvantage is the additional weight for transport.

Vacuum cooling
One of the most rapid and uniform methods of cooling is vacuum cooling. It involves decreasing the pressure around the produce to a point at which the boiling point of water is reduced. The consequent evaporation of the water absorbs heat. This is most efficient with produce that has a large surface area to volume like leafy crops such as lettuce, spinach and cabbage. Adequate cooling can normally be achieved with no more than about 3% water loss but this can be reduced by spraying the produce surface with water prior to cooling.

7.9 Maintaining the quality of fresh produce: prestorage treatments

7.9.1 Surface coatings and wraps

Many fruits and vegetables benefit from a surface coating which can slow down the loss of water (Kester and Fennema, 1986). This is particularly true for crops which are washed, because hot water or the inclusion of detergents can remove natural waxes from the fruit surface. Coatings can also reduce the movement of O_2 and CO_2 in and out of the fruit, respectively. This internal atmosphere modification can slow down respiration; however, the layer must not be too thick or O_2 levels may fall too low and lead to fermentation problems. Many of the coatings applied are derived from plant extracts, for example carnuba or sugar cane waxes or polymers of sugar esters; however, petroleum-based products such as paraffin wax may be added to improve water loss control. An alternative approach to controlling water loss in fresh produce is to shrink wrap the product individ-

ually in plastic films. High density polyethylene (HDPE) is highly suitable for this as it can be applied in a very thin layer, which is a good water vapour barrier but does not affect the movement of respiratory gases and the danger of off-flavours developing (Ben Yehoshua, 1987).

7.9.2 Curing of roots and tubers

Some root and tuber crops, for example sweet potato and Irish potato, retain an ability to heal minor wounds after harvest provided conditions are correct (Burton *et al*., 1992; Morris and Mann, 1955). This involves the development of a new periderm layer at the wound site. As these crops are highly susceptible to physical injury during harvesting and handling, it is generally beneficial to encourage wound healing before storage. This process is known as curing and requires the produce to be held at elevated temperatures and high relative humidity (RH) for a period of time. The actual conditions used depend on the likelihood of disease development. At higher temperatures, curing will be faster but bacterial infection becomes more likely. Irish potato tubers are typically cured at 15–25°C, RH 85–98% for 7–15 days. There is evidence, however, that curing at lower humidities may reduce the incidence of superficial infections (Hide and Caley, 1987). Sweet potato roots are typically cured at 29–32°C, RH 85–98% for 4–8 days.

7.9.3 Dehydration ('curing') of bulb crops

Bulb crops, that is onions and garlic, are unusual among fruits and vegetables in that some water loss is highly desirable in preparation for storage. This dehydration process is known as curing but is a quite different process from curing of roots and tubers. For bulb crops, the aim of curing is to lose water from the outer scales and stalk remnant. In temperate climates, artificial curing is often carried out (although field curing may still be carried out in some countries). Onions are topped and placed in store. Hot air is blasted over them. Temperatures are initially 30°C until the outer scales are dried. The temperature is then dropped to 27°C for about 4 weeks before storing the bulbs at low temperatures (O'Connor, 1979).

7.9.4 Chemical control of fungal and bacterial pathogens

In many instances, the fresh produce is washed prior to grading, processing and packing. The quality of the water is extremely important, particularly if it is recycled. Bacteria and fungal spores can build up in the water and become an excellent source of inoculum unless they are controlled. The most common control method is the addition of chlorine at an active level of between 50–200 ppm. Ozone is also being used in some parts of the industry (Beuchat, 1992).

As described in section 7.5, a number of pathogens that cause significant post-harvest losses in fresh produce are pre-harvest in origin. There are many ways of limiting the extent of pre-harvest infection that are beyond the scope of this book.

The use, however, of resistant cultivars, good crop sanitation, and measures which maintain crop vigour and hence their natural resistance to infection and the application of fungicides will all go a long way to minimising post-harvest disease problems. The use of antibiotics for bacterial control in crops is not accepted in many countries, owing to fears concerning the possibility that any antibiotic resistance arising from field applications might be transferred to human pathogens (Lund, 1983).

After harvest many crops which are to be stored are treated with one or more fungicide. There are about 20 types of fungicide with approval for use on fresh produce (Eckert and Ogawa, 1990), although approval varies from country to country. Fungal resistance to the benzimidazole-based fungicides, such as benomyl, thiabendozole and thiophthanate methyl, is extremely widespread and has led to an increasing use of the ergesterol biosynthesis inhibitors (EBIs) such as imazalil, etaconizole, bitertanol etc. Application methods are highly dependent on the fungicide type and the crop type. Fruits such as apples, pears, mangoes, citrus and various root crops are often either sprayed or dipped in fungicide baths. Some fungicides may be incorporated into waxes for surface application on, for example, citrus. Where it is undesirable for the product to be wetted, fumigants may be used, for example, potatoes may be fumigated with 2-aminobutane to control gangrene and skin spot and sulphur dioxide is applied to control grey mould on table grapes (Eckert and Ogawa, 1988). Many crops such as strawberries are not treated with any post-harvest chemical despite their high perishability which is caused by pathogens.

7.9.5 Sprouting suppressants for root, tuber and bulb crops

Control of sprouting in root and bulb crops can be carried out by pre-harvest applications of maleic hydrazide. The compound must be applied to the foliage three to six weeks before harvest. Root crops can also be treated post-harvest with various sprout suppressants (Burton *et al*., 1992), for example, propham/chlorpropham (IPC/CIPC) which is normally applied as a mixture at about 10 g/t. These compounds must be applied after curing as they suppress wound healing. Tecnazene (TCNB) is a commonly used alternative, which has some advantages over IPC/CIPC in that it has little effect on wound healing and also has some fungicidal properties. Application rate is about 135 mg active ingredient per kg. There are a wide range of alternative chemicals which have sprout-suppressant properties but they all have limitations compared to the conventional compounds described above (Prange *et al*., 1997).

7.9.6 Post-harvest chemical treatments to reduce disorders

Superficial scald is a skin disorder of certain apple cultivars which develops during storage and is due to the oxidation of a natural compound in the skin called α-farnesene. Commercially, the antioxidant compounds diphenylamine and ethoxyquin can be applied as a post-harvest dip to control this disorder (at

0.1–0.25% and 0.2–0.5%, respectively). Diphenylamine may also be applied in wax formulations or in impregnated wraps (Snowdon, 1991).

Another important post-harvest treatment of apples is the use of calcium, either as a pre-harvest spray or as a post-harvest dip, to control the storage disorder, bitter pit (Anon, 1984). Although calcium treatment can improve storage quality of many other fruits, it has not been developed owing to problems in getting sufficient calcium into the tissue by infiltration without causing fruit damage.

7.9.7 Irradiation

Many benefits of applying ionising radiation (X-rays, γ-rays or high energy electrons) to fresh produce have been shown, including sprout inhibition in root, tuber and bulb crops, control of some fungal diseases and increased storage potential through delays to the ripening processes of fruits (Dennison and Ahmed, 1975). A range of treatments have been approved in many countries, including the UK; however, consumers have shown considerable reluctance to accept irradiated food (Foster, 1991). In practice, very little fresh produce is actually irradiated owing to both these consumer concerns and legislative restrictions.

7.10 Maintaining the quality of fresh produce: refrigerated storage

7.10.1 Introduction

As discussed in section 7.8.2, the storage/shelf-life of fresh produce is considerably extended if respiration can be slowed down using refrigeration. Lists of recommended storage conditions for a wide range of fruits and vegetables are given in a number of publications (Kader, 1992; Snowdon and Ahmed, 1981; Thompson, 1996). Following precooling, it is important that the cold chain is maintained throughout the life of the product. This means that refrigeration should take place throughout transportation (Eksteen, 1998) and storage and preferably be maintained during retailing and in the home of the consumer. Typically, road and sea containers are refrigerated, as are the storage units at exporters, importers and retail distribution centres. Air freight is rarely cooled and relies on adequate precooling, good pack insulation and the speed of transport to maintain adequate quality (Frith, 1991). The cool chain tends to be broken in the retail store where fruits and vegetables are rarely displayed in chilled cabinets.

7.10.2 Control of humidity

Most cool stores or refrigerated containers are refrigerated by a direct expansion system (Thompson, 1992). Fans are usually necessary to circulate the storage air over the evaporator coils and then through the produce in the cooling space. Heat is removed from the cooling space, when the refrigerant gas is allowed to expand in the evaporator coils. The temperature gradient between the coil and the produce

is accompanied by a vapour pressure deficit, which increases water loss from the produce. To reduce water losses during longer term storage it is important to have as small a difference between coil temperature and produce storage temperature as possible. For produce particularly susceptible to water loss, for example leafy vegetables, an indirect cooling system may be used. Storage air is cooled to about 1–2°C and humidified to a RH of over 98% by passing it through a shower of cold water that has been cooled by mechanical refrigeration.

7.10.3 Control of ethylene

The presence of ethylene can stimulate senescence and give rise to a number of disorders as described in section 7.6.1. Good store management is needed to ensure that ripening fruit is not stored together with unripe fruit or other produce which is sensitive to ethylene (Dover, 1989). Exhaust gases from vehicles contain ethylene and must be kept well apart from produce stores. For fruits and vegetables which only produce low levels of ethylene, adequate ventilation from a clean air source is usually sufficient to keep ethylene at safe levels. Where ventilation is not sufficient to manage ethylene levels, ethylene can be destroyed by oxidation. Store air can be passed over the oxidising compound, potassium permanganate held on an inert substrate. Alternatively, ultraviolet (UV) light is in use commercially to destroy ethylene. The UV generates ozone production. It is believed that the ethylene is destroyed by active intermediates produced during the formation of the ozone (Reid, 1992). Ethylene can also be destroyed using catalytic converters by heating the air to over 200°C in the presence of a suitable catalyst such as platinum (Knee *et al*., 1985).

7.10.4 Control of chilling injury and low temperature sweetening

Chilling injury in tropical and sub-tropical crops may limit the use of refrigeration to temperatures well above freezing. Chilling injury is dependent not only on the temperature but the length of exposure at that temperature. The early stages of chilling injury are believed to be reversible and some produce can tolerate chilling temperatures for short periods of time without development of symptoms. A range of methods is available to limit chilling injury (Wang, 1991). These include stepwise reduction in storage temperature, or intermittent warming during storage (e.g. nectarines and peaches). Some fruits may become less susceptible to chilling when held under appropriate modified atmospheres, for example mango, avocado.

7.11 Maintaining the quality of fresh produce: controlled atmosphere (CA) storage

Respiration can also be controlled in many crops by reducing the levels of oxygen in store and/or by raising levels of carbon dioxide. This is known as controlled

atmosphere (CA) storage and its use with fruits and vegetables is reviewed by Thompson (1998). Lists of recommended CA conditions for a wide range of crops are provided in a number of other publications (Kader, 1997; Meheriuk, 1990). CA has long been in use as a means of extending the storage life of apples well beyond that achieved just by refrigeration, up to 10 months for some cultivars such as Granny Smith (Meheriuk, 1990). CA can also be useful for chilling sensitive crops, where refrigeration alone may not give adequate storage life. Transport of bananas is increasingly being carried out under CA (typically O_2 3% and CO_2 5%) giving reduced levels of premature ripening and controlling crown rot disease. CA storage of onions can give substantial extension of storage owing to its inhibitory effect on sprouting. The technology is, however, quite expensive to install and needs well trained technical staff to be operated effectively.

High levels of CO_2 can also have a direct inhibitory effect on certain pathogens. The upper limit for CO_2 levels depends on the sensitivity of the crop. Many berry crops have a high tolerance for CO_2, for example, blackcurrants destined for processing into juice are often held under 40% CO_2. Levels above 15% will significantly reduce incidence of grey mould on strawberries, raspberries, cherries and grapes (Kader, 1997) and small scale CA storage structures are in increasing use with these crops.

7.12 Maintaining the quality of fresh produce: packaging

7.12.1 Conventional packs

It is essential to minimise physical damage to fresh produce if it is to have optimal shelf-life. The use of suitable packaging is vital in this respect (Thompson, 1996). The most common form of packaging in this sector is the use of the fibreboard carton; however, for most produce, additional internal packaging, for example tissue paper wraps, trays, cups or pads, is required to reduce damage from abrasion. For very delicate fruits, smaller packs with relatively few layers of fruits are used to reduce compression damage. Moulded trays may be used which physically separate the individual piece of produce. Individual fruits may also be wrapped separately in tissue or waxed paper. This improves the physical protection and also reduces the spread of disease organisms within a pack. Detailed box designs are described in ITC (1988).

7.12.2 Modified atmosphere packaging (MAP)

Polymeric films have been used to package fresh produce for over 35 years, with a number of benefits, including control of water loss, protection from skin abrasion and reduced contamination of the produce during handling. They also provide a barrier to the spread of decay from one unit to another (Kader *et al*., 1989). These films will also affect the movement of respiratory gases depending on the relative permeability of the film. This can lead to the development of lowered O_2 and raised CO_2 levels within the package and, as with CA storage,

this can reduce the respiration of the produce and potentially extend shelf-life. Bananas are commonly transported in sealed polyethylene bags. It has been shown that if a stable gas content of 2% O_2 and 5% CO_2 can be achieved, the shelf-life of bananas can be extended five-fold (Shorter *et al*., 1987).

A modified atmosphere can be created within the pack in two ways. Active modification involves the pulling of a slight vacuum within the pack and then replacing the atmosphere with the desired gas mixture. Absorbers of CO_2, O_2 or ethylene may be included within the pack to control the concentration of these gases. In passive modification systems, the atmosphere is attained through the respiration of the commodity within the pack. The final equilibrium atmosphere will depend on the characteristics of the commodity and the packaging film (Kader *et al*., 1989). Temperature control is extremely important with MAP, as this will influence the gas permeability properties of the film as well as the respiration rate of the product. One of the main drawbacks to MAP is the potential for O_2 levels to fall too low and cause the production of undesirable off-odours caused by fermentation of the tissues.

7.13 Future trends

7.13.1 Minimally processed products and MAP

One of the fastest growing trends in food retailing is that in ready prepared foods. In the fresh produce sector, this is observed in growing sales of so-called fresh cut or minimally processed salads. New developments are having to be made in MAP to prevent the rapid deterioration which occurs once fresh produce has been cut open (Day, 1996; Day and Gorris, 1993). Up to now, the development of new MAP solutions has remained something of an art, with selection based on trial and error. Attempts to put MAP design onto a more theoretical basis have led to a number of models being developed. However, the general applicability of these models has been limited by the complexity of the systems involved (Kader *et al*., 1989). With the continued expansion in computing power available, eventually models which can be used successfully to predict suitable MAP solutions will be developed.

These developments in MAP will be accelerated by the commercial availability of films for so-called ‘active packaging’, for example, polymer films which become more permeable to respiratory gases at higher temperatures (Day and Gorris, 1993). Packaging may include components which remove aroma or off-flavours, scavenge O_2, ethylene or water vapour or emit CO_2 or other preservative vapours (Robertson, 1991; Wills *et al*., 1998). Novel gas combinations such high O_2, argon or neon may have useful applications in this field (Day, 1996).

7.13.2 On-line technologies for non-destructive grading and shelf-life evaluation

Another market of growing importance is the ‘ready-to-eat’ market where the consumer is led by the product label to expect a fully ripe fruit for immediate

consumption. To guarantee good eating quality while minimising post-harvest losses, the development of robust non-destructive quality testing equipment for use on packing lines is required. This type of equipment will also be used for the detection of external and internal defects, thus reducing labour costs in the packhouse.

The physical science behind many non-destructive techniques for evaluating internal quality of fresh produce such as the use of near infrared, X-ray scattering, acoustic resonance, etc. is well understood (Chen and Sun, 1991). The goal of turning the science into technologies which can be applied commercially within the fresh produce sector has proved somewhat elusive. Flavour factors such as sugar content may eventually be routinely measured using near infrared (Peiris *et al*., 1999). Aroma profiles of fruits may be assessed using electronic nose technology based on polymer arrays which are sensitive to volatile compounds (Russell, 1995). At the time of writing, the response time of this equipment is too slow to be of practical use, that is, it is in the order of minutes rather than seconds. Some of this additional information could be incorporated on to labels applied on-line, perhaps indicating the expected shelf-life and percentage sugar content of each individual product.

Machine vision applications for the detection of external blemishes are rapidly making progress towards commercialisation (Tillett, 1991; Yang, 1992). Among the novel techniques being developed for the non-destructive detection of internal defects are computer-aided X-ray tomography and nuclear magnetic resonance (NMR) imaging. These are based on the measurement of differences in tissue density or proton mobility respectively and can be used, for example, to detect cavities or tissues disruption caused by insects, disease development or developmental disorders (Wills *et al*., 1998).

7.13.3 Replacements for post-harvest chemicals

In many countries there is a strong trend towards reducing the use of chemicals in horticulture, including post-harvest fungicides, sprout suppressants and antioxidants for scald control. Increasingly, consumers are prepared to pay for organic products and the retail sector is encouraging the trend (Geier, 1999). Another and perhaps more significant factor in the trend to reduce usage of post-harvest chemicals is the escalating costs to the agrochemicals industry of the registration of new pesticides or reregistration of currently used pesticides (Crossley and Mascall, 1997). Post-harvest use of pesticides on fruits and vegetables is an extremely small market compared with pre-harvest applications on major world crops such as cereals and oilseed crops. Many chemicals are now being voluntarily deregistered by their producers for post-harvest use. Others have been deregistered by regulatory bodies on the basis of new health and safety data. In 1994 the EU began the process of harmonising maximum residue levels (MRLs) for each crop/pesticide active ingredient combination in use across EU countries. Where the chemicals have been found to be out of patent and where no chemical company is willing to pay the cost of the new data requirements, the active ingredient is being or has been

banned. The implications of this pesticide 'harmony' in Europe are potentially serious for the European horticulture industry as well as international growers exporting to Europe (Aked and Henderson, 1999).

It is clear that the fresh produce sector urgently needs alternatives to post-harvest chemicals and developments of these technologies will grow in the future. Among the technologies already in use or in development are controlled and modified atmosphere storage, for example, to manage scald in apples (Dover, 1997) and physical treatments such as heat (Barkai-Golan and Phillips, 1991), the use of biocontrol agents (Koomen, 1997), 'natural' chemicals such as plant extracts and methods to stimulate natural disease resistance in crops such as UV applications (Joyce and Johnson, 1999).

One new chemical which may gain future approval for use on fresh produce is the gaseous inhibitor of ethylene action, 1-methylcyclopropene (1-MCP). 1-MCP inhibits ripening in climacteric fruit and ethylene-stimulated senescence and is active at very low (ppb) concentrations (Serek *et al*., 1995).

7.13.4 Increased emphasis on the health aspects of fresh produce consumption

Consumers have long been encouraged by government health advisors to increase their consumption of fresh produce on the basis that these food products are vital dietary sources of certain minerals and vitamins. However, it is now widely believed that high levels of fresh produce consumption may ward off many fatal diseases such as cancer and heart disease (Joshipura *et al*., 2001; Wallstrom *et al*., 2000). As further advances are made in understanding the links between diet and disease, it is likely that the nutritional value of fruits and vegetables will become an important quality factor. Thus the maintenance throughout storage of key chemical components that are found to have specific health benefits will pose additional challenges to the post-harvest technologist.

7.13.5 Genetically modified (GM) fruits and vegetables

Despite consumer concerns about the desirability of genetically engineered crops, it is likely that new GM products (for example, with altered colour, flavour or nutritional properties) will become available on the market in the future. Novel properties in a product may change its responses to storage and require new approaches to maintaining product quality. Genetic alterations have already been directed to reducing unwanted quality changes. The first GM fresh product to be marketed was the FlavrSavr tomato which was engineered using antisense RNA technology to have reduced levels of polygalacturonase (Fuchs and Perlak, 1992). This increased the shelf-life of the tomato by preventing the excessive softening which accompanies over-ripening. Other fruits such as tomatoes and melons have been manipulated to reduce ethylene synthesis. Such fruits can have extremely extended shelf-lives. Susceptibility to post-harvest damage and disorders has been manipulated in a number of crops, for example, polyphenol oxidase activity has been reduced in potatoes (Bachem *et al*., 1994) removing sensitivity to bruising. Other

research around the world seeks to do the same thing in a diverse range of crops, including pineapples, apples, lettuces and grapes to prevent a range of browning reactions which accompany physical and physiological injury (Thwaites, 1995). There are other ways in which the shelf-life of fresh produce could be extended genetically, for example, by enhancing the synthesis of antimicrobial compounds.

7.14 Conclusions

The fresh produce sector is a growth market driven by improvements in quality, variety and all year round availability. The industry has to satisfy ever higher quality requirements combined with high labour costs, an emphasis on reductions in chemical inputs, both pre- and post-harvest, and market demand for ready prepared products. For growth to continue, the industry has to be prepared to adopt a wide range of technologies to enable extended shelf-life while maintaining product quality. Continued research and development is therefore needed worldwide to find improved ways of increasing the stability and shelf-life of fruits and vegetables. Providing consumer confidence can be gained, genetic engineering may hold the key to dramatic changes in the management of fresh produce shelf-life in the future.

It can be concluded that those who wish to improve the control of fresh produce quality need a broad knowledge base, including aspects of horticulture, physiology, biochemistry, plant pathology and molecular biology. They also need to be familiar with a wide range of technologies and management strategies, ranging from packaging options to cool chain management. Maintaining quality of fresh produce for both the fresh produce markets and processing industries promises to remain a challenging but fascinating activity.

7.15 Sources of further information and advice

7.15.1 Research organisations

Owing to the huge number of organisations that carry out research into fresh produce quality worldwide, the author has limited references to UK establishments only. The following organisations engage in research relevant to the storage and shelf-life of fresh produce. Those who are still funded to some degree by the public sector may provide some advice and information free of charge. Organisations funded to a large extent by industry usually charge for information and may only provide scientific data to paying members.

Campden and Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK: this government and industry sponsored research organisation has research and training programmes in aspects of MAP and HACCP for fresh produce.

Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK: this is a research organisation supported by grants from the Biotech-

nology and Biological Sciences Research Council. It carries out basic and strategic research on food safety, quality, nutrition and chemistry.

Horticulture Research International (Headquarters), Wellesbourne: this is a multisite government research organisation with a number of groups carrying out research to extend the storage potential of UK grown fruits and vegetables.

Leatherhead Food Research Association (Fruit and Vegetable Panel), Randalls Road, Leatherhead, Surrey KT22 7RY, UK: this is an industry sponsored research organisation with a product panel on fruits and vegetables and some training programmes relating to fresh produce processing.

Shipowners Refrigerated Cargo Research Association, 140, Newmarket Road, Cambridge CB5 8HE, UK: this industry sponsored organisation carries out research on shipping of cargo, including fresh produce.

Silsoe Research Institute, Wrest Park, Silsoe, Bedford MK45 4HS, UK: the Institute is government funded with relevant research being conducted on physical properties of fresh produce, non-destructive testing techniques and machine vision technology for harvesting and grading horticultural products.

The following university sector organisations are known by the author to conduct research and/or provide training on aspects of shelf-life extension of fresh produce:

Cranfield University at Silsoe (Postharvest Technology Laboratory), Silsoe, Bedford MK45 4DT; *Natural Resources Institute* (Postharvest Horticulture Group), University of Greenwich, Chatham, Kent ME4 4TB; *Nottingham University* (Plant Sciences Division), Sutton Bonnington Campus, Loughborough LE12 5RD; *Reading University* (Department of Agricultural Botany), Reading, Berkshire RG6 6AS; *Scottish Agricultural College* (Food Systems Division), Craibstone Estate, Buckburn, Aberdeen AB21 9YA; *Writtle College*, University of Essex, Chelmsford, Essex CM1 3RR; *Wye College*, University of London (Department of Agriculture and Horticulture), Ashford, Kent TN25 5AH.

7.15.2 Written and electronic sources

The following books should be referred to for an overview of fresh produce biology and relevant post-harvest technologies for fruits and vegetables (Kader, 1992; Kays, 1991; Thompson, 1996; Weichmann, 1987; Wills *et al*., 1998). The journals *Postharvest Biology and Technology* and *Scientia Horticulturae* (Elsevier) and *Postharvest News and Information* (CABI Publishing) publish scientific papers relating to horticultural produce. Review articles and abstracts of relevant papers can be found in the CAB International publication, *Postharvest News and Information*. The following website is produced by the Postharvest Technology Research and Information Centre, Department of Pomology, University of California, Davis, CA, USA. It provides produce fact sheet, properties and recommended conditions for storage of fresh fruits and vegetables and fact sheets on physiological disorders of fruits and vegetables. http://postharvest.ucdavis.edu/

Extensive postharvest information has been collated at the FAO website. http://www.fao.org/inpho/. Subscribers to the Postharvest Mailing List can exchange information with other users. Contact: posth@hra.marc.cri.nz

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8

Measuring fresh fruit and vegetable quality: advanced optical methods

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8.1 Introduction

Time-resolved reflectance spectroscopy (TRS) has been investigated as a novel non-destructive technique for quality evaluation of fruits. In contrast to conventional optical methods, and widely used for non-destructive tests of fruits and agricultural products, TRS yields a complete optical characterisation of the investigated sample through simultaneous estimation of the absorption coefficient and the transport scattering coefficient. This is accomplished by interpreting the attenuation and broadening experienced by a short laser pulse with a proper theoretical model while travelling in a diffusive medium, such as most fruits. Optical properties of fruits constitute a complex system, therefore light (electromagnetic radiation) is affected by many factors in its interaction with fruit tissues. Absorption and scattering are therefore complex effects. However, to a first approximation, the absorption coefficient is primarily dependent on tissue components (water, chlorophyll, sugars), while the transport scattering coefficient is dependent on tissue microscopic structure (cells, fibres). Moreover, key advantages of TRS applied to fruits and vegetables include insensitivity to skin colour and properties and penetration into the pulp of fruits to a depth of more than 2 cm.

Sections 8.2–8.6 introduces light propagation in diffusive media and the principles of time-resolved reflectance spectroscopy. A description of instrumentation and data analysis for time-resolved reflectance spectroscopy is useful to understand the novel technique completely. Section 8.7 presents the non-destructive optical characterisation of fruits. Absorption and scattering spectra of different fruits are reported. Tissue contents and tissue structure are investigated by interpreting absorption and scattering spectra by Lambert-Beer and Mie theory, respectively. Section 8.8 is a gallery of preliminary applications of the novel

technique. Monitoring of ripening and identification of defects on intact fruits show the potential of time-resolved reflectance. Section 8.9 discusses the relationship between time-resolved reflectance and standard mechanical-chemical tests for fruit quality assessment and the possibility of setting an optical quality index. Section 8.10 gives a survey of research papers, conference proceedings and web sites of interest.

8.2 Advantages of time-resolved optical methods

The internal quality of fruits and vegetables is ordinarily assessed using destructive techniques, based on the evaluation of chemical, physical and mechanical properties, such as acidity or soluble solids (sugars) and firmness, respectively. This necessarily implies that a few samples can be tested and the derived information can then be extended to the whole batch of fruits. Non-invasive methods for the quality assessment could be applied to each single item, even repeatedly if necessary, with evident commercial advantages. Consequently, interest in the development and application of non-destructive techniques for the evaluation of internal quality is growing more and more, not only at a basic research level, but also among people involved in the distribution in the market.

Different non-destructive techniques have been proposed to probe a variety of quality-related factors in fruits.[1] For example, anthocyanins in strawberries have been detected by photoacoustic techniques.[2] The artificial nose, with its potential to detect small quantities of released chemicals, may prove useful for those aspects of quality related to aroma production[3] even though few data on such applications are currently available. Ultrasounds cannot penetrate deeply into the pulp of most fruits owing to the porous nature of the tissue, yet some promising results have been obtained using low frequency ultrasounds.[4] Nuclear magnetic resonance appears promising in terms of specificity and spatial resolution,[5] but is not suitable for in-the-field or mass applications.

Other techniques using ultraviolet (UV, 4–400 nm), visible (VIS, 400–700 nm) or near-infrared (NIR, 700–2500 nm) radiation have been devised based on the measurement of the total diffusely reflected signal at different wavelengths. For instance, UV/VIS fluorescence of chlorophyll compounds is used for investigations of photosynthetic activity since chlorophyll content and photosynthetic capacity are often related to maturity or defects.[1] In the visible region of the spectrum, colorimetry has been used to determine the colour of the skin of peaches[6] and, in the near infrared region, the spectrum of re-emitted light has been studied, mainly to estimate the total sugar content.[7]

Referring to the optical technique, a key limitation is that the intensity of the diffusely remitted light is strongly dependent on the colour of the skin, thus masking information from the pulp. In particular, the total reflected intensity is determined both by the absorption and the scattering properties, in such a way that it is not feasible to separate the effects of these properties. Absorption and scattering contain distinct information on the medium. Absorption is determined

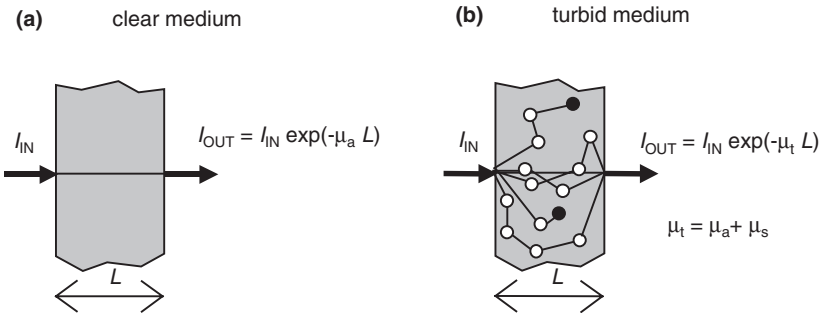

Fig. 8.1 Absorption spectroscopy in (a) clear and (b) turbid media: photon paths, scattering (○) and absorbing (●) centres.

by the pigments and constituents of the pulp that produce characteristic spectral features in the visible and near infrared region of the spectrum. Conversely, scattering is due to the local variation of the dielectric constant inside the medium. Microscopic changes in refractive index caused by membranes, air vacuoles or organelles deviate the photon paths and are ultimately responsible for light diffusion.

When considering conventional absorption spectroscopy measurements in a collimated geometry, results may be confounded by the fact that it is impossible to discriminate between absorption and scattering events. The transmitted intensity through a clear medium can be related by the Lambert law to the absorption coefficient μ_a since the distance travelled by light in the medium equals the source–detector distance L (see Fig. 8.1).

Conversely, in a diffusive medium an intensity measurement yields the attenuation coefficient $\mu_t = \mu_a + \mu_s$ representing the photon loss due to absorption and to photons scattered into directions different from the one of observation. The effect of scattering can be properly taken into account by direct measurements of photon pathlength. Since photon pathlength is directly related to time-of-flight in the medium, the natural choice is to perform time-resolved measurements.

8.3 Principles of time-resolved reflectance

Consider the injection of a short pulse of monochromatic light within a diffusive medium. By using a simplified description the medium can be regarded as consisting of scattering centres and absorbing centres, and the light pulse can be considered to be a stream of particles, called photons, moving ballistically within the medium. Whenever a photon strikes a scattering centre it changes its trajectory and keeps on propagating in the medium, until it is eventually re-emitted across the boundary, or it is definitely captured by an absorbing centre (see Fig. 8.2).

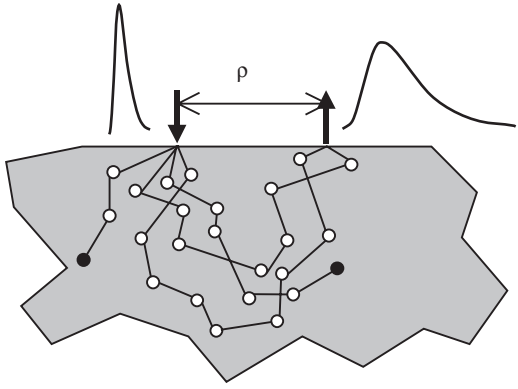

Fig. 8.2 Photon migration in turbid media: photon paths, scattering (○) and absorbing (●) centres.

The characteristic parameters of light propagation within the diffusive medium are the scattering length l_s and the absorption length l_a (typically expressed in units of mm or cm), representing the photon mean free path between successive scattering events and absorption events, respectively. Equivalently, and more frequently, the scattering coefficient $\mu_s = 1/l_s$ (i.e. $\mu s = (ls)^{-1}$) and the absorption coefficient $\mu_a = 1/l_a$ (i.e. $\mu a = (la)^{-1}$) (typically expressed in units of mm^{-1} or cm^{-1}) can be introduced to indicate the scattering probability per unit length and the absorption probability per unit length, respectively. To account for non-isotropic propagation of photons, the effective scattering coefficient $\mu_s' = (1 - g)\mu_s$ is commonly used, where g is the anisotropy factor, that is, the mean cosine of the scattering angle.

In a diffusive medium light scattering in the visible and near infrared spectral region is naturally stronger than light absorption, even if the latter can be non-negligible. This implies that light can be scattered many times before being either absorbed or re-emitted from the medium. The phenomenon is therefore called multiple scattering of light. Multiple scattering of light in a diffusive medium introduces an uncertainty in the pathlength travelled by photons in the medium. Light propagation in turbid medium is therefore addressed by the term photon migration.[8]

Following the injection of the light pulse into a turbid medium, the temporal distribution of the re-emitted photons at a distance ρ (see Fig. 8.2) from the injection point will be delayed, broadened and attenuated. A typical time-resolved reflectance curve is shown in Fig. 8.3. To a first approximation, the delay is a consequence of the finite time light takes to travel the distance between source and detector. Broadening is mainly due to the many different paths that photons undergo because of multiple scattering. Finally, attenuation because absorption reduces the probability of detecting a photon, and diffusion into other directions within the medium decreases the number of detected photons in the direction under consideration.

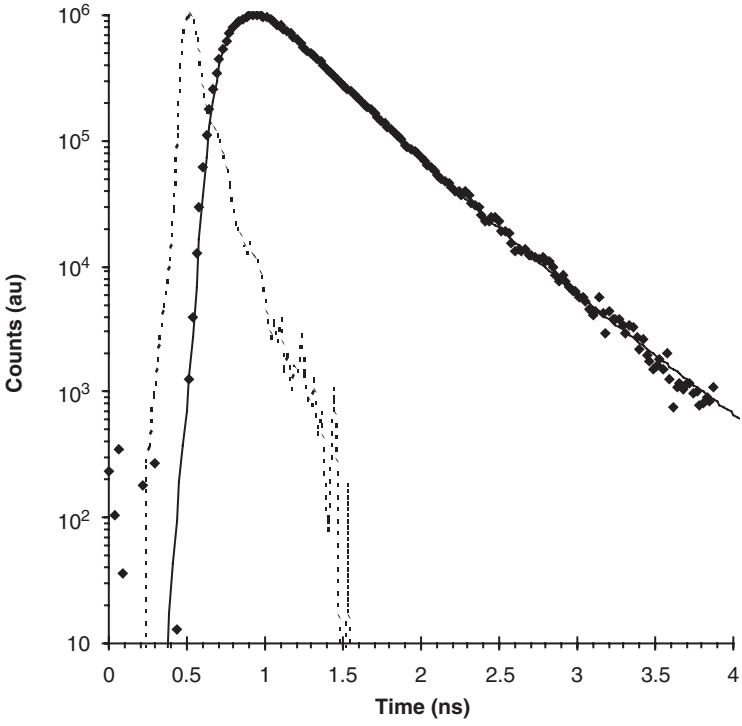

Fig. 8.3 Experimental TRS curve (diamond), IRF (dashed line) and best fit to diffusion theory (solid line).

8.4 Instrumentation

8.4.1 Photon migration

Photon migration measurements in the time domain rely on the ability to extract the information encoded in the temporal distribution of the re-emitted light, following the injection of a short monochromatic pulse in a diffusive medium. Typical values of the optical parameters in the red and in the near infrared part of the electromagnetic spectrum set the timescale of photon migration events in the range 1–10 ns and fix the ratio of detected to injected power at about −80 dB.

The two key points in designing a system for time-resolved measurements are thus temporal resolution and high sensitivity. Temporal resolution is mainly affected by the width of the light pulse and by the response of the detection apparatus. Pulsed lasers, which produce short (10–100 ps) and ultra-short (10–100 fs) light pulses with a repetition frequency up to 100 MHz, and photon detection systems with temporal resolution in the range 100–300 ps, are nowadays available. When concerned with sensitivity, the power of the injected light pulse should obviously be fixed at appropriate values, so as to avoid possible damage or injury to the sample. In the case of biological tissues the safety regulations[9]

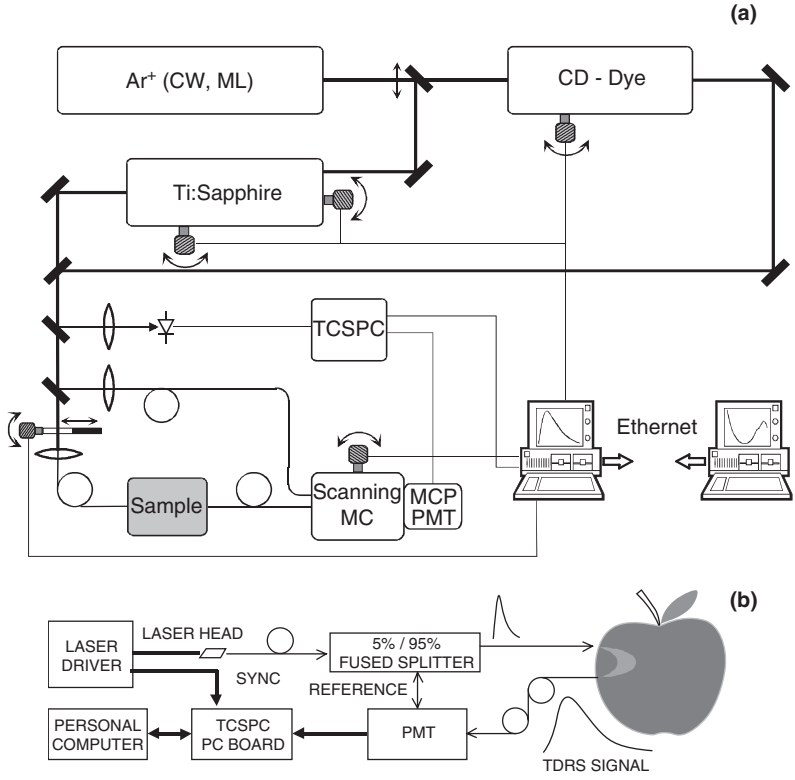

Fig. 8.4 Diagrams of the laboratory system (a) and of the compact prototype (b) for TRS measurements.

set the maximum permissible value to 2 mW mm^{-2} for laser pulses in the wavelength range 600–1000 nm. In the following, two different systems for time-resolved reflectance measurements based on the time-correlated single-photon counting (TCSPC) technique[10] are described (see Fig. 8.4). The first system is a laboratory set-up for broad band absorption and scattering spectroscopy by time-resolved reflectance, whose primary use is for basic studies of tissue components and structures. The second is a compact device working at selected wavelengths, which can be easily moved and therefore used in the field. Results from the two instruments will be presented below.

8.4.2 Time-resolved spectrometer for absorption and scattering spectroscopy in diffusive media

The optimal trade-off between sensitivity and temporal resolution in a TRS system can be achieved using mode-locked lasers as light sources and time-correlated single-photon counting for detection. The sources available are a dye

laser (Mod. CR-599, Coherent, Ca) and a titanium:sapphire laser (Mod. 3900, Spectra-Physics, Ca). Both sources are optically pumped by an argon laser (Mod. Innova, Coherent, Ca) running in mode-locking or continuous wave (CW) regimes, respectively. The dye laser is operated with a DCM (4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran) dye that permits tunability between 610 and 700 nm. Synchronous pumping mode-locking together with a cavity dumper yield pulses shorter then 20 ps (full width at half maximum, FWHM) at a repetition rate of about 8 MHz with an average power of 10 mW. The titanium:sapphire laser is tunable between 700 and 1010 nm using two different mirror sets. The laser structure is properly modified to produce a mode-locking regime by means of an acousto-optic modulator, with pulses of about 100 ps (FWHM), a repetition rate of 100 MHz, and an average power of 100–1000 mW over the entire spectral range.

The laser light is injected to, and collected from, the sample by means of 1 mm core 1 m long plastic-glass fibres set on the fruit surface at a relative distance of 1.5 cm. An appropriate fibre holder keeps the fibres in contact with the sample, one parallel to the other, which avoids collection of directly reflected light. The distal end of the collecting fibre is placed at the entrance slit of a scanning monochromator (Mod. HR-250, Jobin Yvon, France), coupled to a double micro-channel plate photomultiplier (Mod. R1564U, Hamamatsu, Japan). A small fraction of the main laser beam is split off by means of a glass plate, and detected by a fast PIN (P-type doped, intrinsic, N-type doped silicon) photodiode, which provides a triggering (reference) signal. Also, some laser light is coupled to another optical fibre and fed directly to the photomultiplier to provide an on-line monitoring of the system behaviour.

An electronic chain for time-correlated single-photon counting then processes both the photomultiplier signal and the triggering signal. The signals are first delayed by stages, and then preformed by constant fraction discriminators (Mod. 2126, Canberra, Co). The relative delay between the signals is then converted into a voltage signal by a time to amplitude converter (Mod. TC862, Oxford, TN), which is processed by a multichannel analyser (Mod. Varro, Silena, Italy). The temporal width of the instrumental transfer function is <120 ps (FWHM) as measured by connecting the injection and collection fibres.

The whole system of measurements is driven by a personal computer that automatically controls laser tuning, light attenuation, scanning of the monochromator, data transfer from the multichannel analyser, data visualisation and eventually data storage for further processing.

8.4.3 Compact prototype for time-resolved reflectance measurements

The system employs two pulsed diode lasers (Mod. PDL 800, PicoQuant GmbH, Germany) at 672 nm and 800 nm with a pulse duration of about 100 ps, a repetition rate up to 80 MHz and an average power of 1 mW. The pulsed diode laser is coupled into a multimode graded-index fibre (Mod. MMF-IRVIS-50/125, OZ Optics, Canada).

The signal is then split into two fibres by a fibre optic splitter (Mod. FUSEDIRVIS 5/95, OZ Optics, Canada). The first fibre receives a small fraction (5%) of the power and is fed directly into the photomultiplier to account for eventual time drifts of the instrumentation and to provide a time reference. The other fibre receives most of the power and delivers light to the sample. The re-emitted light is collected from the sample by 1 mm plastic fibres (Mod. EH4001, ESKA) in reflectance geometry.

The TRS curves are detected by a metal-channel dynode photomultiplier tube (Mod. RS5600U-L16, Hamamatsu, Japan) and are measured by a time-correlated single-photon counting PC board (Mod. SPC300, Becker&Hickl GmbH, Germany) with 1 MHz acquisition frequency and 25 ps temporal resolution. Custom made software, written in LabWindows and ANSI C languages, control data acquisition and analysis.

The typical instrument response function, obtained facing the injection fibre and the collection fibre, has a FWHM of about 200 ps for both wavelengths.

8.5 Data analysis

The temporal profile of the time-resolved reflectance curve is analysed using a solution of the radiative transport equation under the diffusion approximation for a semi-infinite homogeneous medium[11,12]

$$R(\rho,t) = \frac{1}{2}(4\pi\nu)^{-3/2} t^{-5/2} e^{-\mu_a \nu t} e^{-\frac{\rho^2}{4Dvt}} \left(z_0 e^{-\frac{z_0^2}{4Dvt}} - (z_0 + 2z_e) e^{-\frac{(z_0+2z_e)^2}{4Dvt}} \right) \quad [8.1]$$

where $R(\rho,t)$ is the number of photons per unit time (t) and area re-emitted from the tissue at a distance ρ from the injection point. ρ is the source–detector distance (or interfibre distance), $v = c/n$ is the speed of light in the medium, n is the refraction index, $D = (3\mu_s')^{-1}$ is the diffusion coefficient, $z_0 = (\mu_s')^{-1}$ is the isotropisation length, z_e is the extrapolated distance which takes into account the refraction index mismatch at the surface.

The experimental curve is fitted with a convolution of the theoretical function with the instrumental response function (IRF). The best fit is reached minimising the χ^2 varying both μ_a, and μ_s' using a Levenberg–Marquardt iterative procedure. Owing to the lower accuracy of the models in earlier times, the range of the fit includes all the points on the experimental curve with a number of counts higher than 80% of the peak value on the rising edge of the curve and 1% of the peak value on the falling edge. Figure 8.3 shows the best fit of a typical experimental curve. The instrumental transfer function is also shown for comparison (dashed line).

The fitting procedure can automatically analyse a full batch of experimental curves on a standard PC (Athlon AMD, 1 GHz) at a speed of 10 curves per second. Synchronisation of the analysis and measurement PCs over the network permits on-line processing of the experimental data, so that the absorption and

scattering coefficients are shown on the screen in real time while the measurement is in progress.

8.6 Effect of skin and penetration depth

8.6.1 Skin

Measurements were performed on apples (Golden Delicious, Granny Smith and Starking Delicious), peaches, nectarines, kiwifruit and melons. The tests proved that TRS allows the assessment of the internal optical properties and that the optical properties of the skin do not prevent the assessment of information on the bulk, at least for fruits with thin skins.

For apples, no significant change in the measured optical properties (both absorption and scattering) is caused by skin removal. This is proved by the experimental finding that in none of the cases considered did skin removal alter the results significantly, despite the different optical properties of the skin in each distinct situation, for example a yellow-skinned apple (Golden Delicious) compared with a red-skinned one (Starking Delicious), as shown in Fig. 8.5. Similar outcomes were obtained for peaches and nectarines (data not shown). The peeling of the skin did not alter markedly the results, confirming that TRS is most sensitive to the internal features.

The situation is different for thick-skinned fruits. In particular, for kiwifruit where peeling led to a 20–25% increase in the absorption coefficient over the entire NIR range examined (720–840 nm). However, this effect concerns only the absolute estimate of the optical properties. The spectral line shape is not significantly altered. Consequently, even though the skin influences the results, it does not necessarily make TRS measurements inappropriate for the assessment of internal quality of thick-skinned fruits. For melons (Cantaloupe) measured in the bed region, the skin removal significantly reduces the chlorophyll absorption, while it has no significant effect on the NIR absorption. In both wavelength ranges, a 15–25% decrease is observed in the measured values of the scattering.

8.6.2 Penetration depth

In a further experiment, the penetration depth of a TRS measurement was determined. It is well known that the volume probed by a TRS measurement is a 'banana shaped' region connecting the injection and collection points.[13] It is not easy to define the measurement volume, since the photon paths are more densely packed in the banana region but can be distributed in the whole medium. Attempts were made to determine the maximum depth in the pulp that can give some detectable contribution to the TRS curve. A series of measurements were performed on a Starking Delicious apple where slices of pulp were cut from opposite sides of the measurement site. Spectra were taken of the whole apple, and then slices were removed to yield a total thickness of 4.1, 2.7, 2.1 and 1.5 cm.

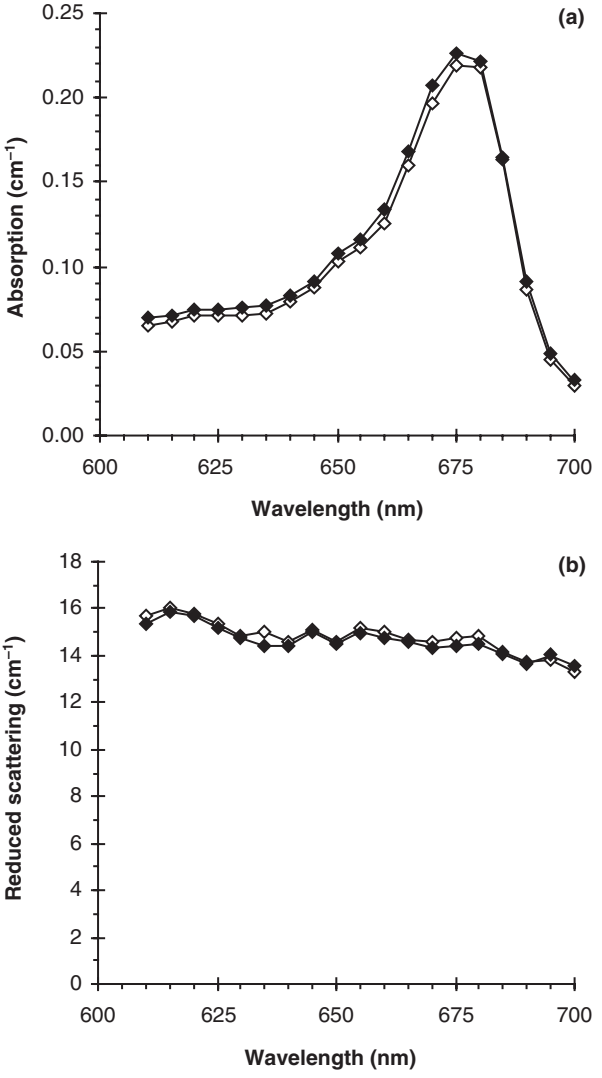

Fig. 8.5 Influence of skin on TRS measurements: absorption (a) and transport scattering (b) spectra of a Starking Delicious apple before (closed symbols) and after (open symbols) peeling.

The fitted absorption and scattering spectra are shown in Fig. 8.6. For the absorption measurement, μ_a is unchanged down to a thickness of 2.7 cm. For the 2.1 cm thick slice, μ_a starts deviating from the measurement of the whole apple with a discrepancy of 25% at 680 nm, while for a thickness of 1.5 cm the discrepancy increases up to 50%. The highest variations are observed on the tails of the spectrum, where the absorption is lower. The results for the scattering coefficient show

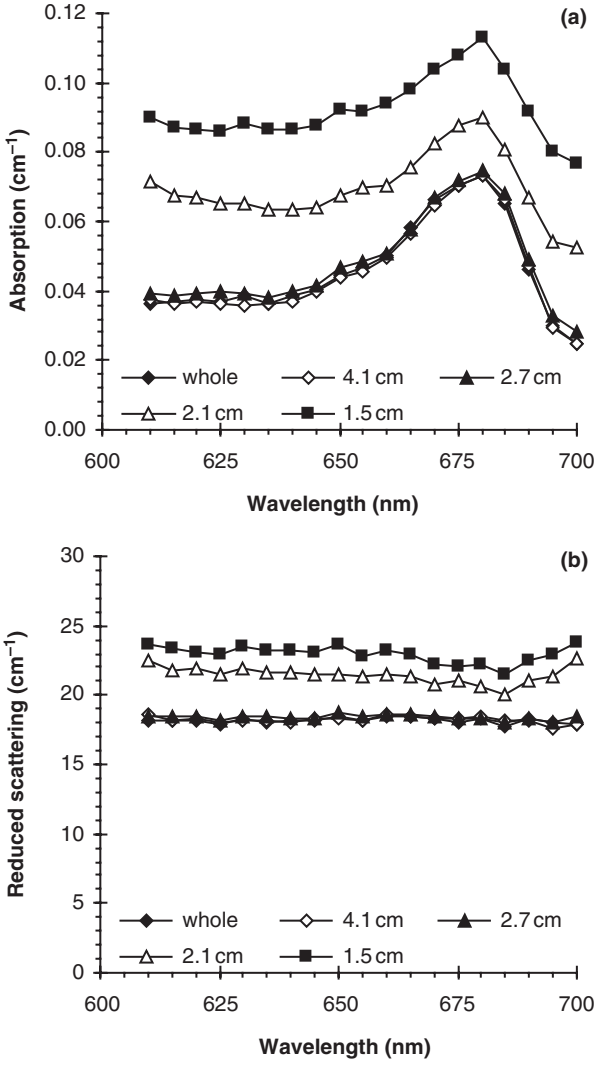

Fig. 8.6 Absorption (a) and scattering (b) spectra of a Starking Delicious apple. Different curves correspond to measurements on the whole apple, and on slices of the same apple obtained by cutting the fruit on the opposite side of the measurement site.

a similar behaviour, with almost no changes down to a thickness of 2.7 cm, and discrepancies of 15% and 25% for a 2.1 and 1.5 cm thickness, respectively. Overall, these data show that the TRS measurement is probing a depth of at least 2 cm in the pulp. Of course this is a rough estimate, yet it confirms that the TRS measurement is not confined to the surface of the fruit. Moreover, the penetration depth can be somehow dependent on the optical properties, and deeper penetration is expected in less absorbing and/or scattering fruit.

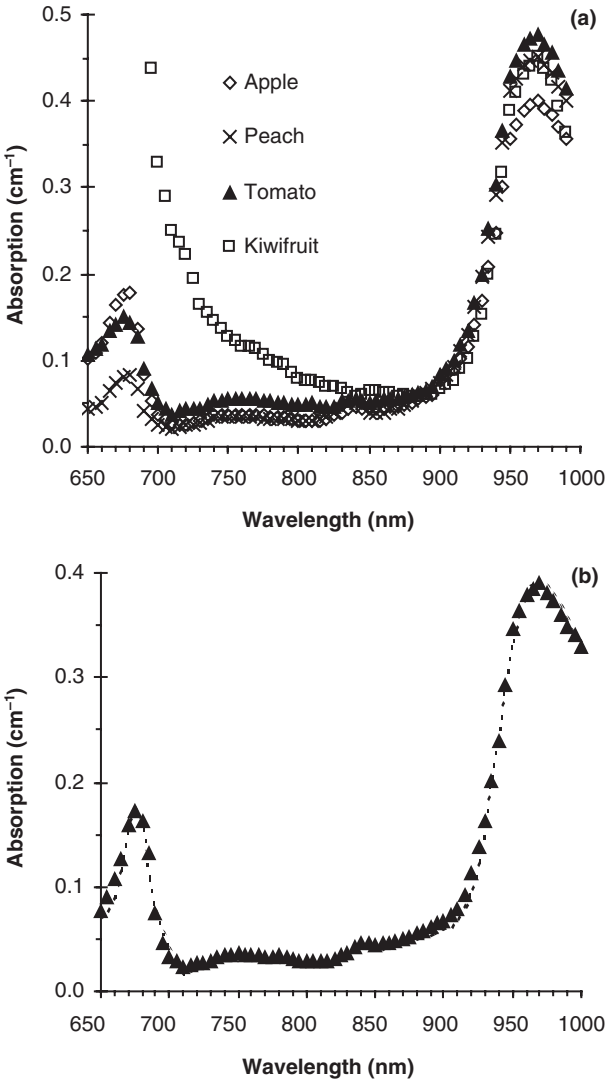

Fig. 8.7 (a) Absorption spectra of apple, peach, tomato and kiwifruit. (b) Best fit of chlorophyll-a and water line shape to the absorption spectrum of a Starking apple.

8.7 Optical properties of fruits and vegetables

8.7.1 Absorption and tissue components

Typical absorption spectra of different fruits (apple Starking Delicious, yellow peach, tomato and kiwifruit) are reported in Fig. 8.7(a). The absorption spectrum of the apple is dominated by the water peak, centred around 970 nm, with an absolute value of about 0.4 cm^{-1}. Minor absorption features of water are usually

Table 8.1 Chlorophyll-a and water content in different fruits

Fruit	Chlorophyll-a (μM)	Water (%)
Apple (Starking Delicious)	0.96	82.6
Peach	0.49	93.8
Tomato	0.52	95.0
Kiwifruit	6.91	98.8

detected around 740 and 835 nm, where the absorption coefficient is low (0.05 cm^{-1}). A significant absorption peak (0.12–0.18 cm^{-1}) at 675 nm, corresponding to chlorophyll-a, is found. Both the line shape and the absolute value of the absorption spectra of peach and tomato are quite similar to those of apples. However, for kiwifruit, as expected from the visual appearance of its flesh, chlorophyll-a absorption is considerable, with a maximum value up to 2 or 3 times the water maximum in the infrared.

Information on the water content can be obtained by considering the absolute values of the absorption at 970 nm. In agreement with the different water/fibres ratio in distinct species, a higher absorption was detected in tomatoes (~0.5 cm^{-1}), than in peaches and kiwifruits (~0.45 cm^{-1}), and in apples (~0.4 cm^{-1}). The absorption at 675 nm provides information on the chlorophyll-a content and preliminary data obtained from apples suggest that this could be a useful parameter to test the ripening stage. A series of measurements performed on the same fruits showed a progressive decrease in red absorption, in agreement with the gradual reduction in the chlorophyll content with post-harvest ripening.[14]

To quantify the percentage volume of water and the chlorophyll-a content in the bulk of the intact fruits, a best fit of the absorption spectrum with the line shape of water[15] and of chlorophyll-a[16] was performed. To account for the presence of other chromophores of fruits, such as carotenoids and anthocyanins, which exhibit characteristic peaks at shorter wavelengths than 650 nm, a flat background spectrum of arbitrary amplitude was used as a free parameter in the fit.

Figure 8.7(b) shows a typical example of fit for the absorption spectrum of a Starking Delicious apple to the line shape of water and chlorophyll-a. Table 8.1 reports the chlorophyll-a and water content in different fruits. In all cases a 0.02–0.03 cm^{-1} contribution was added by the flat background spectrum.

8.7.2 Scattering and tissue structure

The scattering properties for all the species considered showed no particular spectral features. The value of the transport scattering coefficient decreased progressively with increasing wavelength. Typical examples are shown in Fig. 8.8(a) for a Starking Delicious apple, a peach, a tomato and a kiwifruit. The transport scattering spectrum of the kiwifruit was noisier than the spectrum

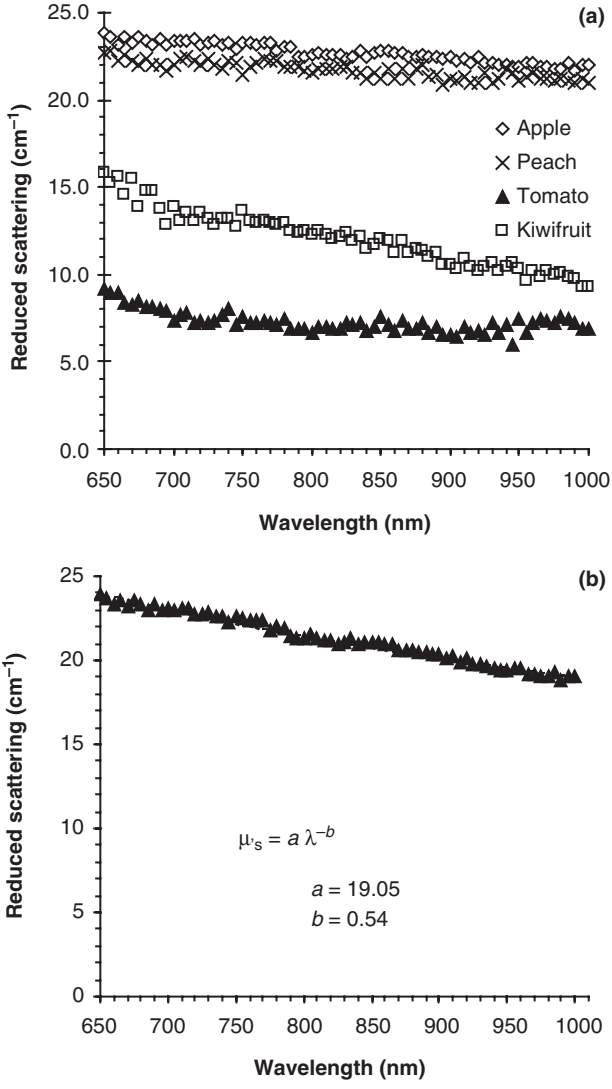

Fig. 8.8 (a) Scattering spectra of apple, peach, tomato and kiwifruit. (b) Best fit of Mie theory to the scattering spectrum of a Starking apple.

of other fruits, particularly in the 675 nm region where the high absorption of chlorophyll reduced the accuracy of the evaluation of transport scattering by TRS measurements.

Even though marked variations in the absolute values were noticed depending on variety and ripeness, kiwifruits and tomatoes are usually characterised by a lower scattering than other species.

Table 8.2 Parameters *a* and *b* for different fruits

Fruit	a (cm^{-1})	b (cm^{-1})	r (μm)
Apple (Starking Delicious)	17.4	0.12	0.759
Peach	14.4	0.20	0.740
Tomato	2.9	0.48	0.591
Kiwifruit	4.5	0.95	0.266

Further information could be obtained by interpreting the transport scattering spectra with Mie theory. For a homogeneous sphere of radius *r*, Mie theory predicts the wavelength dependence of the scattering and the relation between scattering and sphere size. Under the hypothesis that the scattering centres are homogeneous spheres behaving individually, the relationship between μ'_s and wavelength (λ) can be empirically described as follows:[17]

$$\mu'_s = ax^b \qquad [8.2]$$

where the size parameter x is defined as $x = 2\pi r n_m \lambda^{-1}$, with the refraction index of the medium n_m chosen to be 1.35, and a and b are free parameters. In particular, a is proportional to the density of the scattering centres and b depends on their size. Moreover, b can be empirically expressed as a third order polynomial function of r, therefore the estimate of b can yield the sphere radius r.[18]

Figure 8.8(b) shows a typical transport scattering spectrum of a Starking Delicious apple and the best fit to Mie theory. The estimated average size of scattering centres in different fruits is shown in Table 8.2. It was observed that a and b varied in the range 2.9–17.4 cm^{-1} and 0.12–0.95, respectively. This suggests that different fruits have different density and average dimensions of scattering centres (the range for r is 0.15–0.78 μm). It is worth noting that, as the tissues are a complex distribution of cells and fibres, these parameters do not assess the real size of scattering centres in the tissue, rather they are average equivalent parameters, which could eventually be related to physical or chemical fruit characteristics such as firmness or sugar content.

8.8 Applications: analysing fruit maturity and quality defects

8.8.1 Picking date experiment

To prove the applicability of the technique in real life applications, the compact prototype for TRS measurements was sent to Horticulture Research International and there tested on a picking date experiment to check the tracking of maturity stages in apples.[14] Fruits of the Gala variety were harvested from the same orchard at three different picking dates (pick 1 = 15 September, pick 2 = 25 September and pick 3 = 9 October), stored under controlled atmosphere at 1.5°C for 7

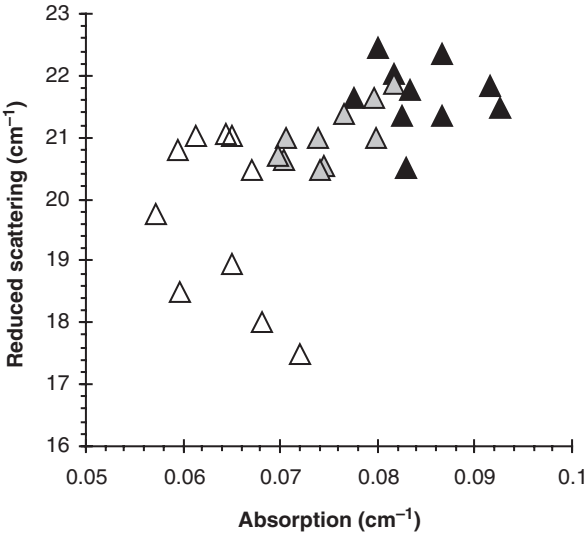

Fig. 8.9 Plot of the absorption and scattering measurements of 30 apples taken from a Gala cultivar at successive harvest dates: pick 1 (black triangle), pick 2 (grey triangle), pick 3 (white triangle), and measured all together with the prototype after 7 months' storage under controlled atmosphere.

months, and then measured all together using the prototype. For each fruit, four equally spaced positions on the equatorial plane were measured and averaged. Results are presented in Fig. 8.9, where every fruit is coded by its μ_a and μ_s' at 672 nm. The measured μ_a decreases passing from pick 1 (black triangle) to pick 2 (grey triangle) and to pick 3 (white triangle), indicating a decrease in chlorophyll (CHL) content. Also the scattering coefficient is somehow related to the picking date with a general decrease for latest harvest.

Similar results were found for peaches. The technique is not only able to distinguish between different batches of fruits but can also monitor small variations due to shelf-life storage.

8.8.2 Detection of defects

Encouraging results have been obtained by applying TRS to non-invasive detection of defects in fruits. Preliminary measurements show that TRS can discriminate mealiness,[19] watercore and bruise in apple, and brown heart in pears.[20]

Brown heart (BH) is an internal disorder sometimes shown by pears during controlled atmosphere (CA) storage. The symptoms are in no way recognisable from the outside of the fruit and are visible only after cutting the fruit. The aim of this work was to test TRS for analysing pears at risk of being affected by BH, in order to check if internal browning can be detected in the intact fruit by non-

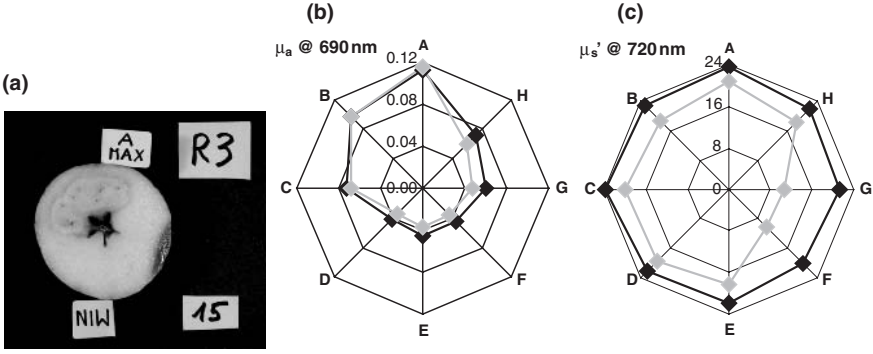

Fig. 8.10 Absorption coefficient at 690 nm (b) and transport scattering coefficient at 720 nm (c) as a function of the position around the equator of a partially BH pear picked at late harvest. Reported measurements were performed at the end of storage (black diamond) and at the end of shelf life (grey diamond). A photograph of the equatorial section of the fruit is shown in (a). Units for absorption and scattering are cm^{-1}.

destructive means. 'Conference' pear fruits at low risk (early harvest, low CO_2 CA storage) and high risk (late harvest, high CO_2 CA storage) for BH were measured with TRS at 690 nm and 720 nm on eight points around the equator. BH was detected in pears by a significant increase of the absorption coefficient μ_a at 720 nm. The absorption coefficient μ_a at 690 nm responded by both increasing in the presence of BH in affected fruits and decreasing with ripening in sound fruits, so it alone cannot have a unique interpretation. The decrease of the absorption coefficient μ_a at 690 nm in sound fruits can be attributed to degradation of chlorophyll, which has an absorption peak at 675 nm. The scattering coefficient μ_s' at 720 nm was influenced by translucency of soaked looking tissue, as in overripe fruits and in bruised regions. This technique allows a description of the virtual appearance of the internal tissue in the intact fruit to a depth of 2 cm, of the presence of defects and of their position inside the fruit, as it can be visually confirmed only after cutting the fruit.

An example is reported in Fig. 8.10, where the plots of the absorption coefficient at 672 nm and of the scattering coefficient at 720 nm are compared with the photograph of a partially BH pear.

8.9 Future trends

The use of the optical properties of the pulp of fruits and vegetables for the assessment of the internal quality of fruit has still to be investigated. More studies are required to correlate the measured optical properties with other chemical or physical parameters of the fruit such as soluble solids (sugar), acidity or firmness.

Since TRS permits the measurement of the absorption spectrum of the pulp independent of the scattering properties, it may be possible to detect absorbing substances such as chlorophylls and anthocyanins in the visible region or sugar and water in the NIR region. This technique might be suitable for following the ripening process pre-harvest, or for monitoring fruit changes during long-term storage. Scattering inside a fruit is mainly due to refractive index mismatches between liquids and membranes. Thus, the mean scattering coefficient could provide information on the internal structure, as suggested by a study on kiwifruits. In our work, changes in the scattering coefficient were related to the stage of maturity and to the ripening process, and could contribute to monitoring them.

Clearly, many technical aspects need still to be solved before an industrial application can take place. Most of all, the fruit characterisation in terms of pulp optical properties has to be compared to the presently accepted estimators of fruit quality, that is, sugar content, acidity and firmness.

A possible criticism of the usefulness of TRS for applications in agriculture is the cost and complexity of the instrumentation, especially whenever more than one wavelength is needed. However, rapid progress in optoelectronics, particularly in telecommunications, has led to considerable growth in instrumentation for time-resolved measurements, so that the development of a compact and low-cost time-resolved instrument is now feasible. A first prototype, working with semiconductor lasers, a compact photomultiplier and all-fibre optics that can be used as a stand alone portable instrument, was built in our laboratory. The compact prototype is characterised by ease of use and portability and a relatively low cost (about 20 000 euro before assembly).

Post-harvest selection of fruit at industrial level employs automated machines for grading and sorting of fruits based on external parameters (colour, size) and weight. Typical speed for in-line analysis is 5 fruits per second. The acquisition time of TRS measurements can be as low as 500 ms per point in the wavelength range 700–800 nm on most fruits. In this respect TRS measurements are not far from being applicable in on-line analysis. However, in view of a possible application of the TRS technique at industrial level, it is necessary to address several factors like acquisition time, number of measurement points, use of multichannel acquisition, and contact between fruit and optical probe. Detection of an internal disorder may in fact require mapping of the fruit to localise the defect. Moreover, in performing a non-contact measurement which could speed up the measurement time, care should be taken to reject background light and to enhance the signal. On the other hand, the TRS technique could be useful in the orchards, in the packing house or in the marketing chain as a complementary tool for non-destructive characterisation of fruits.

8.10 Sources of further information and advice

The study of light propagation in diffusive media, or photon migration, is a recent and open field of physics and optics. A limited number of books deal with this

issue and most of the support material should be found in the scientific literature, that is in journal and conference proceedings. Most applications fall within the biological, medical and clinical application of lasers and optics, therefore research and interest groups are to be found in these communities.

8.10.1 List of books

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Optical Society of America (OSA): *Applied Optics – OT & BO division, Optics Letters, Optics Express, Journal of the Optical Society of America A* (http://www.opticsinfobase.org/)
The International Society for Optical Engineering (SPIE): *Journal of Biomedical Optics* (http://ojps.aip.org/journals/doc/JBOPFO-home/)
Institute of Physics (IOP): *Physics in Medicine and Biology* (http://www.iop.org/Journals/pb).

8.10.3 List of Conference Proceedings

Trend in Optics and Photonics OSA (http://www.osa.org/pubs/tops/)
Proceedings of the SPIE (http://bookstore.spie.org/publications).

8.10.4 List of web sites

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9

Applying advanced instrumental methods: mealiness in fruit

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9.1 Introduction: defining mealiness in fruit

Much effort nowadays is directed towards increasing the consumption of fruits because of their health benefits. The attractiveness of fruits with respect to the purchasing behaviour of consumers is affected by visual appearance, but the expected internal quality is of equal importance. Mealiness is just such an important internal quality parameter, which is characterised by texture deterioration of the fruits during inappropriate storage, resulting in soft, dry and mealy fruit. This phenomenon is of particular significance for fruits such as apples, peaches, nectarines and tomatoes that are characteristically juicy when fresh. In this chapter, only apples will be considered further.

Mealiness is a term which is commonly used by consumers but which is not defined very well in the literature. As a common useful definition is required, the consumer's perception of mealiness is investigated in section 9.2. It will be established how consumers define mealiness and whether there is a common perception across a span of European countries. Sections 9.3–9.11 deal with instrumental techniques to measure mealiness. These techniques range from a histological analysis of mealiness by means of light microscopy to more advanced ones like ultrasonic wave propagation and magnetic resonance mapping tomography. In section 9.12 a dynamic model will be presented to relate the attributes associated with mealiness, like crispiness, hardness and juiciness, to physiological properties such as water status and to describe their evolution as a function of time. This chapter will conclude by indicating some further trends in this research area, with sources of further information and advice.

9.2 Sensory evaluation and consumer's expectations

Although awareness of texture appears to be present on a subconscious level, it plays an essential role in determining people's feelings about foods. Very often flavour overshadows texture at the conscious level. People simply take the texture of a food for granted, considering it an integral part of the nature of the food. They do not distinguish it as a separate and distinct characteristic. This is reflected in the limited ability to verbalise when talking spontaneously about texture. Texture awareness is increased when expectations are violated, associations are made with non-food items or unpleasant mouth sensations are experienced. Szczesniak (1971) observed that sex and socioeconomic group are factors which influence awareness of texture. In general, women were more texture conscious than men. Better educated people were more texture conscious and showed a better understanding of the idea of texture because of better education and experience in dealing with generalised concepts and applying abstract ideas to real life cases.

Sensory evaluation has been defined as 'a scientific discipline used to evoke, measure, analyse, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing' (Andani, 2000). Bourne (1982) summarised it as follows 'there is no instrument available that has the sophistication, elegance, sensitivity, and range of mechanical motions as the mouth or that can promptly change the speed and mode of mastication in response to the sensations received during the previous chew'. Texture was found to be a clearly perceived attribute of foods. Most of the sensory results published on apples are based on preference or difference testing, related to pleasure or acceptability, rather than to intensity of defined attributes (Lapsley, 1989).

Sensory analyses can be conducted at different levels, each resulting in specific information about fruit quality attributes such as texture and flavour. An analytical sensory panel, trained in descriptive sensory analyses, will provide detailed information about the sensory attributes of the product. In consumer preference tests, the general descriptive quality perceptions of the consumer will be studied. Repertory grid methods can be interpreted as an intermediary technique. Using consumer expectation theory, information can be gathered about what the consumer expects of the product and how this will influence his or her purchase intent. Each of these methods provides answers to different questions, as will be illustrated below. For each type of test, an example in the framework of texture and mealiness in apples will be discussed.

9.2.1 Analytical sensory panel

In the early 1970s, Civille and Szczesniak (1973) developed a tool to describe and quantify textural characteristics of foods. This technique was not an instrumental technique but involved a panel of people trained to assess texture. Nicot (1992) summarised all the practical aspects which have to be considered when

performing a sensory study with a trained panel. The experimental design depends on the nature of the product, the environmental factors which have to be controlled in the room where the tests are performed, the way the samples are presented to the panel and how they are encoded (Williams and Carter, 1977; Dürr, 1979). There are many problems associated with the sampling of the fruit, such as variation within one fruit and between fruits of the same batch. Moreover, there may be wide variability between the subjects who rate the samples. The differences between human beings reflected, for instance, by the equipment they have available for the process of chewing, their neurophysiology for conveying the information to the brain and their capacity for describing experiences to the investigator, should be taken into account or corrected for during the analyses of the data. Another source of variability is the response of the panellist and the method used by the investigator to elicit and record the response. Some individual differences are unavoidable while others to some extent are under the experimenter's control.

In the framework of a European project (EU FLAIR CT95-0302) on mealiness of fruits, a panel was trained at the Institute of Food Research (Reading, UK) and the IATA (Valencia, Spain) to assess mealiness in apples (Nicolaï *et al*., 1999). Different degrees of mealiness were induced by storing Granny Smith, Golden Delicious, Late Top Red, Cox Jonagold and Boskoop apples in air and 95% relative humidity at 20°C for variable times. A discussion session was held to select descriptors that would characterise the samples. Remarkably, mealiness was not one of the descriptors used by the trained panel as it was not a term they could comprehend. First the extent to which the peel influenced perceptions was evaluated. Removing the peel had some effect on attributes related to taste, but did not seem to affect the ratings of the texture-related attributes. It was decided to restrict further sensory work to peeled samples. The attribute scores observed for the texture attributes pulpy, granular, floury, juicy, hard and crispy for the different cultivars and storage conditions demonstrated that these descriptors were used to describe different degrees of mealiness. Hence, mealiness was not perceived as a one-dimensional parameter but as an umbrella term which encompassed the descriptors floury and granular. The latter attributes were found to be negatively correlated to attributes such as hardness, crispiness and juiciness (Nicolaï *et al*., 1999).

9.2.2 Consumer preference patterns

In contrast to a trained analytical panel, consumers often do not have a standardised vocabulary to describe objectively what they are perceiving when assessing product attributes. Instead of describing their perceptions by means of specific product attributes, because of their limited vocabulary, they use hedonically based terms like 'nice' and 'tasty' to express their perceptions (Andani, 2000).

Andani (2000) studied whether consumers perceived mealiness in apples as a negative quality attribute, and whether their preference pattern leant more to

'fresh' than 'mealy' apples. The participants were given three apple cultivars (Cox's, Boskoop and Jonagold) at three different stages of mealiness (fresh, mid-point and mealy). To obtain these stages the apples were stored in mealiness inducing conditions (20°C and ± 95% relative humidity, RH) for a certain period depending on the required mealiness level. Each of 150 subjects was asked to rate the apples for how much they liked the fruit on a nine-point hedonic box scale labelled from 'dislike extremely' to 'like extremely'. To analyse and visualise the preference data structure, the preference mapping methodology was applied (Carroll, 1972; Greenhoff and MacFie, 1994). The multidimensional character of the preference mapping technique offers a number of advantages over univariate analysis algorithms. The information from each participant for all assessed products is taken into account in the analysis, the scores for products are not averaged over consumers, but each individual is represented on the map (Earthy, 1996). Hence, no information is lost by averaging, and natural segmentation of consumers over the map is illustrated (McEwan, 1988/9).

Based on this preference mapping methodology, the author observed a separation along the first preference dimension between Jonagold and Cox on the one hand and Boskoop on the other hand. Boskoop had a low consumer acceptance or preference level. The data suggested that preference was driven by dislike of Boskoop more so than liking of Cox and Jonagold. The same apples samples were tasted by the sensory panel to identify the specific product attributes causing the consumer preference segmentation. It was found that the dislike of Boskoop apples was caused by the 'bitter', 'acid' and 'unripe' flavour of the Boskoop variety, suggesting this dislike was more related to flavour than texture. Although less clearly than the cultivar (flavour) segmentation, the consumer preference pattern leant more to non-mealy apples, indicating that the consumers perceived mealiness as a negative quality attribute. Andani (2000) reported that more 'mealy' samples were perceived as having a 'granular' texture. Thus the results supported the hypothesis, which states that consumers would perceive mealiness in apples as a negative quality attribute, and show a greater liking for 'fresh' than 'mealy' fruit. For the same three cultivars, Jaeger *et al*. (1998) looked at cross-cultural differences between British and Danish consumers in relation to preferences for fresh and aged apples. The preference patterns were similar for British and Danish consumers and reflected no cross-cultural differences (Jaeger *et al*., 1998).

In a study among Spanish consumers living in Madrid it was found that the consumer in general sees mealiness as a negative characteristic (López *et al.*, 1996). Flavour is regarded as more important than appearance. Men prefer more sweet apples while in general women prefer more acidic fruits. Young people also preferably eat more acidic fruits.

9.2.3 Repertory grid method

The repertory grid method is another technique to assess how consumers perceive product attributes like mealiness in apples. This technique is used to gather

consumer information about products at a level between the trained sensory panel and the consumer's preference or acceptability judgement. Kelly (1955) was the first to use a repertory grid method. He developed the technique to identify the constructs that people use to structure their perceptions of the social world. The subjects are asked to say in which way two stimuli are alike and different from a third. The process is repeated until the subject does not know any more new items. In 1981, Olsen (1981) first applied this technique to food acceptability. He added a second part to the method by asking the subjects to define a scale to measure the amount of each construct perceived in the objects. Hence, each person uses his own constructs and scales for evaluating the objects.

A repertory grid study was conducted among consumers of four different countries (Belgium, UK, Spain and Denmark) and five different languages (Dutch, French, English, Spanish and Danish) (De Smedt, 2000; Andani, 2000). The participants were given apples (Cox's, Boskoop and Jonagold) of three different mealiness stages (non-mealy, midpoint and mealy). The data in this study were analysed using generalised procustes analysis (GPA). GPA is a member of the family of methods that are concerned with the analysis of data arising from several individuals. The purpose is to know how the individuals differ and, equally, to what extent they may agree in their perceptions of the same phenomena. GPA is an empirical statistical technique which allows the investigator to relate in a multivariate space different sets of attributes or constructs generated by the different consumers with the tasted samples. It also deals with the problem of individual panellists who constantly under or over score an attribute (Dijksterhuis and Gower, 1991). Figure 9.1 gives a plot of the consensus solution of the GPA. The first dimension separates the Boskoop apples from the other cultivars and runs from bottom right to top left. The second dimension runs from the top right to the bottom left and aligns well with the degree of mealiness. On the consensus plot, the distribution of the sample means for each consumer group around the global sample mean can be seen. It can be concluded that no one consumer group was significantly different from the other groups when describing their perception of the samples as the position of the groups around the sample is rather tight.

From this study it could be concluded that there is a consensus among different consumer groups in the way they perceive mealiness. However, the way different consumers describe their perception is quite different. Flemish (Dutch speaking Belgians), Walloons (French speaking Belgians), Danes and Spaniards all use a translation of the word 'mealy'. Apart from this, they all have their own attributes to describe mealiness. English consumers on the other hand do not use the term 'mealy'. It is not a term they can comprehend. They will describe mealy apples as coarse, spongy, dry and crumbly. No clear difference was found between the Flemish and the Walloon consumers in Belgium in their ability to generate descriptors. Both groups generated approximately the same number of descriptors. The Flemish consumers used the mealiness category more widely than the Walloon consumers. In general, consumers from different countries perceive the

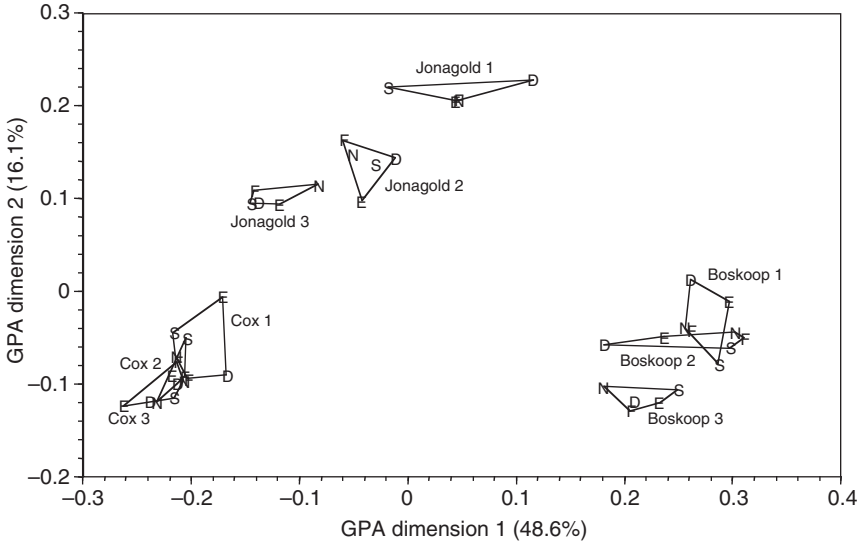

Fig. 9.1 GPA consensus plot showing variation between the different consumer groups (GPA with isotropic scaling) for three apple cultivars (Cox, Jonagold and Boskoop) in three mealiness stages (1: fresh, 2: midpoint, 3: mealy). D: Danish, E: English, S: Spanish, N: Dutch, F: French (source: Andani, 2000).

differences between the samples similarly, which means that there is a cross-cultural consensus with respect to the perception of mealiness.

9.2.4 Consumer expectations and acceptability

Consumers have prior expectations, supported by previous experience, about the quality attributes of a product (Deliza and MacFie, 1996). For fruits, these expectations are based to a large extent on the external fruit appearance, manual texture perception and aroma (Christensen, 1983; Cardello and Segars, 1989), which may be used by the consumer as a guide to freshness, ripeness, quality and variety (Richardson-Harman *et al*., 1998). Different expectation theories are proposed in the literature (Deliza and MacFie, 1996) to analyse the consumer's expectations.

Experiments were organised both in the UK and in Spain to quantify which external features of the apple influence the expected perception of the sensory properties (Andani, 2000; Nicolaï *et al*., 1999). The features were skin colour, density and 'texture' to touch. The participants gave more weight to what they perceived 'in mouth' rather than the external features of the apple. From other studies, it was found that it was not possible to manipulate the subjects' perceptions. It was also confirmed by means of consumer studies that, at least in Spain, there clearly exists a market segment who prefer mealy apples. Andani (2000) studied the relation between gender and the importance of texture in apples. The

results suggested that texture is significantly more important to females (92%) than to males (61%). Up to 69% of the females rated texture to be 'very important' compared to 49% of the males. Neither sex judged the texture to be 'not very important', emphasising the importance of texture and, hence, mealiness, to the consumer.

9.3 Instrumental methods

Attempts to correlate sensory measurements with more objective instrumental measurements go back to the beginning of the century. Researchers have always wanted to relate what a person perceives to the physical variables which can be measured by so-called objective means (Moskowitz, 1983). Szczesniak and Ilker (1988) and Casutt *et al.* (1994) correlated sensory perception of juiciness with experimental parameters. They found that juiciness was a multifactorial attribute positively correlated to the amount of juice present in the food. Sensory perceived juiciness was negatively correlated with mealiness. Finney (1971) used sensory and instrumental tests to evaluate Red Delicious apples. He observed that sensory firmness correlated better with Magness–Taylor firmness than with sonic firmness measurements. Diehl and Hamann (1979) concluded from instrumental and sensory studies on Red Delicious apples that the sensory crispiness was directly related to the modulus values from uniaxial compression tests and torsion tests, while graininess, a measure of mealiness, was inversely related to the modulus values and the shear stress at failure in torsion. In general, graininess was a texture parameter which gave negative correlations with the other sensory parameters used to describe the apples. Harker *et al.* (1997) found a curvilinear relationship between sensory perceived hardness and tensile strength, puncture strength and shear strength for Royal Gala apples. Paoletti *et al.* (1993) tried to discriminate among apple cultivars and different levels of quality for their mechanical and texture characteristics by instrumental and sensory methods. They found high correlation values between most of the instrumentally and sensory measured parameters.

The potential of several instrumental techniques in the study of mealiness in apples will be discussed further. First, microscopic images of mealy tissue will be compared to those of non-mealy tissue. Subsequently, the confined compression test will be described as a destructive measurement technique to measure mealiness. Finally, the potential of several non-destructive techniques to measure or detect mealiness will be assessed: ultrasonic wave propagation, nuclear magnetic resonance relaxometry and imaging, NIR reflectance spectroscopy, aroma analysis, acoustic impulse response technique and electrical impedance.

9.4 Microscopic imaging

It is commonly accepted that mealiness is related to the relative strength of the cell wall compared to that of the middle lamella (Harker and Hallett, 1992). If

the cell wall is stronger than the middle lamella, the tissue will yield between the cells and the cell contents will not be released during mastication. If the cell wall is weaker than the middle lamella, yielding will occur through the cells and as a result the liquid content will be released. In the former case the sensory perception is that of a dry, chalky granular texture, and in the second case, that of a juicy product. Changes in the tensile strength of apple tissue were related to the way in which cells were separated from each other. Application of the tensile test following cool storage of low maturity apples resulted in the rupture of individual cells at the fracture surface and the subsequent release of cell contents and collapse of the cell wall. However, when tensile tests were applied to cool-stored more mature fruit, neighbouring cells were pulled apart, leaving undamaged cells at the surface.

De Smedt *et al*. (1998) made microscopic images to visualise the difference between fresh and mealy apples, and to establish the relationships between mechanical tests and microscopic observations for three commercially important Belgian cultivars: Boskoop, Cox's Orange Pippin and Jonagold. In Fig. 9.2(a) and (b) light microscopic images of the tissue of a fresh and a mealy Jonagold apple are shown, respectively. It is clear that the mealy tissue contains more air voids and the cells are only loosely interconnected. For all three cultivars it was found that in fresh apples the cells tend to break when a force is applied, whereas in mealy apples the cells tend to separate instead of break. Using four cell shape parameters (area, perimeter and two roundness parameters), De Smedt *et al*. (1998) applied discriminant and principal component analysis techniques to discriminate between fresh and mealy Cox's Orange Pippin and Boskoop apples but not between fresh and mealy Jonagold apples. This confirms sensory results which indicate that Jonagold apples become less easily mealy than Cox's apples.

9.5 Confined compression test

Mealiness has been defined as a multidimensional parameter combining the lack of crispiness, hardness and juiciness (De Smedt, 2000). Crispiness can be measured through a shear-rupture or tensile test, hardness and juiciness through a confined compression test (Barreiro *et al*., 1998c). In the latter test a sample is compressed in a cylindrical probe and the breaking force and juice area of the spot accumulated in the filter paper underneath the probe are measured. Based on the instrumental parameters of crispiness, hardness and juiciness, Barreiro *et al*. (1998a, 1998c) developed a nine category mealiness scale for Top-Red apples. This scale was evaluated by De Smedt (2000) for different apple cultivars (Golden and Cox's). It was concluded that the scale was only suitable for the classification of apples which were very mealy or not mealy at all. Moreover, since consumers are not able to classify apples into nine categories, De Smedt (2000), constructed a three category classification system (fresh, mid-point and mealy), relating sensory measurements and objective destructive instrumental

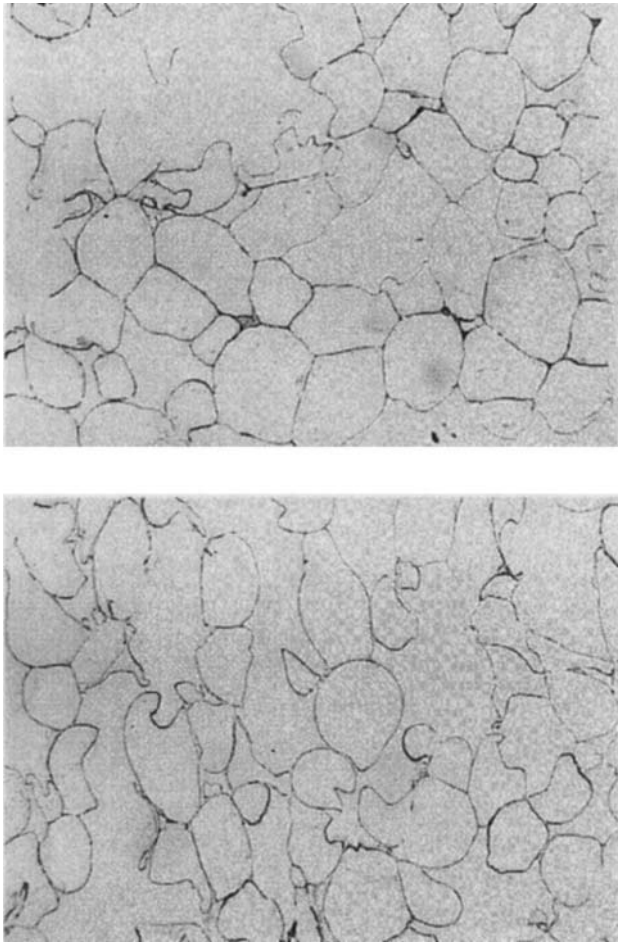

Fig. 9.2 Light microscopic images of the tissue of a fresh (a) and mealy (b) Jonagold apple. The mealy tissue contains more air voids and the cells are only loosely interconnected (source: De Smedt, 2000).

measurements of hardness and juiciness. With this classification system, De Smedt (2000) succeeded in correctly classifying 10 out of 12 batches.

9.6 Ultrasonic wave propagation

Over the last 50 years, ultrasound has been applied frequently in the food industry. Sound waves are produced as a result of the mechanical vibration of molecules and atoms of a material around their equilibrium positions. Once the excitation is removed, the energy stored as ultrasound is dissipated and the molecules return

to their equilibrium position. Any food product with any elasticity can support the propagation of an acoustic wave (Galili *et al*., 1993). De Smedt (2000) evaluated the potential of ultrasonic wave propagation to measure mealiness. Both transmission velocity and attenuation were considered. Although ultrasonic wave propagation is basically a non-destructive technique, the attenuation – even at 50 kHz – was found to be so large that it was necessary to carry out the measurements on fruit samples. De Smedt (2000) used plexiglas adapters to concentrate the wave and obtain a higher input signal. The ultrasound equipment was mounted on a universal testing machine, applying a constant force with the probes to the sample. In this way more consistent attenuation readings were obtained. It was observed experimentally that the sound propagates faster through radial than through longitudinal samples. Measuring the velocity during a storage period of four weeks showed that this characteristic property decreased significantly in time. Although moderate correlation values were found between the velocity and attenuation of the sound and the sensory attributes related to mealiness, De Smedt (2000) concluded that ultrasound cannot be considered as the most appropriate measurement technique for the evaluation of mealiness. The velocity and, to a lesser extent, the amplitude of the ultrasonic waves are a function of the firmness of the fruit, which explains the relationship found between sensory mealiness and the ultrasonic parameters, since mealy apples are in general soft.

9.7 Nuclear magnetic resonance relaxometry and imaging

Nuclear magnetic resonance (NMR) relaxometry and imaging have been evaluated as a technique to measure mealiness. Barreiro and colleagues used magnetic resonance techniques to assess mealiness in apples (Barreiro *et al*., 1999) and peaches (Barreiro *et al*., 1998b). Magnetic resonance techniques rely on the magnetic properties that some atomic nuclei have. When placed in a magnetic field, the natural magnetic dipoles of the nuclei reorient themselves along the magnetic field. After excitation they return to their equilibrium position. The rate at which this happens can be expressed by two relaxation times (T_1 and T_2) and is a function of the texture of the material (Smith and Lange, 1998). Barreiro *et al*. (1999) found that the variability of the T_2 values inside an apple was larger than that between apples. However, a difference between the average T_2 value of fresh apples and that of apples stored in mealiness-enhancing conditions was noticed; minimum T_2 values were shown to be significantly lower for mealy apples than for fresh apples, indicating that a more desegregated structure and a lower juiciness content lead to lower T_2 values. Furthermore, they also found that the T_2 maps of mealy apples showed a regional variation of contrast which was not shown for non-mealy apples. Not all results found for apples were similar to those found for peaches (Barreiro *et al*., 1999; Barreiro *et al*., 2000). This might indicate that the development of mealiness in both fruits is caused by different underlying physiological changes. The magnetic resonance images of mealy apples also showed a regional variation of contrast which was not the case for non-mealy

apples (Barreiro *et al*., 2000). This variation of contrast was similar to the NMR images of apples with internal breakdown although the contrast was smaller.

9.8 Near-infrared reflectance spectroscopy

Near infrared (NIR) spectroscopy has been evaluated in the past as a non-destructive measurement technique for measuring Jonagold apple quality attributes, like soluble solids, pH and firmness (Lammertyn *et al*., 1998). Experiments with NIR reflectance spectroscopy indicated that mealiness could be measured in a non-destructive way (Nicolaï *et al*., 1999). A special fixed sample-presentation module, developed for an existing spectrophotometer (Fig. 9.3) allows rapid acquisition of diffuse reflectance spectra by simply putting the unpeeled apple or another fruit on top of an optical window, supported by self-centring mechanics. However, as reported in Nicolaï *et al*. (1999), the calibration models contain many principal components and need to be improved further for mealiness prediction.

9.9 Aroma, sugar and acid analysis

Because cells of mealy tissue do not break during mastication, the flavour compounds are not liberated. This may explain the fact that the aroma of mealy apples

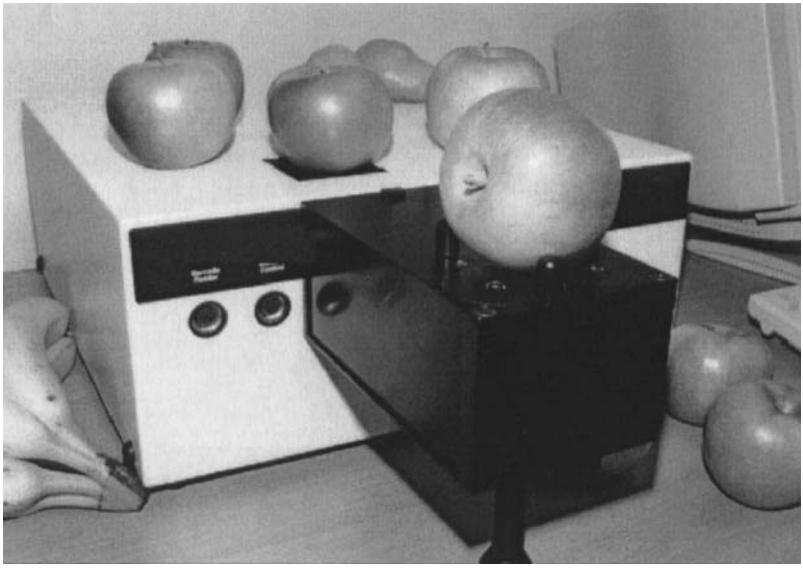

Fig. 9.3 Near infrared (NIR) spectrophotometer with sample presentation accessory.

was perceived by the sensory panels to be at a lower intensity compared to fresh apples. However, gas chromatographic measurements of the headspace when investigating the aroma compounds of Cox and Jonagold apples revealed that the concentration of aroma compounds in the static headspace even increased in mealy apples while the aroma determined by sensory panel clearly decreased. Sugars and organic acids were measured by means of high performance liquid chromatography (HPLC), for both non-mealy and mealy apples. A linear relationship between malic acid content and sensory mealiness was observed (Nicolaï *et al*., 1999).

9.10 Acoustic impulse response technique

The firmness or stiffness of the apples can be measured using the acoustic impulse response technique (Chen and De Baerdemaeker, 1993). The fruit is gently impacted and the response signal is recorded. The frequency spectrum is calculated by means of a fast Fourier transformation. The firmness is then calculated from the mass and the first resonance frequency (Langenakens *et al*., 1997). The apple firmness measured through an acoustic impulse response technique showed a significant correlation with the sensory attributes of juiciness (De Smedt, 2000). The sensory descriptor 'floury' shows a correlation with the sensory attributes dealing with juiciness. This confirms the fact that the floury sensation in the mouth is due to the combination of a loss of texture and juiciness.

De Smedt (2000) established a statistical model between the sensory attributes of crispiness, floury and juiciness assessment at first bite and during chewing, in relation to the readings obtained from the confined compression test and from the acoustic impulse response technique. Reasonable correlation coefficients of 0.85 for juiciness and 0.71 for crispiness were observed. Although she concluded that the statistical models do not allow for continuous prediction of the sensory attributes that define mealiness, the instrumental parameters involved can be used to identify different commercial mealiness stages.

9.11 Electrical impedance

Harker and Maindonald (1994) used electrical impedance measurements to look at the ripening of nectarine fruit. During ripening, the resistance of the cell wall and the vacuole declined and the capacitance of the membranes decreased. A higher resistance of the cell wall was observed in chilling-injured woolly fruits compared to normally ripened fruits. Varlan (1996) performed electrical impedance measurements on apples. She found a very high variance in the measurements. Although it was hard to draw conclusions she noticed some general trends in the electrical impedance parameters during ripening: an increase in low frequency resistance and constant phase angle on the one hand and a decrease in characteristic frequency and high frequency resistance on the other hand. Further

research is needed to find a possible relation between mealiness in apples and the results of electrical impedance measurements.

9.12 Modelling mealiness

To date, qualitative information is available with respect to the development of mealiness in apples as a function of the storage conditions (Harker and Hallett, 1992; De Smedt *et al*., 1998; Andani *et al*., 1999). De Smedt *et al*. (2001) constructed a comprehensive mechanistic model for quantitative prediction purposes. This model describes the changes in the middle lamella, the water transfer through the tissue and their interaction at the cellular level as affected by the relative humidity for both air and low oxygen storage. The model explains the time dependency of the hardness, tensile strength and juiciness of apple tissue. These mechanical parameters have been shown to be directly related to mealiness as perceived by sensory panels (Barreiro *et al*., 1998b; De Smedt, 2000).

Texture properties of apple, such as mealiness, are affected by the mechanical and chemical properties of the cell walls and middle lamellae, by the water status and, in particular, by turgor pressure of the cell. These properties change considerably during post-harvest storage and affect each other. For example, a key transformation in apple is the hydrolysis of pectin which requires water as a substrate. Water is available from inside the cells and is also produced through respiration. De Smedt *et al*. (2001), therefore, decided to include the following general features in the model:

- respiration
- changes of the middle lamella
- transfer of water in the apple
- relationships between fruit texture attributes and the middle lamella and cell turgor.

They assumed that the apple can be considered to be a homogeneous object. The only independent variable left is the time and, therefore, ordinary differential equations are sufficient to define the model structure. They also noted that this model should, hence, be considered as a crude approximation of the reality.

The model is based on a simplification of the histological structure of the apple (Fig. 9.4). The authors assumed that the apple consists of two compartments, the symplast, consisting of the entire network of cytoplasm interconnected by plasmodesmata, and the apoplast, consisting of the cell walls system and the intercellular space (Taiz and Zeiger, 1998). The symplast is separated from the apoplast by a semi-permeable membrane, the plasmalemma. Passive (diffusive) transport of water between both compartments is possible through the plasmalemma. The apoplast can exchange water with the environment via epidermal transfer. The apple skin, with its protective wax layer, is the major barrier to this transfer. The water loss of Cox's apples during a commercial storage period of 6 months at 3°C and 90% RH is typically 5% or greater.

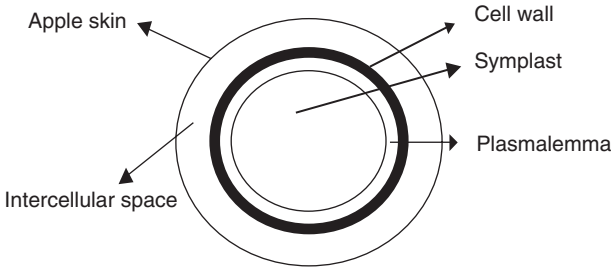

Fig. 9.4 Schematic representation of an apple (reproduced from De Smedt *et al*., 2001 with kind permission of Elsevier Science).

Relative humidity can be considered to be a property of the environment which affects the behaviour of the apple. It is an input variable of the model and is available to the post-harvest technologist to optimise the storage process. In the cell compartment, the respiration process was modelled by two chemical reactions: the hydrolysis of starch into hexose units and water and the oxidation of the hexose units into water and carbon dioxide. In the intercellular space the dissolution of pectins was modelled by a simple hydrolysis reaction.

By specifying mass balances and assuming simple chemical kinetics, De Smedt *et al*. (2001) derived a set of six differential equations that describes the changes in the water concentration in the cells and in the intercellular space, as well as the changes in hexose and starch concentration inside the cells and pectin in the middle lamellae. These state variables were related by simple algebraic relations to measurable quantities such as juiciness, crispiness and compressive hardness together with experimentally obtained values of apples stored under normal air and controlled atmosphere storage conditions.

In Fig. 9.5 the experimental data of the five output variables measured by De Smedt *et al*. (2001) are shown as a function of storage time together with the simulated model values. The symbols represent the averages of 20 measurements. The 95% confidence intervals of the mean are given by vertical bars. By examining Fig. 9.5 it can be seen that the model fits the data very well, although the model slightly underestimates the tensile strength (crispiness) in the case of apples stored in air (Fig. 9.5e). Juiciness and hardness were estimated more adequately (Fig. 9.5c and a). According to the model, the soluble solids for the apples stored under normal air composition kept on increasing after 100 days, while apples stored in CA conditions reached a more or less constant value (Fig. 9.5a). This could not be verified by experimental measurements because the measuring technique did not allow any more juice to be taken once the apples became rather mealy for the normal air storage condition. However, this prediction was plausible because of the concentration effect that can be expected because of the considerable weight loss (Fig. 9.5b). The model fitted the weight loss well.

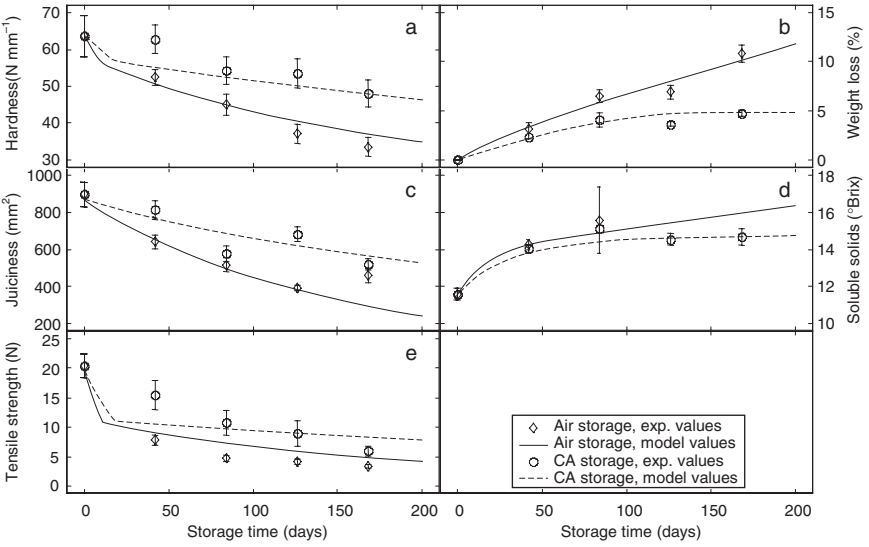

Fig. 9.5 Change in the measured output variables during storage. Error bars denote 95% confidence intervals of the mean of 20 measurements (note, the value of soluble solids after 84 days of air storage is the mean of only three measurements) (reproduced from De Smedt *et al*., 2001 with kind permission of Elsevier Science).

Sensory experiments showed that the apples stored under normal air composition were more mealy than those stored in CA (Andani, 2000). According to this model this can be explained through an accelerated degradation of starch and sugar and a more pronounced dissolution of the middle lamellae.

The model of De Smedt *et al*. (2001) can be used advantageously to evaluate the effect of changes of storage conditions – unintentional or on purpose – and fruit characteristics such as size and maturity on the development of mealiness for cool store management purposes.

9.13 Future trends

As can be deducted from the experimental results presented above, non-destructive instrumental assessment of mealiness is rather difficult at this moment. The best, but very expensive, alternative is the use of sensory panels. To reduce the cost in the future, a relationship should be established between the sensory scores from the panel and the destructive measurements from confined compression or related mechanical destructive tests for various apple cultivars. This has already been done by Barreiro *et al*. (1998a and 1998b) for Starking apples and by De Smedt (2000) for Cox apples.

Further research should also focus on the development of non-destructive instrumental techniques to detect mealiness or related attributes. Since mealiness is situated at the histological level, much can be expected from techniques like

nuclear magnetic resonance relaxometry and imaging, since these techniques allow the microstructure of the tissue to be probed.

The mechanical model of De Smedt *et al*. (2001) discussed above is only a very crude approximation of the reality, since the apple was assumed to be homogeneous. The model parameters were independent of the position in the apple. The model should, therefore, be seen as a starting point for modelling the changes occurring during mealiness development in apples. A future trend in modelling mealiness is certainly situated at the microscopic level. Therefore, the micromechanical properties and the chemical structure of the cell walls, but also the turgor pressure and many other physiological parameters should be measured accurately at the cellular level. A model based on these observations may result in better insight in the physiological processes causing mealiness development.

9.14 Sources of further information and advice

In 1996 a EU project (FAIR CT95-0302) was initiated with the overall objective of enhancing quality of fresh fruit to the consumer by prevention or elimination of mealy products through, respectively, improved treatment and the use of instrumental objective measurement and monitoring techniques. The consortium encompassed five countries and consisted of seven partners, of which there were two universities, one private and three public research institutes and one commercial company. The major part of the results presented in this chapter are collected within the framework of this European project (Nicolaï *et al*., 1999). Apart from peer reviewed scientific articles, conference proceedings and contributions to trade journals, two PhD theses (Andani, 2000; De Smedt, 2000) have been written about these research topics. The reader is referred to this literature to find a detailed description of all topics which have been briefly discussed in this chapter.

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10

Maximising the quality of thermally processed fruits and vegetables

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10.1 Introduction: the development of thermal processing

Thermal processing is one of the conventional preservation methods which assures processed foods to be safe and shelf-stable. The origin of commercial thermal processing dates back to 1809 when the Frenchman Nicholas Appert was awarded a prize by the French government for developing a new and successful means of preserving foods, a method that eventually became known as 'canning'. Appert found a new and effective way to preserve food, but did not understand why it prevented food spoilage. In 1864, Louis Pasteur, another Frenchman, explained that the heating process killed (or inactivated) the microorganisms which limited the shelf-life of foods. This laid the foundation for advances in canning methods that eventually revolutionized the industry. In the 1890s, Prescott and Underwood established the relationship between thermophilic bacteria and the spoilage of canned corn. At about the same time, the same type of spoilage was discovered in canned peas by Russell in Wisconsin and Barlow in Illinois. In the 1910s and 1920s, the basic biological and toxicological characteristics of *Clostridium botulinum* were first determined by several American investigators. The importance of controlling *C. botulinum* in canned foods became clear and the basis for its control was established. Bigelow *et al*. (1920) developed the first scientifically based method for calculating the minimum safe sterilization processes for canned foods. It became known as the 'original' or 'graphical' method. Ball (1923) subsequently developed theoretical methods for the determination of thermal processes. Schultz and Olson (1940) developed a nomographic method for process determinations. Most subsequent developments on the subject have been based on these early concepts. Stumbo (1949) devel-

oped procedures for the calculation of sterilization processes based on integrating lethality values over the entire volume of the container. More advanced mathematical methods which eliminated certain relatively small errors inherent in some of the previous mathematical procedures were developed by Hayakawa (1968). Since about 1970, in addition to Ball, Stumbo and Hayakawa, several researchers have contributed to refining the mathematical models of thermal processing further. These later works (Teixeira *et al*., 1969; Purohit and Stumbo, 1972; Lenz and Lund, 1977; Tung and Garland, 1979, etc.) have led to the use of computers for more accurate, rapid and routine heat process calculations and for monitoring and controlling thermal processes by on-line measurement of accomplished lethality.

10.2 Types of thermal process

Thermal processing of fruits and vegetables can be achieved by a variety of techniques using hot water or steam (cooking, blanching, pasteurization, sterilization, evaporation and extrusion), hot air (drying) and irradiated energy (microwave, infrared radiation and ionising radiation), which are described below.

10.2.1 Cooking

Cooking is a heat-processing technique, the primary objective of which is to improve the palatability of the food. It can be considered to encompass several operations that are commonly carried out in the household: boiling, baking, broiling, roasting, frying and stewing, all of which differ in the method of application of heat. Boiling and stewing are done by placing the product in boiling water (or steam). Baking, broiling and roasting require dry heat and these processes are carried out in hot air ovens to improve and alter the eating quality of foods.

Cooking can be considered to be a preservation technique because many cooked foods can be stored longer under proper refrigerated conditions than their uncooked counterparts, if recontamination can be minimized. Cooking results in the destruction or reduction of microbial load and inactivation of undesirable enzymes, two important requirements of most preservation techniques. It can also inactivate toxins occurring naturally or through microbial contamination (in fresh or processed foods), improve digestibility and alter color, flavor and texture to suit the consumer's need. Again, while imparting these desirable effects, cooking will also result in loss of certain heat-labile nutrients.

10.2.2 Blanching

Blanching is a mild heat treatment used to inactivate the oxidative enzymes in fruits and vegetables prior to further processing (canning, freezing and dehydration), which otherwise will result in undesirable changes in color, flavor and nutritive value of the product during handling and storage. Apart from enzyme

inactivation, blanching also serves several additional functions: it removes the tissue gases (to achieve a better vacuum in cans, reduce the strain on can closures during processing and to create reduced oxygen levels in the can), increases the bulk temperature of the tissue, cleanses the tissue, wilts the tissue to facilitate in packing and, in some instances, assists in improving (fixing) the color of green vegetables.

Of the oxidative enzyme systems, the enzyme peroxidase is considered to be the most heat resistant; therefore, peroxidase inactivation has been traditionally used as an index of blanching adequacy. Steam and hot water blanching are the two most commonly used blanching techniques. These processes are simple and inexpensive but are also energy intensive, result in considerable leaching of soluble components (which occurs both during heating and cooling) and produce large quantities of effluent. The merits and disadvantages of these techniques that are discussed below were summarized by Fellows (2000).

Conventional water blanching has lower capital cost and better energy efficiency than steam blanching but results in larger losses of water-soluble components, including vitamins, minerals and sugars. It also results in larger volumes of effluents and risk contamination by thermophilic bacteria. With steam blanching it is possible to reduce significantly the effluent volume as well as leaching losses if air cooling is adopted instead of water. However, uneven blanching can result if the food is blanched in multilayer piles. The individual quick blanching (IQB) technique (Lazar *et al*., 1971) is an innovation based on a two stage heat-hold principle and has been shown to improve the nutrient retention significantly. Research and engineering efforts led to the development of improved blanching equipment that makes use of steam (saturated or superheated) and recirculating hot water to improve nutrient retention, reduce leaching losses and improve energy efficiency (Cumming *et al*., 1984). Other non-conventional blanching procedures use moisturized hot gas, microwave or ohmic heating techniques generally together with air cooling to minimize leaching. The blanching time (10 s to 15 min) usually depends on the type and size of the fruit or vegetable, the type (water, steam, hot gas or microwave) and temperature of the heating medium, as well as the method of heating.

10.2.3 Pasteurization

Pasteurization is also a mild heat treatment performed on foods to destroy vegetative microorganisms (especially pathogens) and inactivate the enzymes. Because the process is not severe enough to kill the spore formers, pasteurized foods must be stored under conditions of refrigeration to minimize microbial spoilage. Also, because only mild heat treatment is involved, the sensory characteristics and nutritive value of the food are minimally affected. The severity of the heat treatment and the length of storage depends on the nature of the product, pH conditions, the resistance of the test microorganism or enzyme, the sensitivity of the product and the type of application of the heat (Fellows, 2000; Holdsworth, 1997).

10.2.4 Sterilization

Sterilization involves a more severe heat treatment aimed at destroying the pathogens and spoilage-causing microorganisms in a food that is packaged in a hermetically sealed environment to prevent recontamination. The process takes into account the heat resistance of the spore formers in addition to their growth sensitivity to oxygen, pH and temperature. The presence of vacuum in cans prevents the growth of most aerobic microorganisms and if the storage temperature is kept below 25°C, the heat-resistant thermophiles pose little or no problem. From the public health perspective the most important microorganism in low acid (pH > 4.5) foods is *C. botulinum*, a heat-resistant, spore-forming anaerobic pathogen that, if it survives processing, can potentially grow and produce the deadly botulism toxin in foods. Because *C. botulinum* and most spore formers do not grow at pH < 4.5 (acid and medium-acid foods), the thermal processing criterion for these foods is the destruction of heat-resistant vegetative microorganisms or enzymes.

10.3 Principles of thermal processing

Canned foods subjected to thermal processing are not sterile and the processes are not designed to make them sterile. The success of thermal processing does not depend on the complete destruction of all microorganisms which would result in low product quality caused by the long heating required. Instead, all pathogens and most spoilage-causing microorganisms in a hermetically sealed container are destroyed, and an environment is created inside the package that does not support the growth of spoilage-type microorganisms and their spores. Indeed, together with the nature of the food (pH), environment (vacuum), hermetic packaging and storage temperature, the given heat process prevents the growth of microorganisms of spoilage and satisfies public health concerns. Hence, to determine the extent of heat treatment several factors must be known (Fellows, 2000): the type and the heat resistance of the target microorganism, spore or enzyme present in the food; the pH of the food; the storage conditions following the process; the heating conditions and the thermophysical properties of the food and the container shape and size.

10.3.1 Thermal resistance of microorganisms

Oxygen, pH and temperature sensitivity. In foods that are packaged under vacuum in hermetically sealed containers, low oxygen levels are intentionally achieved. Therefore, the prevailing conditions do not support the growth of microorganisms that require oxygen (obligate aerobes) and result in food spoilage or public health problems. Furthermore, the spores of obligate aerobes are less heat resistant than the microbial spores that grow under anaerobic conditions (facultative or obligate anaerobes). The growth and activity of these anaerobic microorganisms are largely pH dependent. From a thermal processing standpoint,

foods are divided into three pH groups: (1) high-acid foods (pH < 3.7), (2) acid or medium-acid foods (3.7 < pH < 4.5), and (3) low-acid foods (pH > 4.5).

The most important distinction in the pH classification, especially with reference to thermal processing, is the dividing line between acid and low-acid foods. It has been generally recognized that *C. botulinum* does not grow and produce toxin below a pH of 4.6. Hence, the dividing pH between the low-acid and acid groups is set at 4.5. In the low-acid foods (pH > 4.5), destruction of *C. botulinum* spores is the primary concern in these processes. However, there may be other microorganisms, for example, *Bacillus stearothermophilus*, *B. thermoacidurans* and *C. thermosaccolyaticum*, that are more heat resistant than *C. botulinum*. These are generally thermophilic in nature (optimal growth temperature around 50–55°C) and hence are of little concern if the processed cans are stored at temperatures below 25°C.

Microbial destruction kinetics. To establish the thermal processing schedule, the thermal destruction rate of the test microorganism must be determined under the conditions that normally prevail in the container so that an appropriate heating time can be determined at a given temperature. Furthermore, because packaged foods cannot be heated to process temperatures instantaneously, data on the temperature dependence of the microbial destruction rate are also needed to integrate the destruction effect through the temperature profile under processing conditions.

Survivor curves and *D*-value. Evidence suggest that the thermal destruction of microorganisms follows a first-order reaction indicating a logarithmic order of death (Fig. 10.1). The microbial destruction rate is defined as a decimal reduction time (*D*-value), which is the heating time in minutes at a given temperature required to result in one decimal reduction (90% destruction) in the surviving microbial population. Graphically, this represents the time range between which the survival curve passes through one logarithmic cycle (Fig. 10.1). Mathematically:

$$D = \frac{(t_2 - t_1)}{[\log(a) - \log(b)]} \qquad [10.1]$$

where a and b represent the survivors following heating for t_1 and t_2 min, respectively.

Thermal death time (TDT) and *D*-value. In food microbiology another term is often employed, thermal death time (TDT), which is the heating time required to cause microbial death or destruction. TDT data are obtained by subjecting microbial population to a series of heat treatments at a given temperature and testing for survivors. TDT represents a time between the shortest destruction and the longest survival times. The death in this instance generally indicates the failure of a given microbial population, after the heat treatment, to show a positive growth in the subculture media. Comparing TDT approach with the decimal reduction approach, it can easily be recognized that TDT value depends on the initial microbial load (while the *D*-value does not).

Temperature dependence and *z*-value. The *D*-value depends strongly on the temperature employed. The temperature sensitivity of *D*-values at various tem-

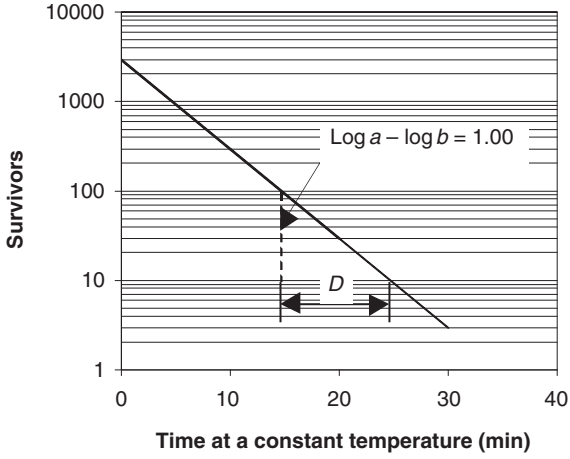

Fig. 10.1 A typical survivor curve.

peratures is normally expressed as a thermal resistance curve with log *D*-values plotted against temperature (Fig. 10.2). The temperature sensitivity indicator is defined as a *z*-value, which represents a temperature range that results in a 10-fold change in *D*-values, or graphically it represents the temperature range through which the *D*-value curve passes through one logarithmic cycle. Mathematically:

$$z = \frac{(T_2 - T_1)}{[\log(D_1) - \log(D_2)]} \qquad [10.2]$$

where D_1 and D_2 are *D*-values at T_1 and T_2, respectively. The *D*-value at any given temperature can be obtained from a modified form of equation [10.2] using a reference *D*-value (D_0) at a reference temperature, T_r, usually 121°C for thermal sterilization):

$$D = D_0 10^{(T_r - T)/z} \qquad [10.3]$$

Lethality concept. Lethality (*F*-value) is a measure of the heat treatment or sterilization processes. To compare the relative sterilizing capacities of heat processes, a unit of lethality needs to be established. For convenience, this is defined as an equivalent heating of 1 min at a reference temperature, which is usually taken to be 121°C for the sterilization processes. Thus the *F*-value would represent a certain multiple or fraction of the *D*-value depending on the type of the microorganism; therefore, a relationship like equation [10.3] also holds good with reference to the *F*-value:

$$F = F_0 10^{(T_r - T)/z} \qquad [10.4]$$

The F_0 in this case will be the *F*-value at the reference temperature (T_r). A

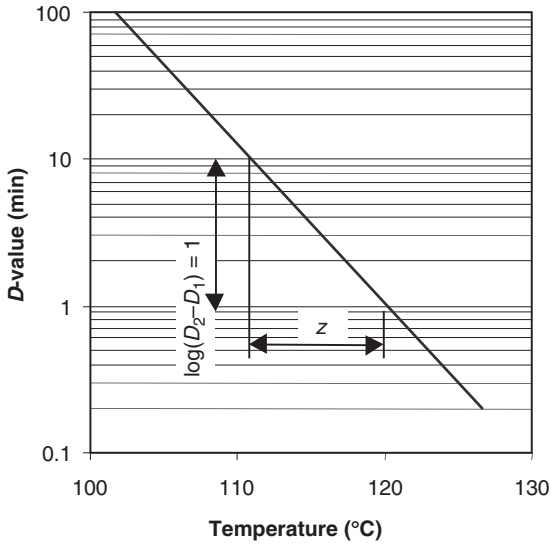

Fig. 10.2 A typical thermal resistance curve.

reference (or phantom) TDT curve is defined as a curve parallel to the real TDT or thermal resistance curve (i.e. having the same z-value) and having a TDT (F-value) of 1 min at 121°C. With a phantom TDT curve so defined, it will be possible to express the lethal effects of any time–temperature combination in terms of equivalent minutes at 121°C or lethality:

$$F_0 = F10^{(T-T_r)/z} \qquad [10.5]$$

For real processes where the food passes through a time–temperature profile, it should be possible to use this concept to integrate the lethal effects through the various time–temperature combinations. The combined lethality so obtained for a process is called process lethality and is also represented by the symbol F_0. Furthermore, with reference to the processing situation, the lethality can be expressed as related to a specific location (normally thermal center) or any other arbitrarily chosen location or integrated over the container. From a microbiological safety point of view, the assurance of minimal lethality at the thermal center is of utmost importance, while from a quality standpoint it is desirable to minimize the overall destruction.

10.3.2 Heat penetration curves

Simple time–temperature curves during heating and cooling by conduction and convection heating are shown in Fig. 10.3. The general and improved general methods of process calculation make use of this type of information. On the other hand, most formula methods make use of heat penetration data obtained from a

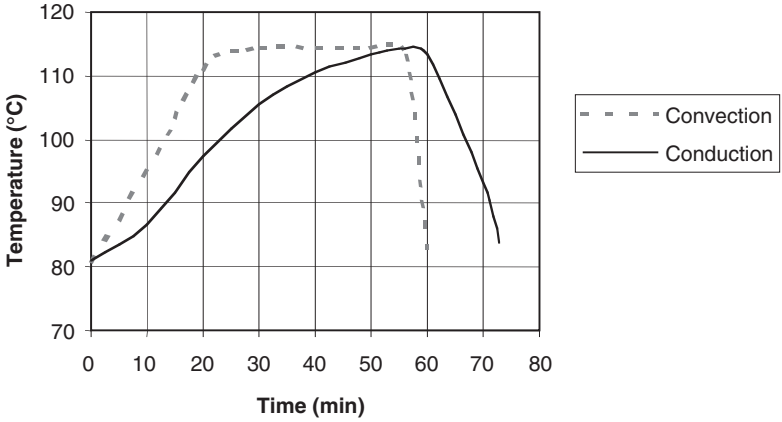

Fig. 10.3 Typical heat penetration.

semilogarithmic plot of the temperature difference ($T_R - T$, on log scale) between the retort (T_R) and the product (T) against time (on linear abscissa) as shown in Fig. 10.4 (Jackson plot). This can be easily accomplished by rotating the semilog paper through 180° and labeling the top line 1°C below the retort temperature, then plotting temperatures directly (Fig. 10.4). The heating rate index (f_h) can be obtained as the negative reciprocal slope of the straight line portion of the curve or time to cross one log cycle. The lag factor j_h is obtained using the following relationship:

$$j_h = (T_R - T_{pih})/(T_R - T_{ih}) \quad [10.6]$$

where T_R is the retort temperature, T_{ih} is the initial product temperature and T_{pih} is the pseudo-initial product temperature.

A similar plot of $T - T_w$, the temperature difference between the product and the cooling water temperature during cooling (Fig. 10.5) is used to obtain the cooling parameters. To plot the cooling curve, the semilog paper is kept in the normal position and the bottom line is marked 1°C above the cooling water temperature and the temperatures are plotted directly. From this plot, the cooling rate index f_c and cooling lag factor j_c can similarly be obtained.

10.4 Thermal process calculations

The purpose of the thermal process calculations is to arrive at an appropriate process time under a given set of heating conditions that will result in a given process lethality, or alternatively to estimate the process lethality of a given process. The method must accurately integrate the lethal effects of the transient temperature response of the food undergoing the thermal processes with respect to test microorganism of both public health and spoilage concern. The desired

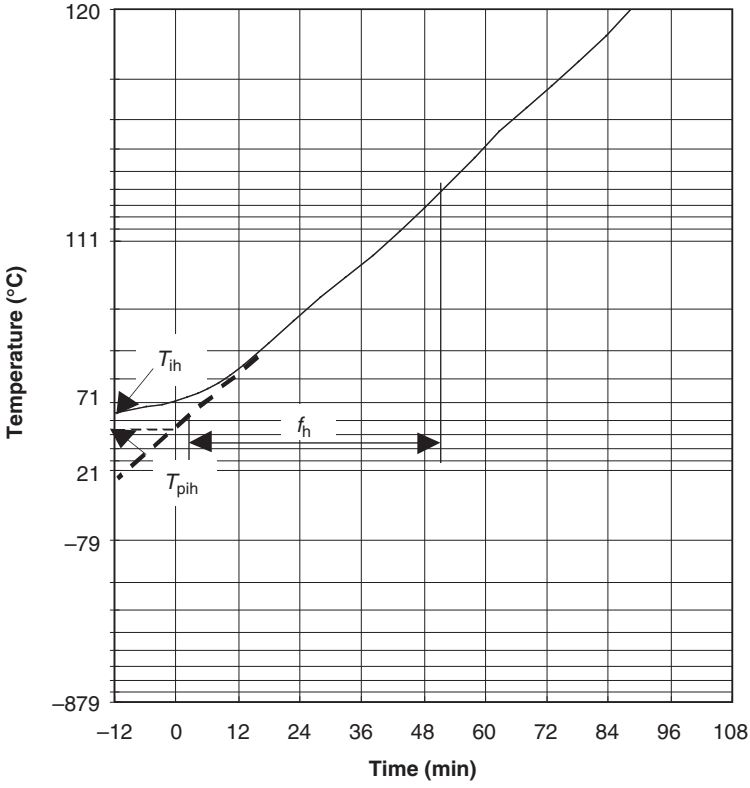

Fig. 10.4 Semilogarithmic plot of heating curves. f_h is the heat rate index (min), T_{ih} is the initial food temperature when heating is started and T_{pih} is the pseudo-initial temperature during heating.

degree of lethality in terms of an equivalent time at a reference temperature (T_r) is generally pre-established and the processes are designed to deliver a minimum of this preset value at the thermal center. The main equation to be solved is the basic integral equation for accumulated lethality value:

$$F = \int_0^t 10^{(T-T_r)/z} dt \qquad [10.7]$$

The process calculation methods are broadly divided into two types: general method and formula methods.

10.4.1 General method

The general method, devised by Bigelow *et al*. (1920), is the simplest and most accurate of all methods, involving graphical or numerical integration of equation [10.7].

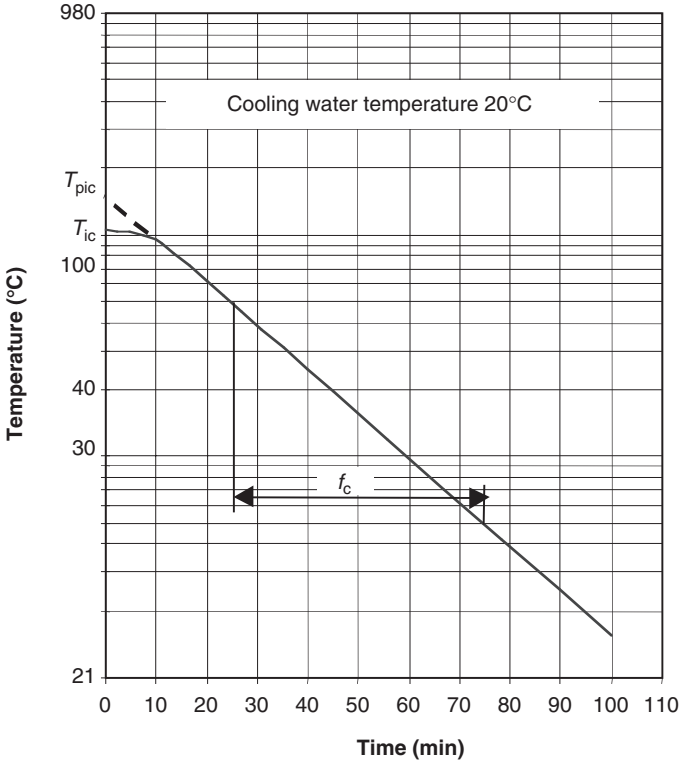

Fig. 10.5 Semilogarithmic plot of cooling curves. f_c is the cooling rate index (min) and T_{ic} is the initial food temperature when cooling is started.

The lethal effects at the different time–temperature combinations in a thermal process are integrated so as to account for the total accumulated lethality, since each temperature is considered to have a sterilizing value. The process time calculation is based on the following formula:

$$\frac{F_t}{F_{T_r}} = 10^{(T_r - T)/z} = \text{TDT} \qquad [10.8]$$

However, to determine the lethal effect at any temperature T, the reciprocal of equation [10.8] is used. The lethality rate (1/TDT) is then used in a graphical integration procedure to compute thermal process times (Lund, 1975). The precision of this method is mainly dependent on how accurate the temperature measurements are, as well as the time intervals for these measurements. This method is known as the improved general method since it is accurate and does not rely on assumptions about the heat penetration, but it is laborious (Stumbo, 1973; Lund, 1975; Fellows, 2000).

10.4.2 Some formula methods

In order to estimate the process time or accumulated lethality under a given processing condition more easily and faster, several formula methods (Ball, Stumbo, Pham etc.) have been developed since the 1920s.

The Ball method is the simplest and most widely used technique for process calculations. It is based on the following equations [10.9] to [10.11] derived from the heat penetration curve to estimate the process time, B (min):

$$B = f_h \log[j_h(T_R - T_i)]/(T_R - T) \quad [10.9]$$

where f_h is the heating index and j_h is the heating lag factor. The temperature difference $(T_R - T)$ at the end of cook is denoted by g_c and is the key to estimating the process time using equation [10.10]. Ball established a relationship between f_h/U and g in the form of a table and a figure, where U was defined as:

$$U = F_0 F_i \quad [10.10]$$

$$F_i = 10^{(121.1 - T_R)/z} \quad [10.11]$$

where F_0 is the desired process lethality and F_i is the number of minutes at the retort temperature equivalent to 1 minute at 121.1°C.

In deriving the above relationship between f_h/U and g, Ball made some assumptions as follows: $f_h = f_c$, $j_c = 1.41$, $z = 10$°C and the cooling curve is initially hyperbolic followed by logarithmic. These assumptions became some limitations to the use of Ball method.

In order to overcome these limitations of Ball method, Stumbo and Longley (1966) published limited tables (z of 12 to 22) of $f_h/U : g$ that accounted for variations in the j value of cooling curves. Relationships for these tables were arrived at manually using a general method of integration. Later, Jen *et al*. (1971) presented representative tables of $f_h/U : g$, the values for which were obtained by the computerized finite difference method of Teixeira *et al*. (1969). Purohit and Stumbo (1972) refined the method of Jen *et al*. (1971) and developed separate tables (57 altogether) covering the z-values from 8°F to 200°F, which make it possible for the Stumbo method to be used for destruction of different microorganisms such as bacterial spores, vegetative cells or nutrients.

Pham (1987) developed two sets of simple algebraic equations and simplified tables for thermal process calculations, one for $U/f_h > 1$ and the other for $U/f_h < 1$. Pham (1987) claimed that his method provides values at least as accurate as Stumbo's and is more versatile because his one table substitutes for the 57 tables published by Stumbo. Pham (1990) amended his equations to cover situations in which the heating and cooling rates differ, i.e. $f_h \neq f_c$.

10.5 Thermal processing and quality

10.5.1 Quality properties of foods

Quality or degree of excellence is a relative term, and as applied to foods it is interpreted as 'those attributes which render the food agreeable to the person who

eats it'. Some quality factors of foods are crucial in determining their safety and acceptability. Some quality loss occurs owing to the processing operations, while additional loss may occur during storage. The shelf-life of a food is the time period up to which a product can be expected to maintain a predetermined level of certain quality factors under specified processing or storage conditions. The quality has been described as a combination of several factors (Kader, 1985): appearance, texture, flavor, nutritive value and safety components. In the discussion below on the quality associated with thermally processed foods, microbiological and enzyme activities, nutritional quality and organoleptic properties are considered.

Microbiological activity

Thermal processes are primarily designed to eliminate or reduce the number of microorganisms to an acceptable level (commercial sterility) and provide conditions that limit the growth of pathogenic and spoilage microorganisms. While pasteurization treatments rely on storage of processed foods under refrigerated conditions for a specified maximum period, sterilization processes are intended to produce shelf-stable products having long storage life. *C. botulinum* is the key organism of public health significance in the sterilization of low-acid foods (pH > 4.5), while other spoilage type microorganisms are important for acid foods.

Enzyme activity

Several enzymes (peroxidase, lipoxygenase and pectinesterase) if not inactivated cause undesirable quality changes in foods during storage even under refrigerated conditions. For thermal processing of acid foods and pasteurization of dairy products, inactivation of heat-resistant enzymes (pectinesterase, phosphatase and peroxidase) is often used as a basis. In conventional thermal processes, most enzymes are inactivated either because the processes are designed using them as indicators or their heat resistance is lower than other indicator microorganisms. Some of these oxidative enzymes have been reported to have a very low temperature sensitivity compared with the microorganisms (Lund, 1975). The characteristic z-value of enzymes is about 30°C compared with l0°C for microorganisms. This means that relative to inactivation at 100°C, if a process temperature of 130°C is used, the inactivation rate of enzyme will increase 10-fold while that of the microorganism increases 1000-fold. Hence, a process successful for both enzyme inactivation and microbial destruction at lower temperature could potentially result in residual enzyme activity if carried out under at elevated temperatures (>130°C). This aspect has caused some concerns in aseptic processes involving particulate matters (Toledo and Chang, 1990).

Nutritional quality

Consumers are becoming increasingly aware of the nutritional content, toxic factors and the microbiological quality of foods when making choices or buying decisions. The general classes of nutrients include carbohydrates, fats, proteins, vitamins and minerals. Nutrients have received wide interest as quality attributes

(Lund, 1982a,b,c; Thompson, 1982), because nutrients are critical for maintenance of health and they can be objectively evaluated by instrumental methods.

Organoleptic properties
Sensory evaluations are used to judge the texture, flavor and appearance of food products. Sensory panels can be composed of either selected judges who detect sensory differences or describe product characteristics, or untrained consumers who give affective responses. Sensory evaluations by trained panelists can assist in evaluating the extent that organoleptic factors are destroyed by thermal processing. Objective methods (instrumental color, flavor and texture measurements) that correlate well with sensory results are now widely employed in measuring and predicting the sensory properties of foods. Textural and rheological properties are also receiving attention because they are found to relate well to the functional properties of food components.

10.5.2 Effect of thermal processing on food quality

As noted before, the application of food-processing techniques that extend the availability of perishable foods also limits the availability of some of the essential nutrients. Maximizing the nutrient retention during thermal processing has been a considerable challenge for the food industry (Lund, 1988). The losses of nutrients as a result of processing have been divided into three categories: intentional, accidental and inevitable (Bender, 1978). Some unwanted parts of the food are intentionally removed, for example, vegetables and fruit are peeled. The accidental or avoidable losses occur as a result of inadequate control and handling of the food materials. The major concern from a food processing point of view is the inevitable losses that represent the loss of heat-labile nutritional elements destroyed to some degree by heat. The extent of these losses depends on the nature of the thermal process (blanching, pasteurization and sterilization), the raw materials and preprocessing preparation, because operations such as size reduction (dicing and slicing) result in increasing losses through increasing the surface-to-volume ratio. All water-soluble vitamins and minerals as well as some parts of soluble proteins and carbohydrates may be susceptible to losses. The major emphasis in food processing operations is to reduce these inevitable losses through the adoption of the proper time–temperature processing conditions as well as appropriate environmental factors (concentration, pH, etc.) in relation to the specific food product and its target essential nutrient.

Effects of blanching
Blanching perhaps represents the least heat severe of the above processes; however, nutrient loss during blanching can occur because of other effects such as leaching. Depending on the method of blanching, commodity and the nutrient concerned, the loss caused by blanching can be up to 40% for minerals and vitamins (especially vitamin C and thiamin), 35% for sugars and 20% for proteins and amino acids (Selman, 1988). In addition to nutrients, the toxic constituents

(e.g. nitrates and cadmium in spinach) naturally present in the vegetable may also be leached and the level of contaminating microorganisms may be reduced, which are advantages gained by blanching. Blanching may result in some undesirable color changes, resulting from the thermal degradation of blue-green chlorophyll pigments to yellow-green pheophytins. These are sensitive to pH and the presence of metal ions. Alkaline pH and chelating agents favor better retention of the green color. Although texture degradation is characteristic of most heat treatments, low-temperature blanching has been shown to improve the texture of some products (carrots, beans, potato, tomato and cauliflower) owing to activation of pectin methyl esterase (Selman, 1988).

Effects of pasteurization

The nutritional and sensory characteristics of most foods are again only slightly affected by the pasteurization process because of its mild heat treatment (Lund, 1988). However, because it is only a temporary method of shelf-life extension, the product quality continues to change (deteriorate) during storage. The shelf-life depends on the post-pasteurization packaging conditions and storage environment. Color changes in fruits and vegetables are mainly caused by the enzyme activity (polyphenoloxidases) and the presence of oxygen. Deaeration prior to pasteurization excludes oxygen, and the heat treatment inactivates the enzyme to minimize color deterioration of fruits and vegetables.

Effects of sterilization

As discussed earlier, sterilization processes are more severe with respect to the heat treatment given generally to achieve commercial stability. Obviously, these products will be subjected to a greater nutrient loss. The following nutrients are more sensitive to destruction by heat: vitamins A, B_6, B_2, B_1, C, D, E, folic acid, inositol and pantothinic acid and the amino acids lysine and threonine. Because of the possibility of using numerous (infinite) time–temperature combinations to achieve thermal sterilization, the influence of the process cannot be easily quantified.

The following ranges for the thermal degradation of nutrients in the canning process have been given (Lund, 1982 a, b and c): vitamin C 33–90%, thiamin (B_1) 16–83%, riboflavin (B_2) 25–67%, niacin 0–75%, folacin 35–84%, pantothinic acid 30–85%, vitamin B_6 0–91%, biotin 0–78% and vitamin A 0–84%. The severity of the heat treatment is determined by the pH of the food (low-acid foods require more heat treatment time to ensure the destruction of *C. botulinum*); the composition of the food (protein, fats and high concentration of sucrose increase the heat resistance of microorganisms); the heating behavior of the food (conduction and convection); the nature, size and shape of the container; and the nature and mode of application of the heating medium. Container agitation and aseptic processing offer additional variables.

Texture is one of the most important quality determinants in fruit and vegetable foods. When fruits and vegetables, for example, lose their texture, that is, become soggy, mealy, chewy or fibrous, they will be rejected by consumers (Ball,

1923). Textural changes in canned meats are caused by coagulation and loss of water-holding capacity of proteins, which produces shrinkage and stiffening of muscles. The texture of fruits and vegetables is usually softer than the unprocessed product because of the solubility of pectic material and the loss of cell turgor (Fellows, 2000).

The losses of vitamins caused by heat depend on (1) the differences in the types of foods, (2) the presence of residual oxygen in the container, and (3) the methods of preparation (peeling, dicing and slicing) or blanching. Retention of vitamin C and flavor are the most important quality factors in fruit juices (Sizer *et al*, 1988); therefore, it is critical that the juices are processed, packed and stored under conditions that maximize the retention of these quality factors. Deaeration of the juice and packaging in an inert environment are essential to exclude the damaging effects of oxygen on the retention of vitamin C, color and flavor.

10.5.3 Kinetics of quality destruction

To accomplish the primary objectives of thermal processing (optimize the retention of quality factors while providing a risk-free food) it is necessary to obtain quantitative data on the thermal degradation of microorganisms, enzymes and quality factors. There have been numerous studies on the degradation kinetics of these components, which have been summarized in several articles (Lund, 1975; Tragardh and Paulsson, 1985; Villota and Hawkes, 1986; Fellows, 2000). Generally, the loss of nutritional value and quality has been found to fit zero or first-order reaction kinetics (Labuza, 1982; Labuza and Riboh, 1983):

$$-(\mathrm{d}C/\mathrm{d}t) = k\mathbf{C}^n \qquad [10.12]$$

where C is the concentration of the desired quality attribute, t is the time, k is the reaction rate constant and n is the reaction order. Assuming a first-order reaction rate, the decimal reduction time D can be found to be reciprocally related to k ($D = 2.303/k$). With the exception of photochemical reactions and some physical reactions, the rate constant of a reaction is strongly dependent on the temperature. The relationship between rate constant and temperature is usually modeled either by the Arrhenius equation:

$$k = k_0 \mathrm{e}^{-E_a/RT} \qquad [10.13]$$

where k is the reaction rate constant at T, k_0 is the frequency factor, E_a is the activation energy, R is the gas constant and T is the absolute temperature, or by the TDT concept:

$$D = D_0 10^{(T_0 - T)/z} \qquad [10.14]$$

where D is the decimal reduction time at T, D_0 is the D-value at a reference T_0 (usually 121.1°C) and z is the temperature range required to change D by a factor of 10.

Caution must be exercised when applying these concepts to foods because of the heterogeneous nature of foods and the phase changes (solid fats chang-

ing to liquid phase) as temperature rises (Labuza, 1982). Moreover, this expression is considered to be an oversimplification because it results in unrealistic reaction rates in many cases, especially in the underestimation of protein denaturation.

Assuming that N_0 and N are microbial counts, C_0 and C are concentrations of a test nutrient before and after processing, respectively; D_{n0}, D_n, D_{c0} and D_c are decimal reduction times for the microorganism (subscript n) and nutrient (subscript c), respectively and the reference temperature is T_0 and process temperature is T, the relative destruction of nutrients with respect to microorganisms can be found using the following equation [10.15] from Ramaswamy and Abdelrahim (1991):

$$\log(C/C_0) = (D_{n0}/D_{c0})\log(N/N_0)10^{(T_0-T)(1/zn-1/zc)} \qquad [10.15]$$

An alternative equation with similar notations, a reaction rate constant k, an activation energy E_a and absolute temperature T, using the Arrhenius approach can be written as:

$$\ln(C/C_0) = (k_{c0}/k_{n0})\ln(N/N_0)e^{(1/T_0-1/T)(E_{ac}-E_{an})/R} \qquad [10.16]$$

Equations [10.15] and [10.16] can easily be used to calculate, at any given processing temperature, the extent of degradation of any nutrient relative to that of a microbial population, provided the kinetic data for both are known. Employing a bot cook (12D for *C. botulinum*, D_{n0} = 0.21 min, Z_n = 10°C) approach, the thiamin retention (D_{c0} = 160 min, Z_c = 25°C) can be calculated to be 51.2% at l00°C compared to 99.5% at 135°C (equation [10.16]), implying better retention of nutrients at elevated temperatures. Similar results are obtained using the Arrhenius approach (k_{n0} = 11 min^{-1}; k_{c0} = 0.0144 min^{-1}; E_{an} = 70 kcal mol^{-1}; E_{ac} = 29.4 kcal mol^{-1}). Although the two approaches (TDT and Arrhenius) have been assumed to be reconcilable over small temperature ranges (Lund, 1975), caution must be exercised in extrapolating the results, especially while using parameters converted from one system to the other. A large discrepancy may result if parameters obtained at lower temperatures are employed under UHT conditions (Ramaswamy *et al*., 1989).

10.6 Principles for optimizing thermal processes

10.6.1 Principles of optimization for food thermal processing

The kinetic parameters (D and z) of microorganisms, enzymes and quality factors of foods are different and this fact is exploited to optimize thermal processes for the elimination of the microbial hazards and retention of nutritional and sensory qualities of foods. An optimal thermal process may be defined as the minimum treatment required to achieve commercial sterility because heating cost and product quality losses increase if the process time is prolonged. The procedure of optimization can be summarized in the following four steps:

1 objective functions and decision variables
2 mathematical models
3 constraints
4 searching techniques.

For thermal processing of foods, the objectives for optimization include maximum average quality retention, surface quality retention and minimum process time that meets the required lethality value at the can center or the coldest spot in the can. The factors affecting these optimal objectives are numerous including food thermal properties, can size and shape, retort temperature, kinetic parameters of quality factors (D and z), desired lethality value and so on, but the decision variables that can be optimized for a given packaged food are usually only retort temperature for the constant retort temperature (CRT) processing or retort temperature profile for variable retort temperature (VRT) processing. This means that the optimization of CRT thermal processing belongs to a single variable optimization while that of VRT thermal processing belongs to a multiple variable optimization since the VRT functions usually have more than two parameters. The second step is to develop mathematical models describing the relationships between decision variable(s) and objectives. Constraints are necessary for some optimization problems, which can be a range of decision variables such as retort temperature or (and) additional objectives. For example, to obtain the maximum quality retention, the desired lethality value and (or) the maximum process time can be used as constraints in searching for the optimal retort temperature. The use of searching techniques for optimization is to assure that the process of optimization is efficient and robust. Different searching techniques are available for the optimization of thermal processing, the details of some of which are given in the following section.

10.6.2 Optimization models

One of the earliest mathematical treatments of the optimization process for thiamin destruction versus sterilization, in cylindrical cans of conduction-heating product, was proposed by Teixeira *et al*. (1969). A finite difference method was used to determine the temperature distribution and the corresponding thiamin destruction, employing first-order degradation kinetics. In the same year, Hayakawa (1969) extended the concept by using a different mathematical technique involving dimensional analysis and the concept of a mass-average sterilizing value. This was subsequently extended (Hayakawa, 1971) to estimate the mass average value for a physical, chemical or biological change resulting from thermal processing. This work led to formulae which could be used to compute values for nutrient retention which were then intended to be used with standard manual procedures.

Barreiro-Mendez *et al*. (1977) derived models for the loss of nutrients during heating and cooling in cylindrical containers using analytical equations. These equations gave the percentage nutrient retention and experimental results

obtained using an analogue system of 6% maize starch and 1.75% carboxymethyl cellulose and these were in good agreement with the predicted results.

Hayakawa (1977) used a computer model to estimate the percentage of thiamin retention in carrot puree, pea puree, pork puree and spinach, and compared the results with experimental determinations. For processes at 115.6°C the results were within ±3%; however, at the higher temperature of 121.1°C for 60 min, the differences varied between 10 and 16%. Spinach gave the worst comparative results, the predictions being up to 16% less than the experimental results.

Lenz and Lund (1977) used a method of lethality calculation which made use of a new dimensionless group, the lethality/Fourier number L where:

$$L = -\frac{\alpha \ln x}{k_r a^2} \qquad [10.17]$$

where α is the thermal diffusivity, x is the fraction of constituent retained (ratio of concentration at any time t to the initial concentration), k_r is the rate constant at the reference temperature T_r and a is the container radius (i.e. half thickness). This was derived by combining the first-order kinetic equation and the Arrhenius temperature relationship and substituting the time from the Fourier number $\alpha t/a^2$. The latter is obtained from the unsteady state heat transfer equation solution for a finite cylinder and cooling is included by solving the equation for the appropriate boundary conditions at the end of heating.

Thijssen and Kochen (1980) developed a method of process calculation which eliminated the use of tabulated data and interpolation. The model used was based on the following equation [10.18]:

$$\frac{C}{C_0} = \int_0^v \exp\left[-\int_0^t k\,\mathrm{d}t\right]\mathrm{d}v \qquad [10.18]$$

where C is the concentration of a specified component at time t, C_0 is the concentration of the specified component at time 0, V is the volume of the pack for averaging purposes and k is a temperature-dependent kinetic factor. For a uniform initial product temperature T_0, a constant temperature of the heating medium, T_h, and a constant temperature of the cooling medium, T_c, the reduction in a heat-labile component is a function of five dimensionless groups: Fourier number, Biot number, residual temperature ratio and two groups related to kinetic factors. The method used the analytical solutions for the heat transfer equation for sphere, cylinder and rectangular bodies, and also other geometrical shapes.

Castillo *et al*. (1980) extended the method of Barreiro-Mendez *et al*. (1977) to deal with rectangular retortable pouches of food. The interesting point which emerges from the use of this model is that the predicted and experimental temperatures at the end of heating were in good agreement. However at the end of cooling differences of up to 16% were observed, probably owing to the assumption of a very high heat transfer coefficient at the surface of the pouch. The predicted thiamin retention after the thermal processing was in good agreement with the experimental results.

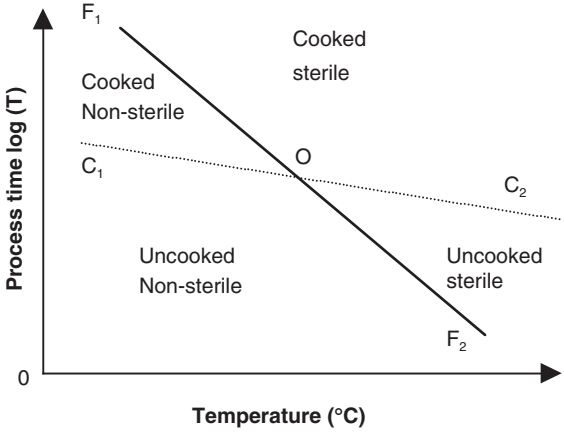

Fig. 10.6 Diagram of *t–T* relationship for microbial destruction, F, and cooking, C.

10.6.3 Searching techniques

Graphical approach

The choice of processing conditions may be determined from a plot of log time versus temperature (Holdsworth, 1997), on which are drawn two straight lines representing constant lethality (F) and cook (C) values, as illustrated in Fig. 10.6. These lines divide the plot into four regions: the line F_1OF_2 marks the boundary between processes which give adequate sterilization and those which do not, while C_1OC_2 marks the boundary between adequate and inadequate cooking. Idealized graphs like this are useful for determining the suitability of various combinations of temperature and time. It should be noted that the graphs are based on instantaneous heating followed by instantaneous cooling of the product, and in particular to thin films of product. Under more realistic conditions it is necessary to include the effects of heat transfer and dimensions of the object being processed. When this is done the straight lines in Fig. 10.6 become curved, and the regions have different boundaries.

Mathematical techniques

According to the type of objective function, optimization problems can be divided into two categories: linear optimization and non-linear optimization problems. Linear programming is a useful tool to deal with the linear optimization problems. Although this technique is considered to be of a limited value because of its assumptions of linearity and infinite divisibility, the technique is very flexible. Moreover, linear programming has the ability to deal with large numbers of constraints in an efficient way, so this technique is very useful for analyzing and optimizing large systems (Saguy, 1983). Many searching techniques have been developed for non-linearity problems including single and multiple variable searching techniques. The former includes the grid search, Fibonacci technique,

golden section method, quadratic techniques and Powell method for the objective function with only one decision variable, while the latter includes alternating variable search techniques, Pattern search, Powell's search, evolutionary operation and response surface analysis, and gradient methods (Saguy, 1983).

Saguy and Karel (1979) used an elegant multi-iterative mathematical technique to optimize thiamin retention in pea puree in a 303×406 can and pork puree in a 401×411 can. The method produced a variable temperature heating profile which optimized the nutrient retention. A constant heating temperature regime was shown to be almost as good as the theoretically derived profile.

Hildenbrand (1980) developed a two-part approach to solving the problem of optimal temperature control. In the first part, the unsteady-state equation for heat transfer into a finite cylinder was solved using Green's functions. In the second part, a method to ensure that the container received the calculated temperature profile was determined. While the approach seems interesting, no further development appears to have taken place. Nardkarni and Hatton (1985) examined the previous work and considered that the methods were not sufficiently rigorous to obtain the best optimization results. These workers used the minimum principle of optimal control theory to obtain optimal solutions. Again simple heating and cooling profiles were better than complex heating profiles.

Banga *et al*. (1991) developed an optimization algorithm, integrated control random search (ICRS), for three objective functions: maximum overall nutrient retention, maximum retention of a quality factor at the surface of the food and the minimum process time. They concluded that the use of a variable temperature profile was advantageous for preserving optimum surface quality.

Artificial intelligence techniques for modeling and optimization

With the rapid development of computer technology and software, artificial intelligence technologies such as artificial neural networks (ANNs) and genetic algorithms have been found to have certain advantages over conventional methods in dealing with system modeling and optimization problems especially for those situations involving non-linear and complicated mathematical approaches.

The neural network is a collection of interconnecting computational elements which mimics neurons in biological systems. It has the capability of relating the input and output parameters without any prior knowledge of the relationship between them. Genetic algorithms (GAs) are a combinatorial optimization technique. They search for an optimal value of a complex objective function by simulation of the biological evolutionary process, based on crossover and mutation as in genetics. An optimal value can be searched for in parallel with a multipoint search procedure. In addition, GAs can use ANN models as that guiding function. This makes it possible to develop a comprehensive optimal control technique using both ANN and GAs.

ANN and GA as individual functions have been widely applied for different areas but as a combination procedure have only been reported in recent years. Chen (2001) evaluated the application of both ANN and GA for modeling and optimization of thermal processing including constant and variable retort thermal

processing. The results showed that it was reliable to use an ANN model for modeling of thermal processing and use a GA–ANN based optimization method for optimization of thermal processing.

10.7 Future trends

It is clear that the conventional thermal methods can lead to desirable destruction of microbial pathogens and spoilage organisms and endogenous, desirable changes such as protein coagulation, starch swelling, textural softening and formation of aroma components. However, undesirable changes also occur, such as loss of vitamins and minerals, formation of thermal reaction components of biopolymers, and in minimal processing terms, loss of fresh appearance, flavor and texture. The classical approach to overcome or at least minimize these undesirable quality changes in thermal processing is the HTST (high temperature short time) or the UHT (ultra high temperature) concept. These are based on the fact that normally the inactivation of microorganisms has more temperature sensitivity than that of quality factors. High temperature will give rapid inactivation of microorganisms and enzymes, which is aimed for in pasteurization or sterilization, and short times will give less undesired quality changes. Unfortunately, the HTST and UHT concepts are severely limited for solid foods. This is because parts of the food in contact with the hot surfaces will be overheated during the time needed for the heat to transfer to the interior or coldest spot of the food. The surface overheating will give quality losses that in severe cases will counterbalance the advantages of the HTST or UHT concept. Thus, as alternative methods, novel thermal processing techniques, such as ohmic heating, high frequency heating and microwave heating, and non-thermal processing techniques, such as high hydrostatic pressure processing and pulsed electricity method, have been receiving more and more interest from both food scientists and industries.

10.7.1 Novel thermal processing techniques

Ohmic heating

Ohmic heating, also called electric resistance heating, is a direct heating method in which the food itself is a conductor of electricity, taken from the mains that are 50 Hz in Europe and 60 Hz in the USA. The food may also be immersed in a conducting liquid, normally a weak salt solution of similar conductivity to the food. Heating is accomplished according to Ohm's law, where the conductivity, or the inverse, the resistivity, of the food will determine the current that will flow between the ground and the electrode. Normally, high voltages up to 5000 V are applied. The conductivity of foods increases considerably with increasing temperature. To reach high temperatures it is therefore necessary to increase the voltage current or to use longer distances between the electrodes and ground. The best known electric resistance heating system is the APV ohmic heating column,

where electrodes immersed into the food are transported in a vertical concentric tube. The ohmic system of APV has been installed for pasteurization and sterilization of a number of food products with excellent resulting quality. The majority of these installations are found in Japan for the production of fruit products (Tempest, 1996). Other industrial cooking operations for electric resistance heating involve rapid cooking of potatoes and vegetables for blanching in the industry and for preparing foods in institutional kitchens. One of the major problems with these applications is ensuring that the electrode materials are inert and do not release metal ions into the conducting solutions and eventually into foods.

High frequency heating

High frequency heating is done in the megahertz region of the electromagnetic spectrum. Frequencies of 13.56 and 27.12 MHz are set aside for industrial heating applications. Foods are heated by transmitting electromagnetic energy through the food placed between an electrode and the ground. The high frequency energy used will allow transfer of energy over air gaps and through non-conducting packaging materials. To achieve sufficiently rapid heating in foods, high electric field intensities are needed.

High frequency heating is accomplished by a combination of dipole heating, when the water dipole tries to align itself with the alternating electric field, and electric resistance heating from the movement of the dissolved ions of the foods. In the lower temperature range, including temperatures below the freezing point of foods, dielectric heating is important, whereas for elevated temperatures, electric conductivity heating dominates. The conductivity losses or the dielectric loss factor increases with increasing temperature, which may lead to problems of runaway heating when already hot parts of the food will absorb a majority of the supplied energy. The dielectric properties of foods are reasonably abundant in the low temperature range, but few data are available in temperatures above normal room temperature.

The largest application in the food industry for high frequency heating is in the finish drying or post-baking of biscuits and other cereal products. Another application is in drying products such as expanded cereals and potato strips. Previously, defrosting of frozen food using high frequency was a major application, but problems of uniformity with foods of mixed composition limited the actual use. The interest in high frequency defrosting has increased again in the last few years.

Microwave heating

Microwaves used in the food industry for heating are of the ISM (industrial, scientific and medical) frequencies 2450 MHz or 900 MHz, corresponding to 12 or 34 cm in wavelength. In this frequency range, the dielectric heating mechanism dominates up to moderate temperatures. Polar molecules, dominated by water, try to align themselves to the rapidly changing direction of the electric field. This alignment requires energy that is taken from the electric field. When the field

changes direction, the molecule 'relaxes' and the energy previously absorbed is dissipated to the surroundings, that is, directly inside the food. This means that the water content of the food is an important factor for the microwave heating performance of foods. The penetration ability of the microwaves in foods is limited. For normal 'wet' foods the penetration depth from one side is about 1–2 cm at 2450 MHz. At higher temperatures, the electric resistance heating from the dissolved ions will also play a role in the heating mechanisms, normally further reducing the penetration depth of the microwave energy. The limited penetration depth of microwaves implies that the distribution of energy within the food can vary. The control of the heating uniformity of microwave heating is difficult, as the objects to be heated are of the same size as the wavelength in the material. Difficulties in controlling heating uniformity must be seen as the major limitation for industrial application of microwave heating. Thus, an important requirement of microwave equipment and microwave energy application in the food industry is the ability to control the heating uniformity properly (Ohlsson, 1983).

Industrial applications of microwave heating are found for most heat treatment operations in the food processing industries. For many years the largest application has been defrosting or thawing of frozen foods, such as blocks of meat, prior to further processing. Often meat is only partially defrosted (tempered) before it can be further processed. Another large application area is for pasteurization, and now also sterilization, of packaged foods. Primarily ready-made foods are processed. The objective of these operations is to pasteurize the food to temperatures in the range of 75–80°C, in order to prolong the shelf-life to about 3 to 4 weeks. Sterilization using microwaves has been investigated for many years, but commercial introduction has only come in the last few years in Europe and Japan. Microwave pasteurization and sterilization promise to give very quick heat processing, which should lead to small quality changes caused by thermal treatment, according to the HTST principle. However, very high requirements of heating uniformity must be met in order to fulfill these quality advantages (Ohlsson, 1991).

Pasteurization by microwave heating can also be done for pumpable foods. Microwaves are directed to the tube where the food is transported and heating is accomplished directly across the tube cross section. Again, uniformity of heating must be ensured, requiring selection of the correct dimensions of the tube diameter and the proper design of the applicators (Ohlsson, 1990). The destruction kinetics of some microorganisms such as *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Escherichia coli*, as well as inactivation of enzymes under continuous microwave heating, have been reported (Tajchakavit and Ramaswamy, 1997; Tajchakavit *et al.*, 1999; Koutchma and Ramaswamy, 2000).

Further application of microwave heating is for drying in combination with conventional hot-air drying. Often microwaves are primarily used for moving water from the wet interior of solid food pieces to the surface, relying on the preferential heating of water by microwaves. Applications can be found for pasta, vegetables and various cereal products, where puffing by rapid expansion of the

interior of the food matrix can also be accomplished using microwave energy (Tempest, 1996).

10.7.2 Non-thermal processing techniques

New non-thermal processes, such as pulsed electric field (PEF) and high pressure (HP) preservation, have been applied to a variety of prototype food products. These processes are best categorized as pasteurization processes because they are not completely effective in reducing the activity of bacterial spores. Treated and properly packaged foods may have extended refrigerated shelf-life or may be shelf-stable if natural or added acids are present to control spore outgrowth.

High pressure processing (HPP) is gaining in popularity within the food industry because of its capacity to inactivate pathogenic microorganisms with minimal heat treatment, resulting in the almost complete retention of nutritional and sensory characteristics of fresh food without sacrificing shelf-life. Other advantages of HPP over traditional thermal processing include: reduced process times, minimal heat damage problems, retention of freshness, flavor, texture and color, no vitamin C loss, no undesirable changes in food during pressure-shift freezing caused by reduced crystal size and multiple ice phase forms and minimal undesirable functionality alterations.

Changes that may be made improve functional properties of food constituents resulting in value-added products. Minimization of damage during pressure-shift freezing and thawing using HPP, non-thermally induced enzyme inactivation and desirable changes in starch–gelatinization properties are some other examples of potential benefits of HPP. However, spore inactivation is a major challenge for HPP. Methods used to achieve full inactivation of spores using HPP are yet to be developed. In thermal processing, D (time required in minutes to reduce the microbial population 10-fold), z (temperature in °C yielding a 10-fold change in D) and F_0 (the integrated lethal value from all heat received by a treated food with a reference temperature of 121.1°C, assuming a z-value of 10°C) values are standard processing parameters; however, there is a need to develop and standardize HPP process parameters with respect to microbial inactivation, because none exists. This is essential for the commercial success of this technology.

Use of pulsed electric fields (PEF) for inactivation of microorganisms is another promising non-thermal processing method. Inactivation of microorganisms exposed to high-voltage PEF is related to the electromechanical instability of the cell membrane. Electric field strength and treatment time are the two most important factors involved in PEF processing. Encouraging results have been reported at the laboratory level, but scaling up to the industrial level escalates the cost of the command charging power supply and of the high-speed electrical switch. A successful continuous PEF processing system for industrial applications has yet to be designed. The high initial cost of setting up the PEF processing system is the major obstacle confronting those who would encourage the system's industrial application. Innovative developments in high-voltage pulse

technology will reduce the cost of pulse generation and will make PEF processing competitive with thermal processing methods (Jeyamkondan *et al*., 1999).

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11

Safety of cooked chilled foods containing vegetables

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11.1 Introduction

Cooked chilled foods also known as REPFEDs (Refrigerated processed foods of extended durabilities) are becoming increasingly popular for retail and catering in developed countries. Consumers enjoy these foods for convenience, taste, variety of recipes and concept of freshness. Production and sales have shown a marked and continuous increase (Hauben, 1999). For instance, the annual turnover of the cooked chilled food sector in France increased twofold between 1990 and 1994, and in 2000 the UK retail prepared chilled food market represented about £5400 million with a market value increase of 19% between 1999 and 2001 (Falconnet and Litman, 1996; Chilled Food Association, 2001). Cooked chilled foods include a wide range of foods. High organoleptic quality is obtained through mild processing and limited use of additives and preservatives. Cooked chilled foods are non-sterile by design and harbour specific microflora. For safety and stability these foods are kept refrigerated during their shelf-life. The shelf-life of cooked chilled foods is highly variable, but is generally extended when compared to that of fresh unprocessed foods or in the catering industry, and always markedly shorter than that of canned, frozen or dehydrated traditional foods. Growth of some bacterial pathogens is possible at temperature as low as 3°C and needs to be controlled to ensure the safety of the consumer. National and international regulations and recommendations have followed the development of the market. Cooked chilled foods have had up to the time of writing an excellent safety record. However, in many cases, the safety margin is unclear and it remains to be established how far the risk caused by microbial hazards may increase in an expanding market.

The aim of this chapter is to give the main technological and microbiological characteristics of cooked chilled foods and the consequences of these characteristics for food safety and to provide some basic principles of control of microbial hazards in cooked chilled foods, focusing on vegetable-based products.

11.2 The manufacturing process: physical and chemical characteristics

The manufacture of cooked chilled foods follows many different processes, which includes cooking at one or several stages of processing (Fig. 11.1). The time needed to cook vegetables, that is, to achieve the optimal texture, ranges from 4 to 13 min for root vegetables, or ranges for potato from 35 to 50 min at 90°C and from 4 to 12 min at 100°C (Harada *et al*., 1985; Harada and Paulus, 1987). Products may receive an additional heat-treatment in the final packaging after vegetable cooking at a temperature sometimes lower than 70°C, and rarely higher than 100°C. This is quite common in the manufacture of *sous vide* foods and may therefore be compared to a 'pasteurisation' treatment.

Cooked chilled foods are mostly prepared without the additives and the preservatives used in traditional food processing. The pH and the water activity (a_w) of the foods are therefore close to those of raw material. pH is between 5.0 and 7.0,

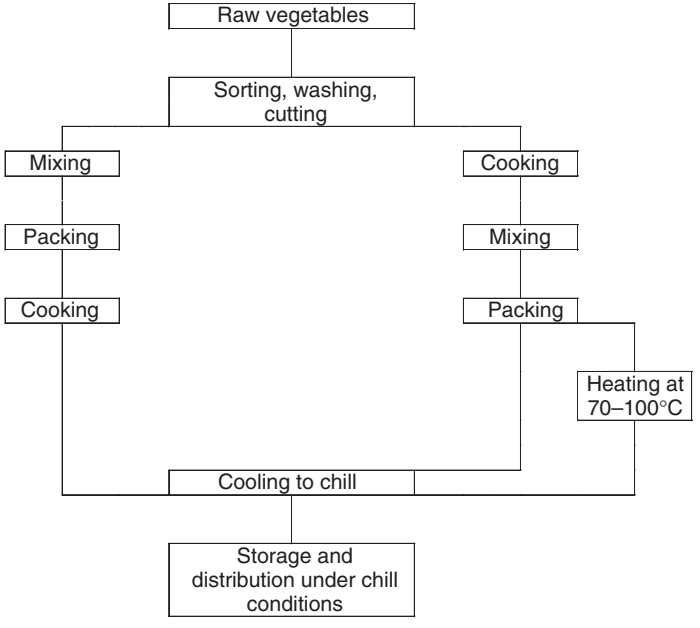

Fig. 11.1 Flow diagram describing the main processing operations in the manufacture of cooked chilled foods made from vegetables.

and mainly between 6.0 and 7.0 and the only exceptions are fruit such as tomato (Lund, 1992). The a_w is high (>0.95, often >0.98) because of high moisture content.

Cooked chilled foods are commonly packaged under low oxygen (O_2) atmospheres obtained by exclusion of air (and then O_2) as in vacuum-packaging or in a oxygen-free modified atmosphere (usually a mixture of CO_2 and N_2) used to prevent either enzymatic oxidation of food material and organoleptic deterioration or microbial growth and spoilage. These contribute to creating conditions close to anaerobiosis, which is favourable to microaerophilic and strict anaerobic microorganisms. In addition, the redox potential of cooked chilled foods, vegetables or processed vegetables may be quite low, that is between −400 and 0 mV (Montville and Conway, 1982; Adams and Moss, 1995; Snyder, 1996), which also creates conditions favourable for these bacteria.

Cooked chilled foods are sold under refrigeration at temperatures mostly lower than 10°C. The shelf-lives of cooked chilled foods are very variable. They range from one week to three months and are commonly close to one month. This depends on product, process and also on national regulations or chill chain conditions.

11.3 Microflora of cooked chilled foods containing vegetables

Vegetables harbour a wide range of microbial species including pathogens. Natural contamination of vegetables with pathogenic bacteria regularly causes outbreaks of food poisoning (Lund, 1992; Beuchat, 1996; Nguyen-the and Carlin, 2000). The heat treatment received during cooking (i.e. a few minutes at temperatures higher than 90°C) will eliminate bacteria, yeasts and moulds from vegetative cells, while heat resistant spores from *Bacillus* spp. and *Clostridium* spp. will, at least partly, survive. Processing of cooked chilled foods does not end with vegetable cooking. Once cooked, vegetables may be contaminated at the mixing stage by cross contamination with other ingredients. Cooked chilled foods are prepared following complex recipes (traditional or ethnic dishes). Dairy ingredients (milk proteins, cream), cheese, meat, spices and technological aids such as thickening agents may be mixed into cooked vegetables bringing their own microflora to the cooked vegetables. In addition, cooked chilled foods are usually prepared in non-sterile environments. Despite a hygienic environment and/or a microbiologically controlled atmosphere, the occurrence of microorganisms on cooked chilled foods may result from the atmosphere surrounding human activity in the factory.

Practical consequences are that:

- the microflora of cooked chilled foods containing vegetables may be markedly different from that of cooked vegetables or from those that may be expected from knowledge of the natural microflora of vegetables;
- microbial hazards must include pathogenic microorganisms which can contaminate the product after cooking.

Table 11.1 Main bacterial species identified in cooked, pasteurised and chilled purées of courgette and selection according to storage temperature (adapted from Guinebretière *et al*., 2001)

Species*	No. (%) of isolates under the following storage conditions		
	21 days at 4°C	21 days at 10°C	5 days at 20–25°C
Bacillus macroides/ B. maroccanus	8 (31)		
Paenibacillus amyloliticus	9 (34)		
Bacillus sp. related to *B. sphaericus*	1 (4)		
P. polymyxa	8 (31)	17 (34)	
Paenibacillus sp.		1 (2)	
Paenibacillus sp. related to *P. azotofixans*		1 (2)	1 (2)
B. cereus		6 (12)	6 (13)
B. pumilus		25 (50)	11 (27)
B. licheniformis			18 (42)
B. subtilis			4 (9)
B. circulans			3 (7)
Total	26 (100)	50 (100)	43 (100)

* Determined by 16S ribosomal DNA gene sequencing.

The microflora of cooked chilled foods is generally poorly documented. The reasons for this are mainly because the development of these foods is relatively recent and because of the absence of generic products, owing to the diversity of recipes and processes. The microbiology of *sous vide* foods, because of the absence of recontamination after cooking or subsequent heating may be representative of the specific microflora of cooked chilled foods containing vegetables.

Extensive work has been done on cooked pasteurised and chilled vegetable purées of commercial origin (Carlin *et al*., 2000b; Choma *et al.*, 2000; Guinebretière *et al*., 2001). In this product it was shown that:

- Initial aerobic counts are generally low (below 10^2 cfu g^{-1}, often below 10^1 cfu g^{-1}).
- Growth is generally slow at 4°C; aerobic counts did not exceed 10^5 cfu g^{-1} after 3 weeks at 4°C, but is markedly increased between 4°C and 10°C.
- The aerobic microflora are dominated by *Bacillus* spp. and similar species and there is a strong selection of bacterial species according to storage temperature (Table 11.1).
- Despite conditions close to anaerobiosis, strict anaerobes (*Clostridium* spp. in particular) were in markedly lower numbers than aerobic bacteria.
- Among the pathogenic bacteria, the prevalence of *B. cereus* was quite high at mild and at abusive (too high for proper storage) temperatures, reaching in

some instances 10^4–10^5 cfu g^{-1} after 20 days' storage at 10°C. Similar features were obtained in gnocchi, an Italian cooked chilled food made from potato (Del Torre *et al*., 2001).

Among non-spore formers, lactic acid bacteria have been also identified as a possible cause of spoilage in *sous vide* foods (Schellekens and Martens, 1993). Because of their lower heat resistance, this is possible only at low temperature and short heat processes.

11.4 Microbial hazards

Microorganisms that present a safety hazard in cooked chilled foods containing vegetables have the following characteristics:

- they are natural contaminants of raw vegetables
- they have been implicated in outbreaks of food poisoning following consumption of vegetable-based foods
- they are able to survive, at least to some extent, the mild heat treatment received by the products during processing
- they are able to grow at temperatures of refrigeration.

The list of the bacteria complying with these conditions is quite large. However *Listeria monocytogenes*, *Clostridium botulinum* and *Bacillus cereus*, according to most experts, are the major concern because of natural contamination, the ability to grow at low temperatures and high resistance to heat (ACMSF, 1992; Peck, 1997; Carlin *et al*., 2000a). These bacteria are widely distributed in the environment and may be isolated from vegetables. Surveys for the presence of *L. monocytogenes* show that 0–85% of fresh vegetable samples are positive, with contamination levels lower than 100 *L. monocytogenes* cfu g^{-1} (Beuchat, 1996; Nguyen-the and Carlin, 2000). This level of contamination is lower than that observed on meat products for instance. Surveys for the presence of *C. botulinum* show 0–100% of fresh vegetable samples are positive, with a maximal contamination level likely to be lower than one *C. botulinum* spore/g (Notermans, 1993; Lund and Peck, 2000). The three species of bacteria have been implicated in outbreaks of food poisoning following the consumption of vegetable-based foods, in both fresh, minimally processed vegetables and heat-processed vegetables. Among non-spore forming bacteria, *L. monocytogenes* is considered to be a relatively heat-resistant organism, when compared to other pathogenic bacteria, and is the most psychrotrophic bacterium among the known pathogens with a lower growth limit at about 0°C. Other pathogens such as *Escherichia coli* and *Salmonella* have a similar heat resistance and a lesser ability to grow at low temperatures. Endospores produced by *C. botulinum* and *B. cereus* confer a high resistance to the heat process applied to cooked chilled foods. Some strains of *C. botulinum* (Group II or non-proteolytic strains) or of *B. cereus* are able to grow at temperatures as low as 3°C and 4°C, respectively (ICMSF, 1996; Lund and

Peck, 2000). Despite being a spore former *C. perfringens* is generally not considered to be a safety concern for cooked chilled foods containing vegetables, because of its poor ability to grow at temperatures lower than 15°C in a vegetable substrate (Labbé, 2000).

L. monocytogenes, *C. botulinum* and *B. cereus* have a strong ability to grow on a vegetable substrate, even at low temperature (Table 11.2). Possible growth of *L. monocytogenes* was shown on a range of raw and cooked vegetable substrates (Farber and Peterkin, 2000). Growth of *B. cereus* and *C. botulinum* was shown in a range of cooked vegetable substrates, at temperatures close to minimal growth temperature. More generally most cooked vegetables at a pH above 4.6 (5.0) appear to be able to support growth and toxin production by proteolytic (non-proteolytic) *C. botulinum*, whatever the supposed nutrient value (Carlin and Peck, 1995, 1996).

11.5 Control of microbial hazards: heat treatment

The heat treatment applied during processing will affect the survival of the bacteria contaminating the foods. The extent of death of bacteria is a function of time and temperature. The rate of destruction, expressed as the decimal reduction time, D, is the time required to destroy 90% of the bacterial population (or to effect a 10-fold reduction or a one log (base 10) reduction). The effect of temperature on the D values, expressed as a z value, is the increase in temperature needed to produce a ten-fold reduction in D values. Both D and z values are available from many sources for all pathogenic bacteria including those of concern for the safety of cooked chilled foods containing vegetables.

The D_{70} value (i.e. decimal reduction time at 70°C) for *L. monocytogenes* is about 0.1–0.2 min (ICMSF, 1996). These values are markedly higher for *C. botulinum*, illustrating the considerably higher heat resistance of bacterial spores when compared to vegetative cells, with a maximum $D_{121.2}$ of about 0.21 min for Group I proteolytic *C. botulinum* and a maximum $D_{82.2}$ of 2.4 for Group II non-proteolytic *C. botulinum*, with z values close to 10°C (ICMSF, 1996). These values are subject to multiple sources of variations, including intraspecies variability, type of foods, preparation of bacterial suspensions, and so on. *B. cereus* is a representative example of strong variations between different strains; D values of 2 min have been reported at 90°C as well as at 121.2°C, with z values from 7 to 14°C (ICMSF, 1996).

Heating these bacteria in vegetable substrates affects heat resistance only marginally, the major decreases being observed in acid vegetable substrate. However some specific effects caused by vegetables are observed on non-proteolytic *C. botulinum*. The measured heat resistance of this bacterium may be increased by addition of juices of vegetable, such as turnip, swede, potato, flat bean or cabbage (Stringer *et al*., 1999). This activity is attributed to an endogenous lysozyme activity, similar to that of hen egg white lysozyme, which is assumed to aid growth from heat damaged spores of non-proteolytic *C. botulinum* (Lund and Peck, 2000).

Table 11.2 Fate of *Listeria monocytogenes*, *Bacillus cereus* and *Clostridium botulinum* in some preparations of cooked vegetables

Bacterium	Food or medium	Temperature (°C)	Parameter	Value	Reference
L. monocytogenes	Vacuum-packed potato	4	Increase in number	+4 log cfu g^{-1} in 14 days	1
	Vacuum-packed potato	15	Increase in number	+4.5 log cfu g^{-1} in 3 days	1
	Vacuum-packed potato	28	Increase in number	+4 log cfu g^{-1} in 12 h	1
B. cereus	Vacuum-packed carrot and potato	4 and 8	Increase in number	0 after 90 days	2
	Vacuum-packed carrot and potato	room	Increase in number	+4.5 log cfu g^{-1} in 3 days	2
	Courgette broth	20	Lag (h), generation time (h)	<5–20, 2.5–3.1[a]	3
	Courgette broth	14	Lag (h), generation time (h)	5–16, 4.4–5.1	3
	Courgette broth	10	Lag (h), generation time (h)	184–333, 3–9	3
	Courgette broth	6.5	Lag (h), generation time (h)	ngo[b]	3
	Carrot broth	25	Lag (h), generation time (h)	7–8, 0.93–1.1	4
	Carrot broth	16	Lag (h), generation time (h)	11–12, 3.2–3.5	4
	Carrot broth	12	Lag (h), generation time (h)	26–28, 7.5–23	4
	Carrot broth	10	Lag (h), generation time (h)	ngo–51, ngo–14	4
	Carrot broth	8	Lag (h), generation time (h)	ngo–110, ngo–15	4
	Carrot broth	5	Lag (h), generation time (h)	ngo–156, ngo–63	4
C. botulinum Group I	Vacuum-packed potato	25	Toxin detection	Samples +ve after 6 days	5
	Vacuum-packed potato	25	Toxin detection	Samples +ve after 7 days	6
	Vacuum-packed potato	20	Toxin detection	Samples +ve after 4 days	7
	Vacuum-packed potato	15	Toxin detection	Samples +ve after 14 days	7
C. botulinum Group II	Vacuum-packed potato	10	Toxin detection	Samples +ve after 9 days	7
	Vacuum-packed potato	4	Toxin detection	No sample +ve after 60 days	7
	Canned peas	8	Toxin detection	Samples +ve after 14 days	8
	Mushroom purée	8	Lag (h), generation time (h)	146, 9	9
	Mushroom purée	5	Lag (h), generation time (h)	304, 12	9
	Cauliflower purée	8	Lag (h), generation time (h)	288, 11	9
	Cauliflower purée	5	Lag (h), generation time (h)	383, 9	9
	Potato purée	8	Lag (h), generation time (h)	628, 10	9
	Vacuum-packed potato, pH 4.8	10 and 20	Toxin detection	Samples +ve after 21 days	10
	Vacuum-packed potato, pH 5.2	7 and 10	Toxin detection	Samples +ve after 21 days	10
	Vacuum-packed potato, pH 5.2	20	Toxin detection	Samples +ve after 14 days	10

[a] Several strains tested; [b] ngo, no growth observed.

1, Juneja *et al.*, 1998; 2, Picoche *et al.*, 1993; 3, Choma *et al.*, 2000; 4, Valero *et al.*, 2000; 5, Lund *et al.*, 1988; 6, Dodds, 1989; 7, Notermans *et al.*, 1981; 8, Gola and Mannino, 1985; 9, Carlin and Peck, 1996; 10, Baumgart, 1987.

11.6 Control of microbial hazards: storage temperature

Refrigeration delays spoilage of products and growth of spoilage organisms. This has been clearly shown on cooked pasteurised and chilled purées of vegetables. On a courgette purée, the time to growth of 10^5 cfu g^{-1} increased from 5 days to 15 days and the time to the appearance of noticeable off-odours (the first detected sign of spoilage) increased from 12 days to 36 days when the temperature decreased from 10°C to 4°C (Carlin *et al*., 2000b). The growth of most pathogenic bacteria is inhibited at low temperature. The minimal growth temperature of proteolytic (Group I) *C. botulinum* is 10°C, that of *E. coli* (including enterohaemorrhagic *E. coli*) and *Salmonella* is 7–8°C and of psychrotrophic strains of *B. cereus* is 4°C. Only non-proteolytic (Group II) *C. botulinum* and *L. monocytogenes* are able to grow at lower temperatures (3°C and 0°C, respectively) (ICMSF, 1996).

When growing in vegetable substrate or in foods, significant delays or absence in growth are generally observed even above the minimum growth temperature. Psychrotrophic isolates of *B. cereus* were unable to grow in a courgette broth at 7°C, while growth was observed in a nutrient broth (Choma *et al.*, 2000). Many strains of *C. botulinum* failed to grow in vegetable substrate at temperatures (15°C for Group I proteolytic *C. botulinum* and 10°C for Group II non-proteolytic *C. botulinum*) substantially higher than the minimum growth temperature, while growth was detected in nutrient broth after a few days of incubation (Carlin and Peck, 1996; Braconnier, 2001). These differences were not simply due to pH, as vegetables with similar pHs showed marked differences in growth potential of the bacterium. However, time to toxin production of *C. botulinum* in vegetable-based foods is in the range reported for other food groups (e.g. meat, fish and poultry).

Slight differences in temperatures can induce high differences in the populations of bacteria. For instance, *B. cereus* was never detected in cooked pasteurised and chilled purées of vegetables stored for 46 days at 4°C, whereas 17 out of 50 samples were positive after a storage of 20–32 days at 10°C, with some counts being higher than 10^5 *B. cereus* cfu g^{-1} (Choma *et al*., 2000).

Some outbreaks have been caused by products exposed for a prolonged time at ambient temperature (20–30°C), when they should have been kept refrigerated. Challenge tests with such incubation temperatures have therefore been performed and show growth to critical levels and/or toxin production of *L. monocytogenes*, *B. cereus* and *C. botulinum* within 1 to 5 days. At the same time spoilage was not observed in many instances and the product was still acceptable to the consumer (Notermans *et al.*, 1981; Lund *et al.*, 1988).

In conclusion, the growth potential of pathogenic bacteria in cooked chilled foods containing vegetables depends strongly on storage temperature and on the nature of the vegetable substrate, interactions between both factors being more significant at low temperatures.

11.7 Control of microbial hazards: heat treatment combined with refrigeration

Processes applied to cooked chilled foods include heating and incubation at low temperatures (Table 11.3). Challenge testing that combines both operations realistically mimics the real situations in cooked chilled food industry. These experiments show that heat treatment markedly affects the ability of inoculated test strains to recover during incubation at low temperature. In a range of purées of vegetables where survival is expected to be similar in each matrix, shown by evidence of growth at high incubation temperature, the probability of growth after heat treatment is markedly delayed (Carlin *et al*., 2000a).

11.8 Control of microbial hazards: other techniques

Cooked chilled foods are usually prepared without preservatives or additives. The natural acidity of the product may be an additional barrier to bacterial growth. *L. monocytogenes* did not grow at 5°C or decreased in numbers at 21°C in a range of commercial tomato products, and time to visible growth in a range of cooked puréed vegetables was related to the pH of vegetables, being longer in the vegetables with the lowest pH (Beuchat and Brackett, 1991). In the same way as for temperature, *L. monocytogenes*, *C. botulinum* and *B. cereus* have their own minimal pH for growth which are close to 4.6, 4.6 and 5.0, respectively (ICMSF, 1996).

Acidification of foods may be used in some specific applications to prolong shelf-life, or to give an additional safety factor to products potentially exposed to mild or severe temperature abuse. The organic acids that can be used are not

Table 11.3 Shortest time to visible growth in a nutrient broth, potato purée or broccoli purée inoculated with 10^7 spores per tube of non-proteolytic *Clostridium botulinum* following no heat treatment or heat treatment in a water bath at 80°C for 10 min (adapted from Carlin *et al*., 2000a)

Incubation temperature (°C)	Shortest time to visible growth (days) in indicated substrate following heating at 80°C for stated heating time (min)					
	Nutrient broth		Potato		Broccoli	
	Heating time		Heating time		Heating time	
	0	10	0	10	0	10
20	2	3	3	6	3	11
10	4	7	16	>56	13	38
8	5	10	20	>56	15	>56
6	8	19	>56	>56	56	>56
4	>56	>56	>56	>56	>56	>56

specific to cooked chilled foods containing vegetables. However some applications in this context are, at least at the experimental stage, possible. Use of sorbic acid in association with citric acid or lactic acid to lower the pH to 5.0 was shown to reduce *B. cereus* growth at 8°C and 12°C in gnocchi, while sorbic acid alone was not efficient (Del Torre *et al.*, 2001). Growth at 12°C of psychrotrophic strains of *B. cereus* in a carrot juice was inhibited for 60 days by acidification of the product to pH 5.0, whereas growth was possible at 5°C at the natural pH of the product (6.2) (Valero *et al.*, 2000). According to the authors, the product was still acceptable to the consumer. Combinations of different organic acids (sorbic, ascorbic and citric acid) were also efficient in inhibiting *C. botulinum* growth in vacuum-packed potato. Some browning inhibitors (sulphite, mixtures of organic acids and antioxidants) inhibited (delayed) growth of *L. monocytogenes* at 4°C (15–28°C) in vacuum-packed potato (Juneja *et al.*, 1998).

Addition of salt has a major disadvantage. Reduction in a_w controls the development of pathogenic bacteria at only relatively high concentrations which are detrimental to the sensory quality of the food. For instance, in a mashed potato purée, addition of 2% sodium chloride was ineffective in controlling growth of *B. cereus* at 30°C and delayed slightly growth at 10°C (Mahakarnchanakul and Beuchat, 1999). This salt concentration in foods is higher than is generally tolerated by consumers. Interactions between pH and a_w in vacuum-packed potato was shown to result in a significant delay in growth of and toxin production by *C. botulinum* (Dodds, 1989). However an efficient sodium chloride concentration was here also relatively high (about 2% and above).

11.9 Current guidelines and regulation

The preservation of cooked chilled foods is based on a combination of preservative factors, some controlled (storage temperature, heat process, eventual addition of preservatives, reducing pH or a_w) and some not controlled (natural sub-optimal pH of foods, balance in nutrients and antimicrobials, addition of herbs and spices). However the controlled factors must be monitored and the effects of the non-controlled factors must be tested to guarantee the safety of cooked chilled foods.

Cooked chilled foods are made from ingredients that are heated in a container or are assembled from heated ingredients under particular hygienic conditions. Those containing raw or very low processed ingredients may occasionally contain vegetative bacteria, including pathogens such as *L. monocytogenes*. According to North American guidelines, a major part of these foods are designated as 'potentially hazardous', because of low acid (pH > 4.6), high moisture content (a_w > 0.85) and packaging in hermetically sealed packages (Farber, 1995). They require refrigeration for microbiological safety and preservation of quality. Regulations and guidelines include general information about hygiene in factories and for employees, quality of raw materials used for processing, and so on. Specific recommendations mainly deal with the determination of a safe shelf-life, assuming

in particular that products will be kept refrigerated. In Europe, shelf-life is based on product formulations and processing parameters for defined chill storage conditions. Such recommendations have been proposed by the UK Advisory Committee on Microbiological Safety of Foods (ACMSF, 1992), with non-proteolytic (psychrotrophic) *C. botulinum* as the target bacterium.

The safety of chilled foods could be ensured by one of the following (Lund and Peck, 2000):

- storage at <3.3°C
- storage at ≤5°C and a shelf-life of ≤10 days
- storage at 5–10°C and a shelf-life of ≤5 days
- heat treatment at 90°C for 10 min or equivalent lethality (e.g. 1 min at 100°C or 51.8 min at 85°C according to the European Chilled Food Federation, ECFF) followed by storage at <10°C
- a pH of <5.0 throughout the food combined with storage at <10°C
- NaCl concentration of >3.5% throughout the food combined with storage at <10°C
- water activity of <0.97 throughout the food combined with storage at <10°C
- other combinations of heat treatment and preservative factors together with storage at <10°C that can be shown consistently to give a protection factor of 6 (i.e. a reduction in the probability of survival and growth by a factor of 10^6).

Similar guidelines have been proposed by the ECFF for non-proteolytic *C. botulinum*, but also include guidelines relative to *L. monocytogenes*, recommending a reduction in numbers by at least 6 logs (or a 6*D* reduction) which may be achieved by a thermal process of 70°C for 2 min or equivalent (i.e. 9.3 min at 65°C, or 5 s at 80°C) (ECFF, 1996).

A draft code related to cooked chilled foods has been proposed by the Codex Alimentarius and would contribute to implementation of these recommendations and legislation worldwide (Codex Alimentarius, 1998).

11.10 Use of microbiological risk assessment

Data published in the scientific literature and reviewed in this chapter indicate the possible presence of pathogenic bacteria, the survival of mild heat-treatment and the growth of pathogenic bacteria, in particular the spore-forming pathogens *C. botulinum* and *B. cereus*, in cooked chilled foods. In contrast cooked chilled foods have an excellent safety record in Europe. However in many cases the current safety margins are unclear and it remains to be established what the risks caused by spore-forming bacteria (SFB) in cooked chilled foods may become in the future expanding market.

Microbial risk assessment of foodborne hazards is a process increasingly applied to food safety. Recent examples include *E. coli* in beef burgers, *B. cereus* in pasteurized milk and *L. monocytogenes* in cheese made from raw milk (Notermans *et al*., 1997; Bemrah *et al.*, 1998; Cassin *et al.*, 1998). In the context

of cooked chilled foods, microbial risk assessment is the probability and severity of food poisonings with which cooked chilled foods may be associated.

Microbial risk assessment consists of the following steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. A formal risk assessment on cooked chilled foods made from vegetables confirmed *C. botulinum* and *B. cereus* as the main hazards, because of (for *C. botulinum* specifically) a high fatality rate, high number of reported cases, the ability of at least some strains of both species to grow at refrigeration temperatures and wide relationship to vegetables either by natural contamination or outbreaks linked to the consumption of processed vegetables (Carlin *et al.*, 2000a). Non spore-forming and psychrotrophic pathogens (such as *L. monocytogenes*) will be considered to be a very low risk in foods heated in the final package, or high if the final products contains raw ingredients. These foods will not be considered here to represent the majority of cooked chilled foods and therefore the discussion will focus on spore-forming pathogens.

Exposure assessment is the quantitative and/or qualitative evaluation of the likely intake of biological agents via foods. In the context of cooked chilled foods this means the number of SFB or the amount of toxin produced by SFB that is identified as hazardous, in a given time. In a simple way this depends on (i) the natural contamination of foods by hazardous bacteria, (ii) the effect of heat processing, and (iii) the growth of hazardous SFB during retail and domestic storage. This information is integrated into risk characterisation to provide an estimation of the probability of food poisoning caused by *C. botulinum* and *B. cereus* in the population of consumers of cooked chilled foods that contain vegetables. This estimate results from a combination of probabilities (including uncertainty and variability) for multiple parameters that affect the behaviour of the microorganisms during the production process and distribution and storage of the products.

The approach still suffers from many weaknesses which are not specific to its application to cooked chilled foods. First of all consider the poor quality of dose response assessment, that is, the relationship between the magnitude of exposure to the microbial agent and the severity and/or frequency of associated adverse health effects. For instance, what is the probability of becoming ill after ingesting 10^5 or 10^7 cells of *B. cereus*? How is this probability related to age, sex or health status? Data on consumer behaviour are also relatively poor. In particular cooked chilled foods are subjected to reheating at home, which can affect survival of pathogens or toxin activity. Growth models do not account for complex factors, such as the interactions between pathogens and saprophytic microflora. The microflora of cooked and pasteurised chilled vegetable purées, for instance, comprise some *Paenibacillus polymyxa* which show some antimicrobial activity against *C. botulinum* (Girardin *et al*., 2002). Finally the microbial data are generally poorly adapted to microbial risk assessment, because they do not often include the variability and uncertainty of the model parameters.

Despite these limitations, the approach has shown some promising results, when applied to cooked and pasteurised chilled vegetable purée, for instance, and the risk due to *B. cereus*. Experimental work has shown that mesophilic strains

of *B. cereus* have a higher probability of contaminating the final product than psychrotrophic strains (Guinebretière, 2001). Microbial risk assessment showed that the level of *B. cereus* at the end of processing was a bad predictor of the level in a package of product after storage (Nauta, 2001). Despite a rather high probability of storage at a mild temperature for prolonged times during retail and in the consumer's home, as shown in many EU countries, the probability of obtaining critical numbers of mesophilic *B. cereus* was markedly lower than that of psychrotrophic *B. cereus*. The model also underlined the importance of monitoring temperature along the chill chain, from the food manufacturer to the consumer.

11.11 Conclusion

The market in cooked chilled foods is in continuous expansion and the proportion of vegetable-based dishes will probably be maintained and possibly increase in the market for cooked chilled foods. Almost any traditional, ethnic, novel and, of course, vegetarian dishes contain vegetables; because of low fat, nutritional value, protective effects (fibres, natural antioxidants, etc.) vegetable-based foods follow consumer requirements for more nutritional and healthy foods. The diversity of tastes and flavours among vegetables may help to suggest a variety of recipes.

Diversity of recipes also means diversity of processes and for each of them the microbial pathogen(s) of concern must be determined with care. The safety problems of vegetable-based foods were often underestimated, because most of them are consumed fresh and unprocessed, or because traditional processed vegetables (canned and frozen products) were actually safe. However epidemiological data remind us that fresh and processed vegetables may be implicated in a number of outbreaks of food poisoning, that they may harbour a wide range of pathogenic microorganisms and that the nutrient value of vegetable substrate is great enough to support growth of pathogens, in the same way as in meat, fish or dairy foods which are generally considered to be better substrates for microbial growth. For these reasons the safety of cooked chilled foods made from vegetables deserves the same consideration as that of any cooked chilled foods.

What improvements should be considered for the safety of cooked chilled foods in the future? Most contamination with pathogens occurs from the field. Control of contamination from the field where the food was grown has shown some success for *E. coli*, *Salmonella* and *L. monocytogenes*, but there is still a lack of plausible evidence of the pathogenic spore formers, the main concern for cooked chilled foods. Technologies preventing contamination of the products which are not given a heat treatment in the final package (clean room technologies), or technologies able to give a heat treatment in the final package, such as microwave technologies, as an alternative to vacuum packaging, are of considerable interest to the cooked chilled food industry. Cooked chilled foods are the products of complex processes combining mixture of different ingredients,

different heat treatments, retail conditions and a shelf-life imposed by the consumer. Risk assessment may help to identify which stages along these complex processes are critical to food safety and therefore to propose efficient and relevant mitigation strategies, such as (without limitation) appropriate shelf-life, information to consumers or increases in heat treatment.

The current situation in the cooked chilled foods industry is safe. However because of the expansion of the market, continuous improvement in the safety of the products must be effected through better control of the fate of the bacterial pathogens along the processing chain and during shelf-life.

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Part 3

New technologies to maximise quality

12

Measuring and improving the natural resistance of fruit

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12.1 Introduction: plant defence mechanisms and post-harvest quality

Plant science and, more specifically, plant defence is one of the most exciting and fast moving fields in modern biology. The study of plant defence mechanisms is currently based on two well-established and interconnected approaches, namely, the genetic and the physicochemical view. The former relies on advances in plant molecular biology, through the sequencing of the plant's genome to identify genes concerned with plant disease resistance. Knowledge of the physiology of defence mechanisms is also crucial to develop new methodologies not only for improved monitoring of the health status of post-harvest fruit but also to find new ways to improve shelf-life. The latter goals are the basis of the physicochemical approach, which is discussed in this chapter.

The natural products of a plant's secondary metabolism have been used in 'natural' medicine since the early times of human history. Because the basic function of these chemicals is to protect the plant from attack, a good strategy would necessarily require the identification of the components of natural defence response in plants. Therefore modern physicochemical technologies, especially laser-based techniques of the type described in this chapter, can be very useful, not only as early and sensitive indicators for spoilage, but also to enhance the natural resistance of crops.

12.2 Plant defence mechanisms: ethylene, phytoalexins and other compounds

At the time of writing, probably one of the most studied plant defence molecules is ethylene, a plant hormone that plays an important role in the regulation of many environmentally and developmentally induced processes such as pathogen infection responses, stress resistance, seed germination, pollination and wilting of flowers, fruit ripening and degreening, senescence, leaf and fruits abscission, and so on.[1–6] Although the emission of ethylene shows a huge variation from one organ to other and among different species, it has been widely demonstrated that the chances of survival of a stressed plant strongly depend on its ability to initiate ethylene-related reponses.[7,8]

A second group of important secondary metabolites are the so-called 'phytoalexins', antipathogenic compounds produced by plants after infection or elicitation by abiotic agents.[9] Phytoalexins were widely studied during the second half of the twentieth century, involving many areas of plant science, including biosynthesis, chemosystematics, natural products chemistry, molecular biology, pharmacology or fungal genetics.[10–13] In general, they are non-volatile compounds with low molecular weight (below 1000 amu), that is, pathogenesis-related peptides and proteins produced by the plants are not included in this category. Phytoalexins present a great chemical diversity and, while many plant families produce phytoalexins with similar chemical structures, a plant can produce a phytoalexin totally unrelated to the ones produced by another plant of the same family. Selected examples of compounds with demonstrated phytoalexinic character are: flavonoid and isoflavonoid derivatives, stilbenes, sesquiterpenes, phenylpropanoid derivatives and polyketides.

In addition to this chemical variety, there are many other difficulties in determining whether a given compound is a phytoalexin or not. Although the production of phytoalexins after infection suggests that some pathogen compound or some of the products arising from the host–pathogen interaction (known as elicitors) trigger the phytoalexin biosynthesis, the biosynthetic pathway is not always easy to elucidate. Thus, some compounds can act as a preformed antifungal constitutive compound in one family and phytoalexin in another; even more, in the case of rice, it has been shown that momilactone A is a constitutive compound in husks and stems, but it is a phytoalexin in leaves.

Thus, although difficult to define through their chemical structure or their synthetic pathway, phytoalexins are well defined by the dynamics of their biosynthesis and their functions within the plant. It is already clear that the induction of phytoalexins is not just a response to infection, but it is one of the main strategies of the defence mechanism of plants against their pathogens.[14]

Besides phytoalexins and signalling substances like ethylene, other defence molecules induced in plants by the action of biotic or abiotic elicitors are classified as pathogenesis-related proteins, cellular barriers (lignins, extensins, callose) and antioxidative systems. In all cases it is necessary for there to be present in the plant some receptor for these elicitors, which are responsible for the initial

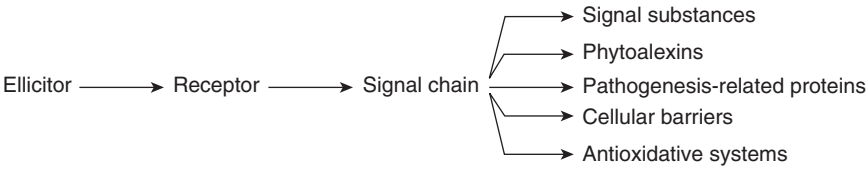

Fig. 12.1 Main plant defence mechanisms induced by biotic and/or abiotic elicitors (adapted from Sandermann *et al.*[18]).

signal (in many cases activated oxygen species) that provokes the production of a specific defence molecule.[15–17] The general scheme for the action of such elicitors[18] is summarised in Fig. 12.1.

The main objective of this chapter is to deal with the basic question of how our current knowledge of plant defence mechanism, including the huge variety of types of chemical warfare on pathogens, can be exploited to increase resistance in fruits. Several pertinent questions are related to this basic one. What level of resolution and sensitivity can be reached by modern techniques to monitor the health status of fruits? Can the internal fruit concentration of these 'natural' pesticides be increased so as to enhance their resistance to spoilage? Can these natural pesticides be externally applied to improve the shelf-life of plants and fruits? If so, can they be biological, ecological and commercially acceptable? What can be learned from the plant defence physiology which, ultimately, could even be commercially used to maintain the post-harvest fruit quality?

Progress made in answering these questions, together with a discussion of the new methods developed in this interesting field is the subject of this chapter. To this end, sections 12.3 and 12.4 deal with the application of highly sensitive analytical methods for the detection and monitoring of natural defence compounds in plants, particularly ethylene and the phytoalexin resveratrol. Sections 12.5 to 12.9 present selected examples of different approaches to improving the natural resistance of plants by using the plant's own defence molecules; thus, the likely future major areas of research devoted to improving the natural resistance in fruits is given. Finally the main sources of further information and advice are listed.

12.3 On-line detection of plant stress: volatile compounds

The identification of the natural defence response in plants relies on the application of highly sensitive analytical methods. This section reviews the development and application of new laser-based techniques to enable detection of natural defence (volatile) molecules with unprecedented sensitivity, versatility and reliability.

Many of the components of the natural defence response in plants are volatile organic compounds that are emitted as a response to pathogen attack. The detection of these compounds presents several problems especially because of

their great variety, low concentration (generally in the ppb (10^{-9}) or ppt (10^{-12}) range) and the rapidity of the processes involved, which can occur in a matter of a few minutes, as it has been demonstrated in the case of the plant response to stress.[19]

The techniques developed for on-line detection of volatile compounds in other fields have to fulfil a number of specific requirements for application in either plant physiology or plant pathology investigations, namely:

- high sensitivity to detect ppb and ppt concentrations
- high selectivity allowing clear differentiation between several compounds and the ability to analyse different gases simultaneously using a single instrument
- excellent time resolution for real-time measurements
- automatic operation allowing day and night analysis.

Typically, the methods used for trace analysis of volatile compounds can be separated into spectroscopic and non-spectroscopic techniques. Of the non-spectroscopic techniques, the most used are chemiluminiscence, mass spectrometry (MS) and gas chromatography (GC). While the former two techniques have been used mainly as laboratory tools, GC has achieved outstanding features for a wide variety of gases at detection limits as low as a part per trillion (pptv) with a high degree of reliability, especially with the implementation of commercial GC–MS instrumentation. In plant science it has been used, for example, for the detection of the ethylene emission as a stress response in more complex plants.[4,20] The main drawback of GC is that previous sample preparation or preconcentration is usually needed which, together with the slow time response of the technique, limits the temporal resolution of the analysis. Moreover, the system is generally not automatic.

Spectroscopic techniques are generally based on absorption measurements, especially in the infrared (IR) wavelength region. IR gas analysers with broadband thermal sources of radiation have been used in investigations of plant defence molecules, but these are generally industrial analysers and designed to detect one single particular gas. Thus, the simultaneous measurement of different gases, which is necessary in the study of many plant processes, is not allowed. The availability of tunable laser light sources has favoured the development of many spectroscopic techniques, among them are differential optical absorption spectroscopy (DOAS),[21–23] light detection and ranging (LIDAR),[23–26] Fourier transform IR spectroscopy[27–29] and tunable diode laser absorption spectroscopy (TDLAS).[30,31] These techniques have been applied in the detection and analysis of volatile organic compounds, especially in environmental applications, but any one of them presents several drawbacks for the detection of natural defence molecules in plants, particularly their lack of sensitivity and/or selectivity.

One of the most interesting developments in the detection of volatile compounds released by the plants during the past few years has been so-called laser photoacoustic spectroscopy (LPAS) which has allowed the identification of many key molecules and the unravelling of signalling plant defence mechanisms, as described below. The technique is based on the photoacoustic effect, that is, the

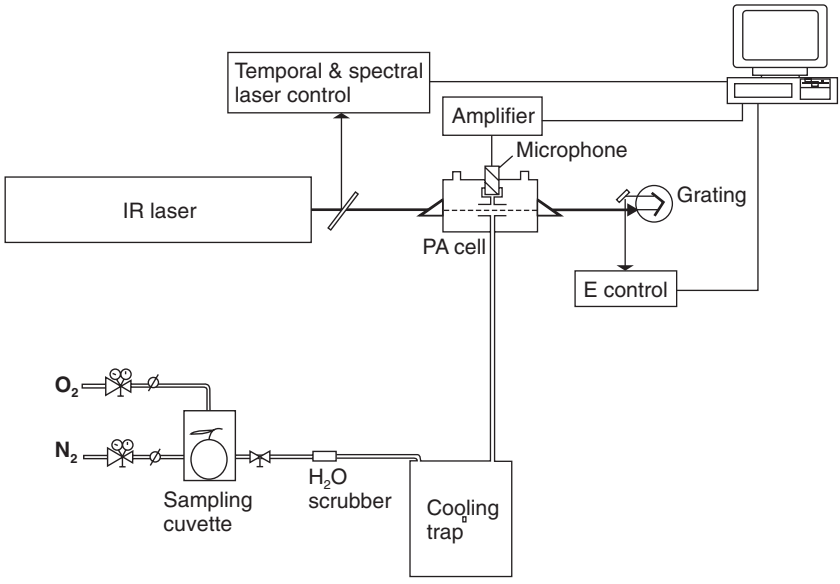

Fig. 12.2 Schematic representation of the LPAS set-up for the detection of plant volatile emissions.

generation of acoustic waves as a consequence of light absorption as was first reported by A. Graham Bell in 1880.[32] A comprehensive description of the physical principles behind LPAS is out of the scope of this chapter and can be found elsewhere.[33–35] Thus only a basic explanation is given.

The effect is originated by the absorption of photons of a suitable wavelength and energy by the gas molecules, which then become excited to a higher rovibrational state. Neglecting spontaneous radiative decay, the absorbed energy is subsequently transferred by intermolecular collisions to translational energy, and thereby to heat. When a gas sample is collected in a closed cell, the heating of the gas molecules will produce an increase in the cell pressure. By modulating the light intensity (e.g. turning the light source on and off) pressure variations are produced which create a sound wave susceptible to detection by a sensitive microphone. Figure 12.2 shows a schematic view of the LPAS experimental system.

The microphone signal depends on (1) the number of absorbing molecules present in the gas, (2) the absorption strength of the molecules at a specific light frequency, and (3) the intensity of the light. Thus, for practical trace gas detection, the light source must satisfy two conditions: it should be narrow banded and tunable in order to reach the specific wavelength of the molecule and it should have high intensity because the absorption signal is proportional to it.

As the absorption processes of interest are related to rovibrational transitions, it is necessary to work in the IR region, where each molecular gas has its own

'fingerprint' absorption spectrum whose strength can vary strongly over a short wavelength interval. Specifically, the range preferred for spectroscopic applications varies between 3 and 20 μm. Although in some cases a high intensity continuous lamp is used as IR source,[36,37] an infrared laser provides both high intensity and narrow band tunable light and is therefore ideal for photoacoustic (PA) detection techniques. CO_2 and CO lasers are commonly used as a light source for PA detection of gases[38,39] because they provide relatively high continuous wave (cw) powers, typically 100 W and 20 W, respectively, over this wavelength region. Pulsed laser sources have been also used for LPAS investigations, but there is much less work published on pulsed photoacoustic.[40]

The main disadvantage of CO_2 and CO laser sources is that their tunability is only moderate. They are only line tunable, which may cause interference problems, with a rather large spacing between the laser lines and cover a relatively short range of wavelengths. Several alternatives have been proposed to overcome these limitations: the use of other CO_2 isotopes or high pressure CO_2 lasers for CO_2-LPAS and a CO overtone laser for CO-LPAS are the more relevant suggestions. Moreover other laser sources have been used in order to implement a broadly tunable source with a narrow bandwidth into PA systems, especially with the rapid development of solid state lasers; among them, tunable III–V diode lasers, diode-pumped solid state lasers or distributed feedback diode lasers, allow the development of compact tunable IR laser radiation with a variety of applications in LPAS.[41,42] Finally, several applications of LPAS using an optical parametric oscillator system has been reported by different groups[43–45] and is certainly the most promising technique for the enhancement of the tunability of PA systems.

Despite the disadvantages mentioned, CO and CO_2 lasers are still the most commonly used IR light sources in photoacoustic spectrometers. In order to show the versatility and main features of this equipment, Table 12.1 shows the limits of detection (LoD) for several compounds reached by the LPAS technique in the Department of Molecular and Laser Physics at the University of Nijmegen.[46]

Table 12.1 gives a clear idea of the multiple applications of LPAS with respect to the detection and reliable analysis of volatile organic compounds in various fields, like environmental chemistry,[47–51] although one of the main applications of LPAS remains in the field of the plant sciences[52–55] owing to the specific requirements mentioned above. In particular, LPAS is widely applied in monitoring the volatile defence compounds released by the plants.[56–63]

As indicated in the introduction to this chapter, ethylene plays an important role in a number of plant physiological processes. LPAS has proved to provide a reliable method of detecting this plant hormone at ppt levels[35,64] in an instantaneous and continuous manner; as a consequence there are many LPAS investigations of ethylene emission from fruits and plants under different environmental conditions.[61,65–70] Figure 12.3 shows the evolution of ethylene emission of a cherry tomato under different conditions.[68] The experiment starts under anaerobic conditions and at $t = 5.6$ h the normoxic conditions are restored, yielding a sudden and huge increment in ethylene emission during a period of about 45 min. The ability of the technique to follow the process in real-time (data are registered

Table 12.1 Limits of detection for laser photoacoustic spectroscopy

Compound	LoD (ppbv)	Compound	LoD (ppbv)
CO			
Carbon disulphide CS_2	0.01	Methane CH_4	1
Acetaldehyde CH_3CHO	0.1	Dimethylsulphide $S(CH_3)_2$	1
Water (vapour) H_2O	0.1	Ammonia NH_3	1
Nitrogen dioxide NO_2	0.1	Trimethylamine $N(CH_3)_3$	1
Sulphur dioxide SO_2	0.1	Ethanol CH_3CH_2OH	3
Nitrous oxide N_2O	1	Pentane $CH_3(CH_2)_3CH_3$	3
Nitric oxide NO	1	Methanethiol CH_3SH	10
Acetylene C_2H_2	1	Hydrogen sulphide H_2S	1000
Ethane C_2H_6	1	Carbon dioxide CO_2	1000
Ethylene C_2H_4	1		
CO_2			
Ammonia NH_3	0.005	Ethylene C_2H_4	0.01
Ozone O_3	0.02	Hydrogen sulphide H_2S	0.04

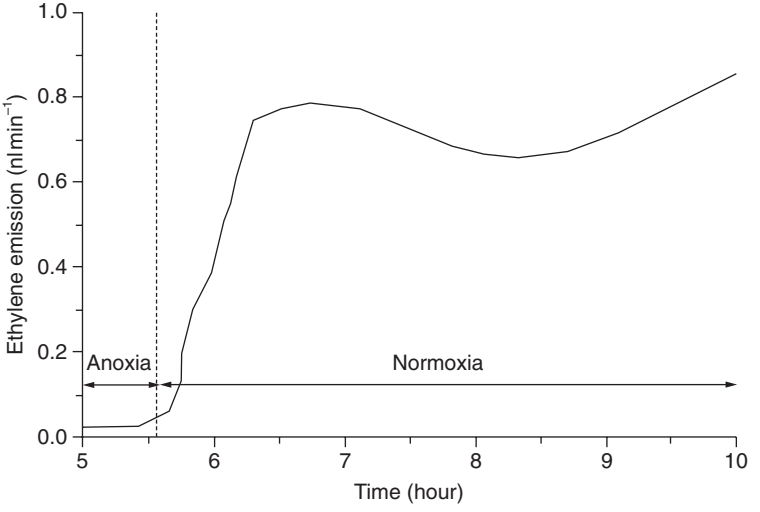

Fig. 12.3 Ethylene emission of a cherry tomato under different anaerobic conditions as measured by the LPAS technique. The rapidity of the plant response and the ability of the technique to follow it are noticeable (adapted from de Vries *et al.*[68]).

every 2 min) together with its high sensitivity (variations of few picolitres per minute can be detected) are remarkable.

LPAS has been also extensively used in the detection of ethanol and acetaldehyde to investigate the rate of alcoholic fermentation in plant tissue during anoxic

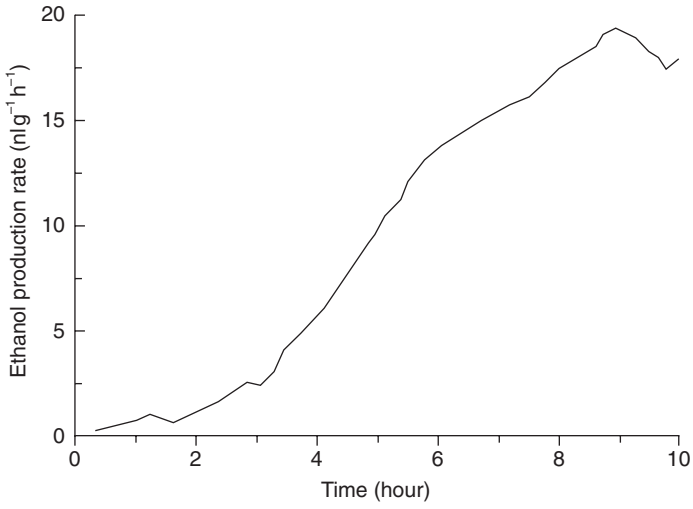

Fig. 12.4 Ethanol and acetaldehyde emission rates from stored pears as measured by the LPAS technique (adapted from Ref. 74).

and hypoxic treatments in several harvested fruits.[19,71–73] Monitoring the trace gases released by the stored fruits and vegetables gives information on the metabolic processes occurring in the crops (rate of fermentation, ripening stage, etc.) and is important in order to optimise the storage conditions. As an example, Fig. 12.4 shows the ethanol production rate from stored pears as measured by LPAS.[74] These studies have opened up new ways of understanding and improving the natural defence mechanisms of stored fruit, as it will be seen in more detail in section 12.6.

12.4 On-line detection of plant stress: non-volatile compounds

One of the crucial problems in this field, especially in the case of fruit and vegetable samples, is the detection and identification of non-volatile organic compounds present at low concentration levels, as is the case for most of the phytoalexins produced by the plants.

The development of new analytical methods based on both mass spectrometry and laser spectrometry is of major interest[75–78] in this field at the time of writing. Although mass spectrometry is widely used in the analysis of these compounds providing exact mass identification, the difficulty lies in volatisation of the sample into the gas phase prior to injection into the analyser. This first step requirement is particularly a problem for thermally labile samples as they rapidly decompose upon heating.

To circumvent this difficulty a wide range of techniques have been developed for non-volatile analysis; the reader is referred to a review[79] for further details and discussion of these new techniques. Techniques that have been applied include fast atom bombardment (FAB),[80–82] field desorption (FD),[82,83] laser desorption (LD),[84,85] plasma desorption mass spectrometry (PDMS)[86,87] and secondary ion mass spectrometry (SIMS).[88,89] Although these methods give significant improvement and extended applicability, they all suffer from the same limitation derived from the fact that both desorption and ionisation cannot be optimised separately, which, obviously, may be critical for many real applications.

Laser desorption methods have been developed in which volatisation and ionisation steps are separated and so higher sample sensitivity is achieved. While they all have the laser desorption step in common, they differ in their ionisation method. A few examples are (a) laser desorption plus electron beam ionisation, (b) chemical ionisation under vacuum conditions, (c) chemical ionisation under atmospheric conditions, (d) laser multiphoton ionisation coupled with time-of-flight mass spectrometry, and in particular resonance enhanced multiphoton ionisation (REMPI)–time-of-flight mass spectrometry (TOFMS) which is considered as one of the most powerful methods for the analysis of trace components in a complex matrix.

The high selectivity of REMPI–TOFMS is given by the combination of mass selective detection with the resonant ionisation process: the ionisation is produced by the successive absorption of two or more laser photons. Thus for efficient ionisation the energy of the first photon has to be resonant with one of the real electronic states in the molecule (resonance enhancement). This condition gives a second selectivity to the technique: laser wavelength selective ionisation. In addition, other clear advantages of REMPI–TOFMS are excellent sensitivity and resolution, major ionisation efficiency, easy control of the molecular fragmentation by the laser intensity and the possibility of simultaneous analysis of different components present in a matrix. As a result, this technique has become well established for spectroscopic analysis, either for fundamental research[90–94] or for applications where very sensitive analysis of trace components in a complex matrix or simultaneous analysis of a large number of components over a large range of concentrations are needed.[95–100]

As an example, the development and subsequent application of a laser technique specially designed to perform fast and direct analysis of non-volatile compounds in fruits and vegetables, particularly *trans*-resveratrol in grapes and vine leaves is presented here. The method is based on the combination of LD followed by REMPI and TOFMS detection. The analytical method can be categorised within group (d) mentioned above but it does not use a supersonic beam. It was conceived for intermediate mass resolution (around $R \approx 10^3$) at an intermediate level of technical simplicity.

3,5,4′-Trihydroxystilbene (*trans*-resveratrol) is an antioxidant compound naturally produced in a huge number of plants, including grapes, as a phytoalexin. Figure 12.5 shows its structural formula. In *Vitis* spp., *trans*-resveratrol is accumulated in vine leaves and grape skin in response to various fungal organisms,

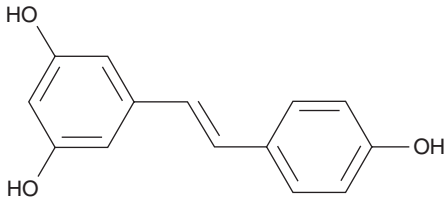

Fig. 12.5 Structural formula of *trans*-resveratrol.

UV radiation or chemicals[101,102] and has been found in wines in concentrations depending on viticultural and enological practices.[103,104] Analytical interest in *trans*-resveratrol was first caused by its natural pesticide properties. Quantitatively, the major component in grapevine phytoalexin response is *trans*-resveratrol, which has been shown to be fungitoxic at physiological concentrations against *Botrytis cinerea*,[105] the causal agent for grey mould, one of the main pathogens in grapes.

Analysis of *trans*-resveratrol is generally carried out by chromatographic methods, gas chromatography (GC)[106,107] and high performance liquid chromatography (HPLC)[108–110] or capillary electrophoresis (CE).[111,112] Regardless of the separation technique, its analysis in grapes and wines requires the use of previous preconcentration and/or multisolvent extraction techniques owing to the complexity of the matrices and to the low concentration of the analyte. The techniques generally employed are liquid extraction with organic solvents or solid phase extraction.

It is generally accepted that the sample preparation is the limiting step in *trans*-resveratrol analysis, not only because of the need for costly and time-consuming operations, but because of the error sources introduced during this operation. This has originated some controversy among different laboratories about their respective sample preparation techniques.[113–115] Several revisions of some of the methods for the analysis of *trans*-resveratrol[116–118] showed a huge variability in the values published, which is attributed to the possibility of isomerisation during the process of derivatisation, important losses caused by oxidation, isomerisation or hydrolysis during the extraction and separation processes, and the presence of some resveratrol derivatives that could interfere in the results obtained. Several sample preparation methods used in the determination of *trans*-resveratrol by HPLC have been also reviewed[119] including a comparison of their main features.

In the case of wine and grape juice samples, several methods have been developed[115,120–122] for the analysis of *trans*-resveratrol by direct injection in the HPLC system, but in most cases this results in chromatograms that are too complex and that sometimes do not allow reliable identification and/or quantitation of the peaks.[119] Direct analysis of *trans*-resveratrol in wines has been also performed by micellar electrokinetic capillary electrophoresis with a clear lack of sensitivity attributed by the authors to the need for preconcentration techniques.[123]

The combination of LD followed by REMPI and TOFMS detection can overcome these error sources, as indicated below. The experimental set-up, schematically shown in Fig. 12.6, has been already described elsewhere,[124] so only a brief report is given here.

Essentially, it consists of two independent high vacuum chambers. The first chamber is used for both laser desorption and laser post-ionisation of the sample followed by the ion acceleration towards the second chamber, basically a time-of-flight unit with a two-microchannel plate detector. A few nanosecond laser pulses from the fundamental emission of a Nd:YAG laser are used for sample desorption. A frequency-doubled dye laser is then used to ionise selectively the desorbed neutral by REMPI. To this end active wavelength laser scanning is achieved with tunability from 230 nm up to 365 nm. In addition to the selective ionisation owing to REMPI, additional selectivity is provided by the use of mass spectrometry, that is, providing mass identification to make the technique more sensitive and universal.

The separation of both desorption and ionisation processes is an important advantage in this arrangement, because it allows the study and optimisation of both processes independently. Consequently, some of the limitations, as for example the low mass peak resolution, of conventional matrix-assisted laser desorption/ionisation (MALDI)[54] were eliminated. In the specific case of *trans*-resveratrol analysis this optimisation has included[125] (1) a 20-fold enhancement in the desorption yield by mixing the analyte with Zn powder (*ME*tal *P*owder *E*nhanced *D*esorption), (2) the determination that *trans*-resveratrol is ionised through a one-colour two-photons process and (3) a resonance ionisation region between 302.5 and 307.5 nm with the maximum at 302.1 nm, which is the optimal wavelength for *trans*-resveratrol analysis in complex samples.

The essential features of the technique are (1) the absence of any separation method for sample preparation; the *trans*-resveratrol is fully extracted from the samples (grape skin or vine leaves) just by cold-pressing using a hydraulic press, (2) enhanced desorption yield given by the mixing the analyte with metal powder and (3) high resolution and sensitivity and a low detection limit caused by laser resonant ionisation and mass spectrometric detection. Thus, the combination of LD followed by REMPI–TOFMS detection can overcome the main error sources present in the chromatographic methods generally employed for resveratrol analysis.

The present method has demonstrated its capability for fast, accurate and reliable analysis of *trans*-resveratrol in agricultural samples, namely grapes and vine leaves, reaching detection limits of only few ppb.[126] As already stated above, *trans*-resveratrol mainly accumulates in the grape skin; this selective accumulation facilitates the analysis as it acts as a natural method of preconcentration of *trans*-resveratrol. The grape samples were prepared by taking the skin off and cold pressing it by means of a hydraulic press. Previously, it was proved that *trans*-resveratrol is completely extracted by this easy procedure.

A batch of 10 grapes was peeled off and the skin pressed obtaining 0.5 ml of essential oil and 580 mg of skin residue. Figure 12.7 shows spectra obtained under

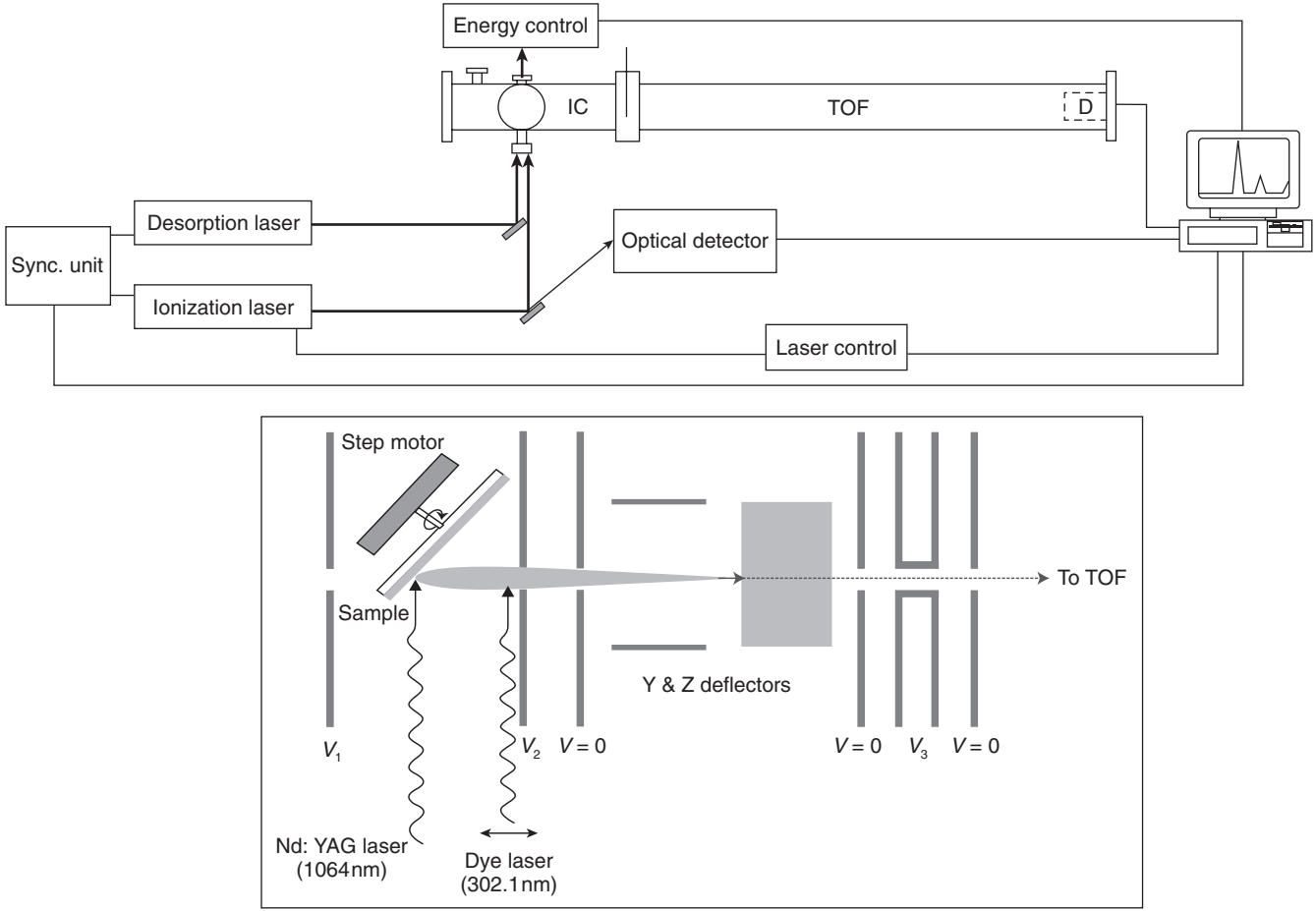

Fig. 12.6 Schematic view of the experimental set-up for the LD + REMPI–TOFMS technique. IC is the ionisation chamber, TOF is the time-of-flight tube. The inset shows the internal parts of the system and the interaction between the two laser beams.

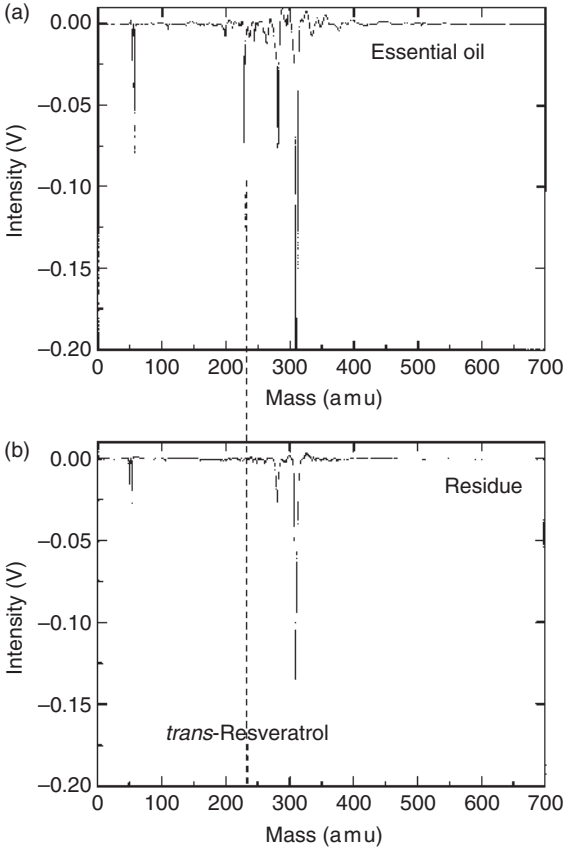

Fig. 12.7 (a) TOF mass spectrum of a sample of essential oil obtained by cold pressing the skin of 10 grapes (0.5 ml). (b) TOF mass spectrum under the same experimental conditions for the residue obtained from this sample after pressing (580 mg).

the same experimental conditions in both cases. While Fig. 12.7(a), obtained from the grape skin essential oil, shows an important signal for *trans*-resveratrol, no significant signal appears in the spectrum in Fig. 12.7(b), corresponding to the skin residue. Both spectra are noisier than usual owing to the fact that the experimental conditions were forced (up to 55 mJ per pulse of desorption energy) in order to be sure that no *trans*-resveratrol remained in the residue. This result confirms the validity of the sample preparation method for *trans*-resveratrol analysis by LD + REMPI–TOFMS in grapes.

Figure 12.8 displays a time-of-flight spectrum obtained from a sample of vine leaves using the same sample preparation method and under the usual experimental conditions. As before, it was confirmed that there is no *trans*-resveratrol remaining in the leaf residue after cold pressing. The resveratrol peak is clearly

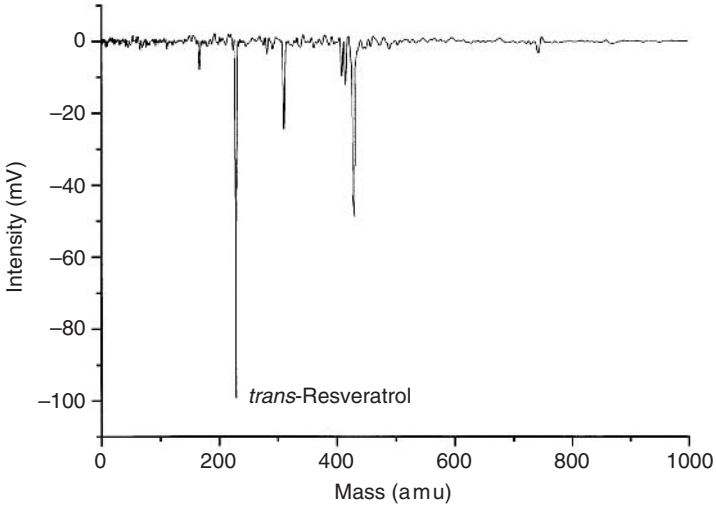

Fig. 12.8 TOF mass spectrum from a sample of grape leaves obtained under the usual experimental conditions ($E_d = 40$ mJ per pulse at 1064 nm; $E_i = 800$ μJ/pulse at 302.1 nm).

noticeable showing how the combination of selective ionisation plus the versatility of the time-of-flight spectrometry, allows clearly identification and analysis of one component without interference from the rest of the sample. This is, of course, one of the major advantages of the present technique.

For this sample the *trans*-resveratrol content has been determined using the standard additions method, that is, adding known quantities of *trans*-resveratrol to several identical samples of leaf essential oil; the value obtained for the intercept with the *x* axis gives the quantity of analyte in the blank. A value of 9 μg of *trans*-resveratrol per gram of leaf was obtained, that is, 9 ppm *trans*-resveratrol. Although this value seems low when compared with other published values, it is not so if the natural evolution of *trans*-resveratrol content in the vine plant is considered. *trans*-Resveratrol is produced at the beginning of spring to protect the plant against infection and declines with the seasonal evolution of the plant; thus, its production is optimum in young leaves during June and July.[127] In grapes, the *trans*-resveratrol content declines with maturity and it is near zero in mature fruit.[128] In this case, the experiments were done in December after the harvesting of the grapes, so it is not surprising to find a very low concentration of *trans*-resveratrol in the vine leaves.

The detection limit for the analysis of *trans*-resveratrol in vine leaves has been calculated from this spectrum. The detection limit of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system; generally this is assumed to be three times the noise level. From here a detection limit better than 0.002 ppm, that is 2 ppb of *trans*-resveratrol in vine leaf, was calculated, which is consistent with the limit previously found for

grape skin. This value is consistent with previous work, also from the authors' group, in which a detection limit of 5 ppb of *trans*-resveratrol in grape skin was reported[125] and appears to mark the current state of the art for thermally labile and non-volatile chemical analysis.

The technique has been applied for screening the post-harvest elicitation of resveratrol in grapes by several external agents. Consequently several experiments were conducted in which exogenous application of resveratrol to several fruits maintained their post-harvest quality, as will be shown in section 12.7.

12.5 Methods for improving natural resistance in fruits

The role of plant pathology in so-called integrated pest management (IPM) is outlined next. Large parts of fruits have to be stored for more or less extended periods of time before they are sold to consumers, causing considerable losses from pathogen attack and natural senescence. Well established solutions for improving this situation based on the use of synthetic pesticides are not free of problems owing to human health risks and environmental effects caused by chemical pesticides. New strategies for solving these problems are based on the development of methods to improve the natural plant resistance by using their own natural processes of pest suppression to control spoilage.

A considerable number of investigations have been conducted on the identification of these secondary plant metabolites and on understanding host–parasite interactions.[129–131] For example, since 1990 many studies have been published on the development of disease-resistant transgenic plants.[132–138] However, a comprehensive genetic analysis of host–pathogen interactions is in many cases still impractical, such that a more classical phytopathologic approach to the activation of plant defence responses is still in use.[139,140]

In what follows, selected examples are presented to provide state-of-the-art in post-harvest science studies using different approaches for improvement of the natural resistance in fruits. The following topics will be covered:

1. stress-induced methods: anoxic and other treatments
2. direct exogenous application of plant phytoalexins as natural pesticides
3. fruit decay inhibition by prestorage heat treatment
4. disease-resistant transgenic plants.

12.6 Anoxic and other treatments

One of the solutions used to decrease post-harvest losses is controlled atmosphere storage; certain crops are stored under different conditions (low oxygen concentration, low temperature, high CO_2 concentration, etc.) to slow down metabolic processes like ripening. With this in view, it is important to monitor the

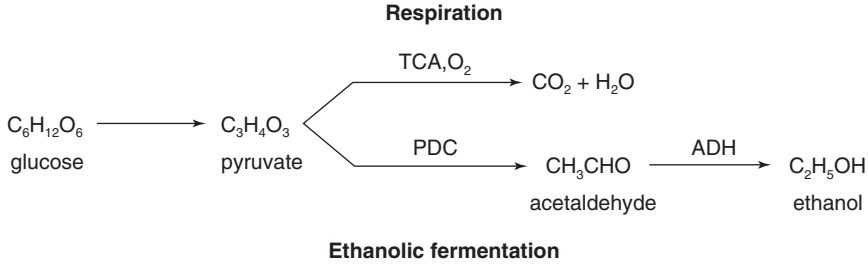

Fig. 12.9 Respiration and ethanolic fermentation processes.

metabolic responses of harvested crops under these conditions in order to develop new storage systems.

One of the processes under study is the balance between respiration and alcoholic fermentation, which is dependent on oxygen concentration conditions.[71,141,142] Under normal aerobic conditions (21% O_2), plants produce energy through respiration by oxidation of pyruvate, through the tricarboxylic acid cycle (TCA) and oxidative phosphorylation in presence of the atmospheric O_2, to CO_2 and water. Under anoxic conditions, the plant has to produce ATP (adenosine triphosphate) with no consumption of O_2 by different fermentation processes; this phenomenon is very common in nature, for example under flooding or ice-encasement conditions, and plants have developed different fermentation pathways that play an important role in survival during long periods of anoxia. One of the most common fermentation pathways is ethanolic fermentation, in which pyruvate is first decarboxylated to acetaldehyde by pyruvate decarboxylase (PDC) and this is then quickly reduced to ethanol by the action of alcohol dehydrogenase (ADH). Both processes are schematically shown in Fig. 12.9.

As the balance between the two processes depends on the oxygen concentration, study of the parameters determining the respiration to fermentation ratio is very important in order to optimise crop storage at low O_2 concentration. For this reason, the emission and effects of ethanol and acetaldehyde under anoxic and/or hypoxic conditions have been of outstanding interest.[143–145]

Acetaldehyde is, in general, toxic to plant cells owing to its high reactivity. Several investigations have been conducted on its effects on the ripening processes by the direct exogenous application of acetaldehyde and/or ethanol.[146] In tomato, low acetaldehyde concentration has been shown to inhibit ripening but the results are dependent on the initial fruit maturity, the applied concentration and the duration of exposure; in contrast, acetaldehyde accelerated senescence in pears and blueberries.

One important factor to have in mind when storing crops in low oxygen controlled atmosphere is the restoration rate of the aerobic conditions. After a period of oxygen deprivation, re-exposure to air can cause important damage to plant

tissues, in some cases being more detrimental than the lack of oxygen itself.[147] The causal agent of this post-anoxic injury in plant tissues is acetaldehyde. During anoxia, the plant obtains its energy through alcoholic fermentation and consequently ethanol is being accumulated in the tissues. When re-exposed to oxygen, the ethanol is oxidised to acetaldehyde, which is believed to be responsible for this post-anoxia injury. Acetaldehyde emission takes place a few minutes after the recuperation of normoxic conditions, as has been demonstrated for red peppers,[19] a clear indication that the ethanol oxidation is due to rapidly formed active oxygen species (AOS) like hydrogen peroxide.[148,149] It has been shown that gradually restoring normoxic conditions could reduce the adverse effects of re-aeration. This has been proved, for example, in red bell pepper by measuring the post-anoxic upsurge in acetaldehyde emission as a function of the restoration rate of the O_2 concentration,[60] as can be seen in Fig. 12.10.

Figure 12.10(a) shows the acetaldehyde emission of a red bell pepper under anoxic conditions; the onset of the fermentation is clearly noticeable by the plateau about 3.5 hour after the insertion of the fruit into the anoxic environment. As indicated above, switching directly to a normoxic atmosphere leads to a sudden rise in the acetaldehyde concentration; in this example an attempt to suppress this acetaldehyde upsurge was made by a post-anoxic addition of only 0.4% O_2, but even this low O_2 concentration yielded to a high release of acetaldehyde for about 20 min, as is clearly shown in Fig. 12.10(a). In subsequent experiments, (Fig. 12.10b) lower O_2 concentrations were used in the re-aeration of the red bell pepper sample. After about 9 hour under anoxic conditions, 0.05% O_2 was introduced into the cuvete, leading to only 20% increase in the acetaldehyde emission in 1 hour; from this point gradually increasing the O_2 content produced a smooth decrease in the acetaldehyde production.

This finding opens the way to subsequent investigations into optimising the conditions under which normoxic atmosphere is restored in low oxygen CA storage facilities, aiming to suppress the acetaldehyde upsurge and, consequently, pos-anoxic injury in fruits and vegetables. In later investigations by the same group, the acetaldehyde upsurge could not be prevented in avocado fruits by slowly restoring normoxic conditions after anoxia.[150] Obviously, further studies are still necessary to devise general methods to suppress post-anoxic injury in plants.

Anoxic treatments have been also investigated in the authors' laboratory aiming to elicit phytoalexin *trans*-resveratrol in post-harvested grapes. The results obtained showed an increase in the resveratrol content with treatments up to 24 hour, but the time course of evolution shows that high resveratrol content is better maintained after short anoxic treatments (i.e. 6 hour).

Finally, a development in the effects of inducing stress in fruits (namely grapes) uses the UV irradiation on the fruit.[151–155] A good example of this method is that developed by Cantos *et al.*[156] in which an 11-fold enhancement of the resveratrol content was achieved 3 days after a very short (30 s) irradiation of the grapes at $\lambda = 534$ nm, leaving the main sensory characteristics of the fruits unchanged. On the other hand, it has been shown that UV irradiation can reduce

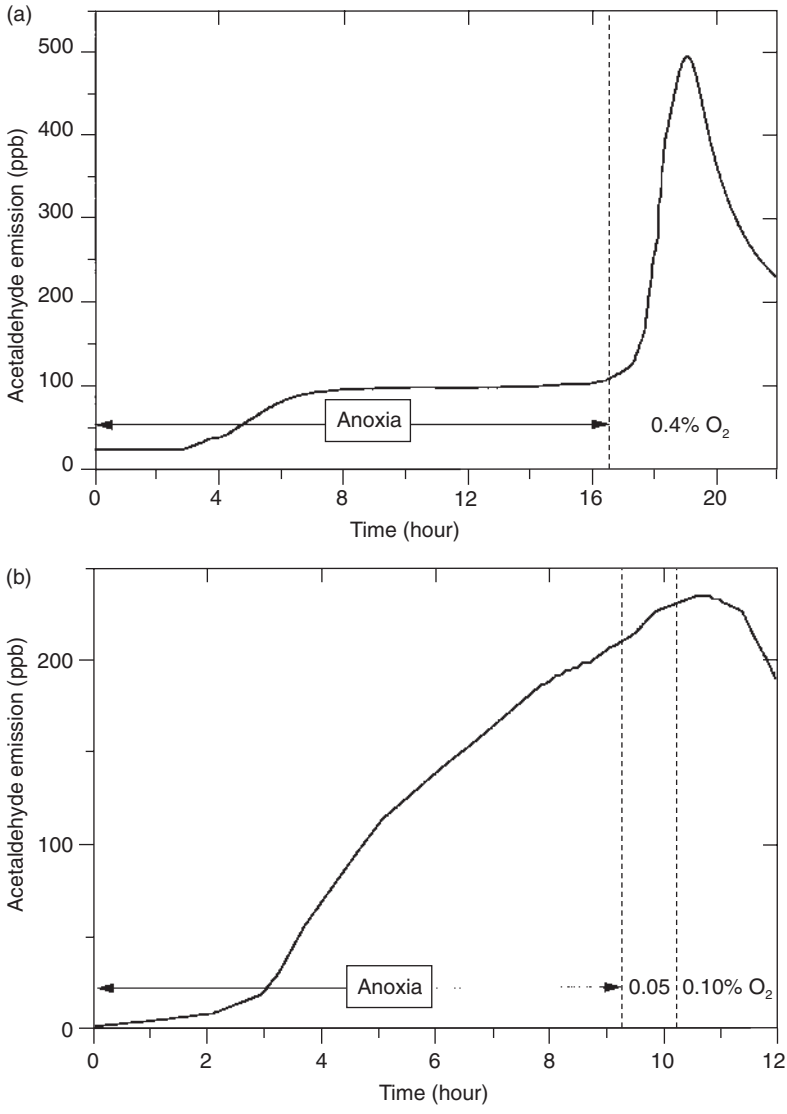

Fig. 12.10 (a) Acetaldehyde release in a red bell pepper under different oxygen conditions. The post-anoxic acetaldehyde upsurge caused the introduction of only 0.4% O_2 is clearly noticeable. (b) Suppression of the post-anoxic acetaldehyde upsurge in a red bell pepper by the gradual restoration of the oxygen concentration (adapted from Oomens *et al.*[60]).

post-harvest decay of table grapes.[157,158] The latter studies only investigated the time evolution of the damaged grapes and no chemical analysis was performed. However, the correlation between the enhancement of the natural resistance of the grapes observed and the elicitation of the resveratrol content is clearly demon-

strated in references 151–156. An example of this correlation is shown in the next section.

12.7 Application of plant phytoalexins

As indicated earlier, at the time of writing tens of thousands of secondary plant metabolites have been identified and there is a growing evidence that most of these compounds are involved in the defence mechanisms of plants, representing a large reservoir of natural pesticides to be used for pest control.[159] In the particular case of vine plants, one of the most important mechanisms for their resistance to fungal diseases involves the synthesis of *trans*-resveratrol as a response to the infection.[160–162]

Most of the investigations on the fungitoxic character of resveratrol have been carried out on its role against *Botrytis cinerea*, but resveratrol has also shown to enhance the resistance of vine plants to other species such as *Rhizopus stonifer*,[155] *Plasmopara viticola*[163] and *Phomopsis viticola*.[164] This rather unspecific antifungal characteristic and the selective accumulation of *trans*-resveratrol in grape skin makes it a good candidate as a 'natural pesticide' against pathogen attack and therefore for improving the natural resistance of grape to fungal infection.

To demonstrate this possibility several grape bunches were immersed for 5 s in a water solution of resveratrol (1.6×10^{-4} M). A similar number of bunches were immersed in bidistilled water for the same time period. After this short treatment, the fruits were kept in open air at room temperature. The results obtained with white grapes (Aledo variety) are shown in Fig. 12.11.[165,166] The picture was obtained ten days after treatment and significant differences can be noticed in the two set of bunches; while the resveratrol-treated bunches still maintained a physical aspect with no sign of losses or deterioration, the untreated ones were not only dehydrated but clearly infected and had deteriorated with local development of fungi.

This result opened the way to subsequent investigations of other fruits. In fact, the phytopathogenic fungus *B. cinerea* can infect a huge range of host plants with no apparent specialisation (berry fruits, horticultural vegetables, monocotyledons, bulbs, ornamentals, etc.), being able to infect more than 235 identified plant species.[167] In addition the grapevine genes encoding for the resveratrol synthase have been transferred to plants which usually do not produce this compound like tobacco,[135,136] rice[137] and tomato[138] with satisfactory results; the antifungal activity of *trans*-resveratrol was transferred to the transgenic lines, obtaining more resistant plants.

In order to demonstrate the capabilities of resveratrol as a natural pesticide, subsequent work was conducted on the application of resveratrol to fruits other than grapes with similar results, except for the decay time. The main results were obtained for apples, tomatoes, avocado fruit and peppers. This interesting finding opens the way to maintaining the post-harvest fruit quality by exogenous application of resveratrol.

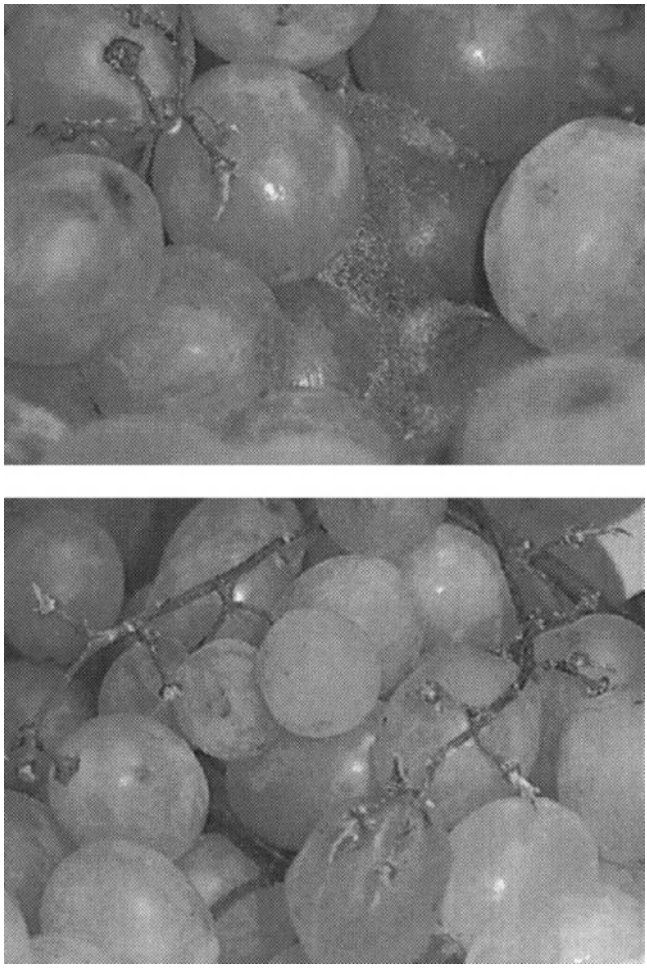

Fig. 12.11 (a) Bunch of Aledo grapes immersed for 5 s in bidistilled water after 10 days of storage at room temperature. (b) Bunch of Aledo grapes immersed for 5 s in a 1.6×10^{-4} M of resveratrol and stored under the same conditions. Their different health status is evident.

It is interesting to note that although some authors have claimed that the risks to human health related to the consumption of natural chemicals in foods are even greater than the risks from pesticide residues,[168,169] the lack of toxicity of resveratrol has been already demonstrated. Effectively, one of the main stages in the development of new natural pesticides is the study of the toxicological and environmental properties of the compound to be used.[159] Biological control agents are some of the more interesting alternatives to the use of harmful chemical pesticides, but they have to be demonstrated to be safe for human consumption. In the case of resveratrol a considerable number of investigations are currently focused

on the health benefits of resveratrol consumption (see references 170–173 for reviews on this subject) giving it an added value as a natural pesticide.

12.8 Prestorage heat treatment

Heat treatment of harvested products has been used for many years for disease control and insect disinfection in fruits and vegetables. Although common on the commercial scale since the beginning of the twentieth century, its use was abandoned with the development of synthetic and chemical pesticides. Nowadays, with increasing consumer awareness about the use of agrochemicals, as already indicated, there has been a resurgence of interest on post-harvest heat treatment, one of the most promising non-chemical treatments for preservation and maintenance of the quality of horticultural products.[174]

In general, classical heat treatment can be divided into short treatments (up to 60 min in water at 45–60°C) or long treatments, also called 'curing treatments' (12 hour to 4 days in vapour or dry air at 38–46°C).[175] Although curing treatments were the first to be used and their beneficial effects in reducing crop decay and improving its shelf-life have been widely demonstrated, the technique is difficult to implement on a commercial scale owing to the high cost of heating large volumes of fruit for long periods of time. The beneficial effects of prestorage hot water dipping have been also demonstrated in several fruits and vegetables.[176]

A technology implemented in this field is a method for simultaneously cleaning and disinfecting fresh harvested products by a short hot water rinse and brushes (HWRB) treatment.[177] The treatment, which is designed to be a part of the producer's sorting line, consists of placing the crops on rotating brushes and rinsing them with hot water (at variable temperatures depending on the type of fruit) for a very short time (typically 10–30 s).[174]

One of the main effects of heat treatment, including HWRB, is disinfection. Crop decay is inhibited by the direct cleaning action of water plus the lethal action of heat on the pathogens at the surface of the fruit; the latter can be enhanced by the addition of fungicides in the water used for the treatment, thus improving the action of the agrochemical compound and consequently minimising the doses that need to be used.[178,179] However, disinfection does not explain by itself the observed decay inhibition, as it is not enough to prevent further infection during storage. It has been found that heat treatment can also have an indirect effect through the induction of defence responses in the treated fruits.[180–182]

An important part of this enhanced resistance is the improvement in wound healing. Heat melts the fruit epiticular waxes, filling the cracks in the cuticle and avoiding their use as invasion sites by pathogen agents. Moreover, germinated spores in these micro wounds are encapsulated and inactivated by the action of the molten wax.[177,183] Apart from this physical effect, some physiological responses in the fruit after heat treatment have been described; among them are induction of pathogen-related (PR) proteins such as chitenase or β-1,3-glucanase; stabilisation of cell membranes; elicitation of antifungal compounds; inhibition

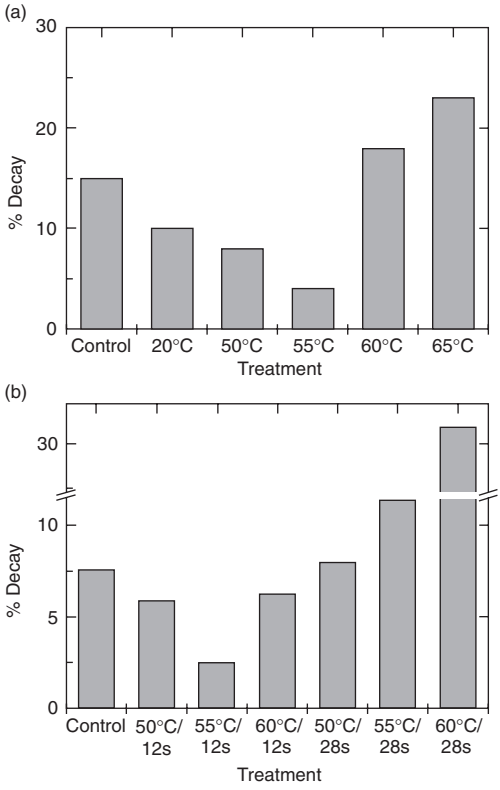

Fig. 12.12 (a) Effect of HWRB treatments at different temperatures on decay incidence in Golden Delicious apple fruits, after 4 months of storage at 1°C and 10 days at 20°C (adapted from Fallik *et al.*[185]). (b) Effect of HWRB treatment at different temperatures and time on decay incidence of sweet peppers, after 14 days of storage at 7°C and 3 days at 20°C (adapted from Fallik *et al.*[177]).

of the synthesis of wall hydrolytic enzymes (polygalacturonases); and/or delay in the degradation rate of preformed antifungal compounds present in the unripe fruits.[174,184]

HWRB treatments have to be optimised for each crop, because the parameters to be used (namely, time and temperature) depend on the type of treated fruit or vegetable. Two examples are shown in Fig. 12.12.

Figure 12.12(a) shows the effects (as measured by the percentage of decay incidence) of 15 s HRWB treatment on apple fruits (Golden Delicious)[185] at five different temperatures; the treatments were done immediately after harvest and the decay was measured after 4 months of storage at 1°C plus 10 days at 20°C. Inhibition of decay increases with the temperature up to 55°C, where 70% more fruit preservation with respect to the control (untreated) group was obtained. At higher temperatures the treatment produced heat damage to the fruits and the decay in these groups was even higher than in the control. Figure 12.12(b) shows

the effects of both temperature and time by demonstrating the incidence of decay in sweet pepper after HRWB treatment at three different temperatures (50, 55 and 60°C) and twice at each temperature (12 and 28 s).[177] Twelve second treatments gave similar results to those obtained with the apples; the optimum temperature was found to be 55°C allowing 73% more fruit preservation than in the control samples (which were only cleaned with dry brushes). In contrast, 28 s treatments gave negative results in all cases mainly caused by heat damage of the fruits produced by the longer treatment. This was especially the case for the samples treated at 55 and 60°C, in which 5% and 40% damage was found, respectively.

Decay percentage is not the only parameter to be considered in assessing the effectiveness of a short HWRB treatment on a given crop; other parameters like fruit quality measurements (firmness, colour, brightness, total soluble solids, titrable acid, heat damage, etc.), respiration (CO_2 production) and ethylene emission, effect of the treatment on fruits after inoculation with the most common diseases, and so on are also being studied. At present the technique has been implemented on peppers,[177] melons,[186] mangoes,[187] litchis,[188] organically grown grapefruit[183] and apples.[185]

12.9 Disease-resistant transgenic plants

A further approach to the reduction of global post-harvest losses caused by pathogens is the introduction of disease-resistant genes in agriculture. Specifically, plant resistant genes (usually referred as R-genes) are the most extensively used genes for the development of disease-resistant transgenic plants.[189] Since the demonstration of the enhanced disease resistance of transgenic plants in the earliest 1990s, there have been many studies on the characterisation of hundreds of R-genes, aiming to optimise R-gene mediated resistance by means of genetic engineering.[131–138,190,191] It is outside of the scope of this chapter to deal with the genetic approach or with the development of genetically modified plants with enhanced resistance to decay (interested readers can refer to reviews on the subject).[192–194] Nevertheless, a few comments are worth making that are essentially related to the production of transgenic plants which may help in the development of physical methods, specifically laser-based detection methods and, subsequently, may contribute to new protocols to improve the post-harvest resistance of crops.

A good example of this strategy is the development of transgenic plants that have altered characteristics of volatile emission. Along this line of research the group of Kuhlemeier in Berne have developed transgenic tomatoes plants that display enhanced production of the volatile compound acetaldehyde. Comparative analysis of such a volatile emission from infected transgenic and wild type tomatoes carried out by the LPAS method clearly indicated that acetaldehyde emission in the transgenic fruit took less time compared with the wild type. This decrease can be understood by considering the possibility that the transgenic fruit uses this component more immediately and more efficiently for other processes

during interaction with the pathogen, in other words, other reactions control the defence mechanism against the pathogen. This is therefore a good illustration where the interplay between the genetic and physical approach contributed to clear identification of acetaldehyde as a potential antibiotic for improvement of plant resistance to pathogenesis.

12.10 Conclusions and future trends

The aim of this chapter has been to give a general view of current concepts of the defence mechanisms in plants and the application of this knowledge to the improvement of natural resistance in fruits. The study of plant defence mechanisms is based on two well-established and interconnected approaches: the physicochemical (classical) and the genetic. The twentieth century began with the discovery that plants can produce specific antifungal substances as a response to fungal attack and ended with the development of disease-resistant transgenic plants. A considerable number of investigations have been conducted on the nature of host–parasite interactions, the identification of the secondary plant metabolites and their specific properties regarding plant health, and on the development of disease-resistant transgenic plants, as has been reviewed here.

However, at the time of writing, crop losses continue to cause reductions of almost 20% in principal crops worldwide. The requirements of modern agriculture are far more restrictive than in the past, namely the inexorable demographic pressure and the need for more environmentally and toxicologically safe pesticides. Although new agrotechnology based on genetic engineering is one of the most dynamic branches of modern biotechnology, the interaction between plants and pathogens is of great complexity and, in many cases, is very specific to a given plant–pathogen combination. Thus, a comprehensive genetic analysis of host–pathogen interactions is in many cases still impractical, such that a more classical phytopathologic approach to the activation of plant defence responses will continue to be used.

The development of new laser-based techniques has had a tremendous impact on plant defence science and consequently on the improvement of natural resistance in fruits. Indeed, the high resolution of these techniques together with their capability to work on-line have made possible plant screening for secondary metabolites with unprecedented sensitivity. This, in turn, has allowed not only the characterisation of genetically modified plants with enhanced resistance to decay, but also the study in real time of the physiology and dynamics underlying the plant–pathogen interaction. Good examples of both types of application have been presented here, namely genetically modified tomatoes which exhibit enhanced antibiotic emission of acetaldehyde and, on the other hand, monitoring of resveratrol in *Botrytis* infected grapes.

Obviously, such a body of knowledge naturally evolves into the development of new treatments and protocols, which can even be commercialised, to improve the post-harvest health status of fruit. The external application of *trans-*

resveratrol to grapes and HWRB of various crops are excellent examples of this 'know-how' in post-harvest treatment.

Clearly the interplay between the so-called genetic and physicochemical approach will lead into vigorous developments in modern biology and more specifically in post-harvest science in the near future.

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13

Improving the shelf-life of vegetables by genetic modification

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13.1 Introduction

The shelf-life of raw or processed food is a measure of the time such products retain their optimum quality, during transport, storage and display at reduced or ambient temperatures. The rapid deterioration of fruit and vegetables during these times is an on-going problem for producers and retailers of food products, particularly of fresh produce, since it results in wastage as products rapidly become unsaleable. Consequently, any extension of shelf-life and improvements in and durability of quality of freshly harvested vegetables is of considerable benefit to both producers and consumers. Genetic manipulation technologies can be exploited to understand and, ultimately, to manipulate both preharvest and post-harvest ripening and senescence of fruits and vegetables.

13.2 Senescence of plant organs

Senescence is the final stage in organ development, involving a series of physiological and biochemical changes. Generally, it is regarded as a form of cell death, characterised by loss of pigments, lipids, total protein and RNA (Smart, 1994). Programmed senescence, in addition to abscission of existing organs, is used to counter the continuous generation of new organs by plant meristems (Bleecker and Patterson, 1997). It is a highly regulated process in which carbon, nitrogen and other nutrients are mobilised and transported to specific parts of the plant, such as seeds, fruits, roots and young leaves (Weaver *et al.*, 1997). This remobilisation allows recycling of the nutrients to accommodate growth and, ultimately, seed production. Consequently, cells of the vascular system through

which nutrients are transported, are the last to senesce (Buchanan-Wollaston, 1997). Senescence of leaves is under strict nuclear control and follows a distinct pattern, in which there is a progressive loss of cellular compartmentalisation.

An early event in cell senescence involves the breakdown of chloroplast membranes, which contain 50% of the protein and 70% of the lipids in leaves. There is also degradation of pigments, particularly chlorophyll, resulting in a yellowing of senescing tissue, together with a progressive loss of other proteins associated with chloroplasts (Bleecker and Patterson, 1997). Such events culminate in a reduction and eventual cessation of photosynthesis. A disproportionate loss of chlorophylls compared to carotenoids accounts for the yellowing of leaves during early senescence (Biswal, 1995). Cytoplasmic volume and the number of cytoplasmic ribosomes decline, ultimately resulting in a decrease in ribosomal RNA and protein synthesis as the endoplasmic reticulum and polysomes disintegrate. Some organelles, such as mitochondria and nuclei, remain intact until late in senescence (Nooden and Guiamet, 1996).

Since senescence requires energy (Buchanan-Wollaston, 1997), cells must have protective mechanisms to maintain their respiratory and transcriptional machinery during this process. Thus, maintenance of mitochondria allows continued respiration to provide energy (Smart, 1994), while nuclei remain intact in order to permit transcription of genes involved in degradative pathways. Such transcription leads to the recovery of cellular components. Eventually, vacuolar membranes degenerate, releasing proteolytic enzymes into the cytosol. This represents one of the final stages of the degradative processes associated with senescence.

13.3 Genetic control of leaf senescence and fruit ripening

Leaf senescence involves profound changes in gene expression. Whilst the expression of most genes is down-regulated during senescence, expression increases in the case of those genes associated with the control of this process (Nam, 1997). A large number of such senescence-associated genes (SAGs) (Gan and Amasino, 1997) have been identified using differential screening, subtractive hybridisation techniques and, more recently, enhancer trap lines of *Arabidopsis thaliana* (He *et al*., 2001).

Some SAGs share sequence similarity with genes expected to be involved in the breakdown and mobilisation of nutrients, such as proteases, RNAses and glutamine synthetases, while the function of others has yet to be determined (Ori *et al*., 1999). In oilseed rape (*Brassica napus*) for example, the SAGs identified to date include the two cysteine proteases, LSC7 and LSC790, an aspartic protease (LSC760), a glutamine synthetase (LSC460), an ATP sulphurylase (LSC680), catalase (LSC650), metallothionein II (LSC210), ferritin (LSC30) and an antifungal protein, LSC212 (Buchanan-Wollaston and Ainsworth, 1997).

Glutamine synthetase converts ammonia to glutamine, ammonia being derived from the deamination of amino acids and the catabolism of nucleic acids during senescence. Glutamine and asparagine are the predominant amino acids in the

phloem during senescence and are regarded as the main transportable amino acids (Buchanan-Wollaston and Ainsworth, 1997). ATP sulphurylase is involved in the biosynthesis of cysteine and methionine. It has been proposed that during senescence the up-regulation of ATP sulphurylase leads to a subsequent increase in the cysteine pool. Cysteine is the precursor for glutathione biosynthesis, a major antioxidant, which, in addition to its role in the recovery of ascorbate and scavenging of reactive oxygen species (ROS), also acts in the transport and storage of sulphur (Rennenberg, 1982), the regulation of cell division and development (Earnshaw and Johnson, 1985), the regulation of gene expression and signalling (Wingate *et al*., 1988; Herouart *et al*., 1993; Moran *et al*., 2001) and the detoxification of xenobiotics and heavy metals (Delhaize *et al*., 1989; Halliwell and Gutteridge, 1986; Timmerman, 1989).

Several differentially regulated isoforms of catalase have been described, many of which exhibit increased expression during senescence (Buchanan-Wollaston and Ainsworth, 1997). In addition to its antioxidant role, catalase has been demonstrated to stimulate respiratory activity, resulting in a net increase in ATP production (Rodriguez *et al*., 2000). Metallothionein, like catalase, may have an antioxidant role, protecting DNA from ROS, the latter being generated as a result of the degradative processes occurring during senescence. Ferritin also has a proposed antioxidant role during senescence.

It has been shown that the production of hydroxyl radicals ($OH^{\bullet}$) depends on the presence of free iron within cells (Halliwell and Gutteridge, 1986, 1999) and that such hydroxyl radicals can result in damage to all classes of biologically important macromolecules, particularly nucleic acids (Deák *et al*., 1999). Thus, the control of free iron concentrations within cells through incorporation into ferritin is of paramount importance if ROS are to remain below lethal concentrations. Most non-metabolisable iron within plant cells is sequestered in ferritin.

Additional SAGs found in *B. napus* include those for metallothionein I (LCC54) (Buchanan-Wollaston, 1994) and a chitinase (LSC222) (Buchanan-Wollaston and Ainsworth, 1997). Three SAGs, namely SENU1, SENU4 and SENU5, have been shown to be up-regulated during foliar senescence in tomato (John *et al*., 1997). While the functions of SENU1 and SENU5 have yet to be determined, SENU4 encodes the pathogenesis-related protein, P6. Seven other SAGs, namely pTOMs 13, 31, 36, 66, 75, 129 and 137, have been identified, which, in addition to being up-regulated in tomato leaves during senescence, are also found to be up-regulated in tomato fruits during ripening (Davies and Grierson, 1989). Consequently, it has been proposed that senescence and ripening may involve the expression of common genes.

The function of pTOM13 has been associated with ethylene synthesis, but the function of the other pTOM SAGs has yet to be determined. However, it has been suggested that the expression of pTOM31, 36, 66 and 129 may be stress related (Davies and Grierson, 1989). Messenger RNA production by SAGs pTOM31, 36, 137, 13, 66 and 75 was also demonstrated to be ethylene dependent.

A SAG encoding the cytoplasmic form of glutamine synthetase was identified in radish (Kawakami and Watanabe, 1988), followed by a dark-inducible SAG,

din1, whose function was described tentatively as being pathogenesis-related (Azumi and Watanabe, 1991). In addition, a SAG encoding another cytoplasmic form of glutamine synthetase has also been reported in rice (*Oryza sativa*) (Kamachi *et al*., 1992). Two SAGs encoding for cytosolic glutamine synthetase (GS1) and chloroplastic glutamine synthetase (GS2) have been shown to be up-regulated in rice, although the abundance of the corresponding polypeptides did not correlate with the abundance of mRNA in rice leaves (Kamachi *et al*., 1992).

A SAG encoding the glyoxysomal enzyme, malate synthase (MS), is present in cucumber (*Cucumis sativus*) (Graham *et al*., 1992), while a senescence-associated receptor-like kinase (SARK) SAG has been described in bean (*Phaseolus vulgaris*) (Hajouj and Gepstein, 2000). SARK gene expression is induced by ethylene, but delayed by cytokinins. Furthermore, since the SARK polypeptide has similarities to other kinase receptors associated with signal transduction pathways, it has been suggested that SARK expression may regulate some pathways of the senescence process (Hajouj and Gepstein, 2000).

In *Arabidopsis*, amongst the many SAGs described, are those encoding a plastidial form of glutamine synthetase, Atgsr2 (Bernhard and Matile, 1994), an RNase, RNS2 (Taylor *et al*., 1993), a polyubiquitin, pSEN3 and a peptide related to endoxyloglucan transferase, pSEN4 (Park *et al*., 1998). Using an enhancer trap approach, 125 potential SAGs have been identified in *Arabidopsis* (He *et al*., 2001), with three of these, Sel25 (SAG103), Sel139 (SAG101) and Sel142 (SAG102), having been cloned. SAG101 has been found to encode an acyl hydrolase; the functions of SAG102 and 103 have not been determined. The mRNA levels of both alphaVPE and gammaVPE, encoding vacuolar processing enzymes specific to vegetative organs, are up-regulated in primary leaves of *Arabidopsis thaliana* during senescence, in parallel to increases in the mRNA level of SAG2 (Kinoshita *et al*., 1999).

In tobacco, plants transformed with the *ipt* gene encoding cytokinin biosynthesis from the T-DNA of the Ti plasmid of the Gram negative soil bacterium *Agrobacterium tumefaciens*, exhibited delayed senescence when the gene was attached to a heat-shock promoter. Such transgenic plants were exploited to isolate senescence-specific cDNA clones expressed at specific stages of senescence (Cooper *et al*., 1996). The gene-expression profile of artificially induced senescence in detached leaves is very similar to natural senescence of intact leaves, with most SAGs exhibiting the same pattern of expression (Hajouj and Gepstein, 2000). This is an important observation, given that most work performed, to date, on transgenic plants to record the events which occur when senescence is delayed has been conducted with assays involving detached leaves, sometimes under conditions of nitrogen starvation.

In fruit, it has been demonstrated that the changes that occur during ripening are due mainly to alterations in gene transcription. Several cDNAs associated with ethylene-driven ripening have been identified in tomato (Gray *et al*., 1992), while in banana (*Musa acuminata*), 11 groups of mRNAs have been documented that are expressed differentially during fruit ripening (Clendennen and May, 1997). Two of these mRNAs encode proteins involved in carbohydrate metabo-

lism, whereas others encode proteins associated with pathogenesis, senescence or stress responses.

Transcripts encoding endochitinase, β-1,3-glucanase, a thaumatin-like protein and ascorbate peroxidase, increased during the ripening of banana fruit, while transcripts encoding starch synthase, granule-bound starch synthase, chitinase, lectin and a metallothinonein II, decreased in abundance (Clendennen and May, 1997). Ripening-associated cDNAs have been isolated from Shiraz grape (*Vitis vinifera*) (Davies and Robinson, 2000). Some of these cDNAs have been shown to code for polypeptides involved in cell wall structure, such as proline-rich proteins, pectin methylesterases and glutamate-rich proteins. Others have been reported to be stress or ripening associated proteins, such as thaumatin-like proteins, metallothioneins, transcription factors, a cytochrome P450 enzyme and proteins induced by water, sugar and cold stress in other species (Davies and Robinson, 2000). In melon (*Cucumis melo*), expression of a variety of ripening-associated cDNAs has been studied in seven varieties which exhibited differences in their ripening behaviour (Aggelis *et al*., 1997). This investigation showed that varieties with delayed expression of 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) mRNAs exhibited delayed softening during ripening, thereby extending the shelf-life of harvested fruit.

13.4 Regulation of leaf senescence

The initiation of leaf senescence is regulated by various internal and environmental factors. Such environmental cues include day length, extremes of temperature, drought, water logging, nutrient deficiency and infection by pathogens (Smart, 1994). Leaf senescence allows reallocation of resources to reproductive organs to ensure that plants complete their life cycles, even under conditions of stress. Internal factors can also induce senescence, including leaf age, reproductive development and the concentrations of phytohormones. Consequently, senescence occurs even in the absence of environmental stress (Gan and Amasino, 1997). A range of phytohormones have been suggested to have a possible role in the initiation of leaf senescence, including auxins, gibberellins, ethylene, abscisic acid and cytokinins (Smart, 1994). Indeed, cytokinins have been implicated for some time in leaf senescence in many species (Nooden and Leopold, 1978; Nooden, 1980), with depletion of cytokinins in these organs being thought to trigger the cascade of events constituting this process.

13.5 Cytokinins and senescence

Cytokinins are believed to delay senescence by maintaining cellular integrity, particularly of the tonoplast membrane. This prevents proteases from the vacuole leaking into the cytoplasm and hydrolysing both soluble proteins and proteins of the chloroplast and mitochondrial membranes. Cytokinins may also act by

inhibiting free radical-mediated oxidation of membrane lipids (Lesham, 1992). Three main approaches have been used to study the effect of cytokinins in plant senecence, these involving the exogenous application of cytokinin solutions, measurements of endogenous cytokinins during senescence and transgene-encoded cytokinin biosynthesis. Assays reveal that the concentrations of endogenous cytokinins in plant tissues decline as senescence progresses (Van Staden and Joughin, 1988). The cytokinin content of the xylem sap in plants such as sunflower and soybean also decreases rapidly with the onset of senescence, which suggests that reduction in cytokinin transport from roots to shoots permits senescence to progress (Nooden *et al*., 1990).

The exogenous application of cytokinins can retard the senescence of detached leaves, although these growth regulators are often less effective in organs attached to the parent plant (Gan and Amasino, 1996). In this respect, external application of cytokinins, such as dihydrozeatin and benzyladenine, has been exploited commercially to extend the shelf-life of freshly harvested fruit, vegetables and cut flowers (Ludford, 1987; Salisbury and Ross, 1992).

An approach to retard senescence has been through the use of transgene-encoded cytokinin biosynthesis. Initially, this was studied in tobacco using constitutive or inducible overexpression of the *ipt* gene which encodes isopentenyl phosphotransferase. This enzyme catalyses the rate limiting step for *de novo* cytokinin biosynthesis (McGaw and Burch, 1995), in other words the addition of Δ^2-isopentenyl pyrophosphate to the N^6 of 5′-adenosine monophosphate to form isopentenyl adenosine 5′-monophosphate (Chen, 1997). Isopentenyl phosphotransferase is highly labile and, to date, has not been purified from plants.

Isopentenyl adenosine 5′-monophosphate is the precursor of all other cytokinins, of which the three most commonly detected and physiologically active forms in plants are isopentenyladenine, zeatin and dihydrozeatin (Salisbury and Ross, 1992; Mok and Mok, 1994). However, whilst overexpression of the *ipt* gene in transgenic plants resulted in elevated foliar cytokinin concentrations and delayed leaf senescence, the high cytokinin concentrations were largely detrimental to growth and fertility (Medford *et al*., 1989; Yusibov *et al*., 1989; Smart *et al*., 1991; Li *et al*., 1992; Hewelt *et al*., 1994; Macháková *et al*., 1997; Wang *et al*., 1997a,b).

13.6 Ethylene and senescence

The plant hormone ethylene controls several developmental processes, including seedling growth and morphology, fruit ripening, abscission and senescence (Hackett *et al*., 2000). Ethylene is synthesised from *S*-adenosyl-L-methionine through the activity of the enzymes 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase. ACC synthase (ACS) converts *S*-adenosyl-L-methionine to ACC, which is then degraded to ethylene by ACC oxidase (ACO). Inhibitors of ethylene biosynthesis and activity, such as amino-oxyacetic acid and silver thiosulphate, delay or prevent senescence (Savin *et al*., 1994). Ethylene has

been demonstrated to induce the expression of SAGs (Davies and Grierson, 1989; Park *et al*., 1998; Weaver *et al*., 1998; Kinoshita *et al*., 1999; Hajouj and Gepstein, 2000). A peroxidase, which is up-regulated during ethylene-induced senescence (Abeles *et al*., 1988; Morgens *et al*., 1990), has been identified in and cloned from cucumber (*Cucumis sativus*). The ethylene-insensitive mutant, *etr1-1*, of *A. thaliana*, has been shown to exhibit a 30% increase in longevity before the onset of senescence, when compared to wild-type plants. This delay in senescence coincided with delayed induction of SAGs and higher expression levels of photosynthesis-associated genes (Grbic and Bleecker, 1995).

13.7 Reactive oxygen species and senescence

There is evidence that peroxidase activity and concentrations of ROS, such as hydrogen peroxide (H_2O_2), increase during senescence and fruit ripening (Hung and Kao, 1998; Lacan and Baccou, 1998; Lin and Kao, 1998; Yamane *et al*., 1999; Lester, 2000; Eskin and Robinson, 2001). In respiring cells, up to 5% of the total oxygen may be reduced to form ROS (Eskin and Robinson, 2001). During post-harvest storage, particularly of processed material, the percentage of oxygen reduced to form ROS increases.

Senescence-induced loss in the chemical composition of chloroplast thylakoids with an associated decline in chlorophyll-*a* fluorescence, may lead to quanta overloading of chloroplast pigments (Biswal, 1995), resulting in photoinhibition and excess electrons being diverted to the formation of ROS, such as singlet oxygen ($^1O_2$). As carbon dioxide is the sink for electrons generated in light reactions in chloroplasts, the formation of ROS is increased by the loss in Calvin cycle efficiency and the degradation of Rubisco during senescence. The alteration of thylakoid structure during senescence results in the release of free chlorophyll and the production of triplet chlorophyll (^{3}Chl*) which, in turn, produces $^1O_2$. Singlet oxygen is known either to oxidise carotenoids directly, or to contribute indirectly to their degradation (Biswal, 1995).

In plants, H_2O_2 inhibits the assimilation of carbon dioxide at low concentrations (Halliwell and Gutteridge, 1999) and is also active with mixed-function oxidases in marking several enzymes for proteolytic degradation. Other ROS, such as superoxide ($O^{\bullet}{}_2^-$) can inactivate some metal-containing enzymes, particularly those containing accessible —SH groups, causing damage to amino acids and loss of protein function (Davies, 1995). Furthermore, H_2O_2 and $O^{\bullet}{}_2^-$ interact via the Haber–Weiss reaction to produce the hydroxyl radical ($OH^{\bullet}$), an extremely reactive ROS. Hydroxyl radicals can initiate self-propagating reactions leading to cellular damage, in particular, the peroxidation of membrane lipids. The latter process has been recognised as a key factor in the loss of membrane selective permeability and fluidity during senescence, leading eventually to loss of cellular integrity (Hong *et al*., 2000).

It has been shown that the maintenance of cellular membrane integrity within mesocarp tissue of both netted and honey dew fruits of muskmelon (*Cucumis melo*)

is critical for regulating post-harvest senescence (Lester and Grusak, 1999; Lester, 2000). A comparison of the two muskmelon varieties, Clipper and Jerac, differing in their shelf-life, indicated that increased antioxidant activity correlated with the maintenance of selective permeability and integrity of membrane lipids, delayed senescence and extended shelf-life (Lacan and Baccou, 1998). Exogenous application of the free radical scavengers, sodium benzoate, propyl gallate and 3,4,5-trichlorophenol to carnation (*Dianthus caryophyllus*) resulted in a delay in the synthesis and the concentration of ethylene (Paulin *et al*., 1986). This was correlated with a delay in the production of peroxidases and the breakdown of membrane lipids resulting, ultimately, in delayed senescence and extended shelf-life. Similarly, exogenous application of the antioxidants L-cysteine, ascorbic acid, reduced glutathione and mercaptoethanol at concentrations of 10^{-3}–10^{-5} M, to spinach (*Spinacia oleracea*) and the three aquatic plants *Potamogeton pectinatus*, *Vallisneria spiralis* and *Hydrilla verticillata* arrested senescence as monitored by the retention of chlorophyll and protein (Jana and Choudhuri, 1987).

The activity of a number of antioxidant enzymes in spinach were assessed under conditions either inducing senescence (ethylene treatment), or those which prevented senescence [10% (v/v) of carbon dioxide, 0.8% (v/v) oxygen and 89.2% (v/v) N_2] (Hodges and Forney, 1999). In order to investigate the role that antioxidants play in the regulation or modulation of the dynamics of senescence in plant tissues, it has been suggested that the decline in the activity of ascorbate, ascorbate peroxidase and catalase over a 35 day storage period, regardless of the composition of the storage atmosphere, is a response to regulation by hydrogen peroxide. As a consequence, it has been proposed that hydrogen peroxide concentrations play an important role in the dynamics and severity of post-harvest senescence and, consequently, shelf-life in spinach (Hodges and Forney, 1999).

13.8 Flavour and shelf-life of vegetables

Organic derivatives corresponding to ROS are produced in cells, mainly in the form of oxidised unsaturated fatty acids during lipid peroxidation. These include lipid peroxy radicals ($LOO^{\bullet}$), hydroperoxides (LOOH) and alkoxyl radicals ($LO^{\bullet}$). Hydroperoxides decompose into a wide range of volatile and non-volatile products, which themselves can undergo further oxidation and/or decomposition, resulting in off-flavours associated with rancid products (Eskin and Robinson, 2001). Lipoxygenases generate flavour and aroma compounds, but also have the ability to form ROS. Consequently, they have also been implicated in the formation of off-flavours during food storage.

13.9 Plant transformation

Numerous protocols have been published for the efficient transformation of a wide range of crop species, with most of the procedures exploiting the use of

biolistics or the natural gene transfer mechanism of *A. tumefaciens* to effect transgene delivery into target plants. The molecular basis of plant transformation has been discussed in several excellent reviews (Pawlowski and Somers, 1991; Christou, 1995; Jaehne *et al*., 1995; Puddephat *et al*., 1996; Tinland, 1996; Wysokinska and Chimel, 1997; Ignacimuthu *et al*., 2000; Newell, 2000), together with transformation protocols for specific crops (Gartland and Davey, 1995; Davey *et al*., 2001, 2002).

13.10 Genetic modification of plants to improve shelf-life

In order to circumvent the detrimental effects of constitutive cytokinin overexpression, Gan and Amasino (1995) devised a strategy, based on autoregulated cytokinin production, which delayed leaf senescence in transgenic tobacco without altering plant phenotype. This strategy exploited the highly senescence-specific promoter, SAG12, from a gene encoding a cysteine proteinase of *A. thaliana* (Lohman *et al*., 1994) fused to the *ipt* gene (synonym *tmr* gene) from *A. tumefaciens* (Hidekamp *et al*., 1983). The chimaeric P_{SAG12}-*IPT* gene was activated only at the onset of senescence in the lower mature leaves of tobacco. This resulted in cytokinin biosynthesis in the leaves, which inhibited their senescence and, consequently, attenuated activity of the P_{SAG12}-*IPT* gene, preventing overproduction of cytokinin. Whilst, theoretically, the feedback system should be tightly regulated, there are reports that the P_{SAG12}-*IPT* strategy may not be so tightly autoregulated as was first expected in tobacco (*Nicotiana alata*) (Schroeder and Stimart, 1998) and lettuce (*Lactuca sativa*) (McCabe *et al*., 2001).

To date, the most detailed studies of the effect of *ipt* expression in P_{SAG12}-*IPT* transgenic plants has been in a limited number of members of the Solanaceae, such as tobacco (*N. tabacum*) (Gan and Amasino, 1996; Schroeder and Stimart, 1998; Jordi *et al*., 2000), although there are reports of the introduction of P_{SAG12}-*IPT* into rice (*Oryza sativa*) (Fu *et al*., 1998), cauliflower (*Brassica oleracea*) (Nguyen *et al*., 1998), *A. thaliana* (Zhang *et al*., 2000) and lettuce (Garratt *et al*., 2000, 2001a; McCabe *et al*., 2001). In particular, this strategy has been successful in the lettuce cv. Evola, delaying senescence during plant development and following harvesting of mature heads. Thus, in four homozygous transgenic lines assessed, senescent leaves were not present on any plants at the seedling stage or during later development. This trait was stably inherited over the three successive seed generations evaluated. In contrast, all corresponding azygous plants and non-transformed plants regenerated from leaf explants exhibited senescent basal leaves. Additionally, apart from retardation of leaf senescence, mature 60-day-old plants, corresponding to the age of plants from which heads are normally harvested commercially, were morphologically normal with no significant differences in head diameter or fresh weight of their leaves and roots. Following harvesting of heads at 60 days after seed sowing and storage for 7 days, the outer leaves of the heads of plants of the four homozygous P_{SAG12}-*IPT* transformed plants retained their chlorophyll. In contrast, the outer leaves of

heads from plants of the four azygous lines were yellow and necrotic after this storage period.

There are a number of potential applications of delayed senescence in P_{SAG12}-*IPT* modified lettuce. Since leaves retain their chlorophyll longer after harvesting, the most obvious application is extended post-harvest quality. Interestingly, homozygous plants also showed a significant reduction in susceptibility to infection by *Botrytis cinerea* (W.J.R.M. Jordi, unpublished) as this pathogen normally targets senescing tissues. Additionally, lettuce plants transformed with the P_{SAG12}-*IPT* gene remained green even when nitrates became depleted in the compost. On this evidence, it was therefore proposed that the expression of this transgene might also provide a strategy for reducing the nitrate content in cultivated lettuce. In this respect, removal of nitrogen from the growth medium 5 or 10 days before harvest of P_{SAG12}-*IPT*-transformed lettuce plants could result in up to 70% reduction in nitrate content with only a slight reduction in growth and no loss of leaf pigmentation and, hence, visual quality. Limits on the nitrate content of lettuce, particularly in Northern Europe, dictate that a reduced nitrate content is an important breeding objective for this crop (Gunes *et al*., 1994).

In addition to the SAG12 promoter, other senescence-specific promoters, such as SAG529 and SAG766A, have been used in the construction of chimaeric genes as part of a delayed senescence strategy for extending shelf-life. Such chimaeric genes have been used to transform broccoli (*B. oleracea*), resulting in the retardation of senescence, as measured by chlorophyll retention, following four days of post-harvest storage (Chen *et al*., 2001). Chimaeric *ipt* genes constructed using heat-shock promoters have also been used to delay senescence in an attempt to extend shelf-life (Medford *et al*., 1989; Smart *et al*., 1991; Smigocki, 1991; Ainley *et al*., 1993; Van Loven *et al*., 1993; Harding and Smigocki, 1994; Veselov *et al*., 1995; Cooper *et al*., 1995, 1996; Kudoyarova *et al*., 1999). However, the heat-shock process itself can affect growth and endogenous cytokinin concentrations (Van Loven *et al*., 1993; Wang *et al*., 1997a,b).

In other investigations, the 35S promoter from cauliflower mosaic virus (CaMV) has been used to control the *ipt* gene in transgenic tobacco and cucumber (*Cucumis sativus*) (Smigocki and Owens, 1988; Makarova *et al*., 1997a,b). However, in these cases, the constitutive expression of the *ipt* gene resulted in developmental abnormalities, including stunted growth and sterility.

Abnormalities have also been observed in plants transformed with the *ipt* gene with other constitutive promoters. For example, when a chalcone synthase promoter (P_{CHS}) from *Antirrhinum majus* was used to drive the *ipt* gene in transgenic tobacco (Wang *et al*., 1997a,b), transgene expression caused inhibition of root development, retardation of leaf senescence, elevation of chlorophyll levels and a delay in flower development and, as a consequence, the onset of flowering. Expression of the P_{CHN}-*IPT* gene also resulted in thicker stems resulting from concomitant enhancement of both cell division and cell expansion. In this respect, such phenotypic abnormalities are similar to those apparent during the later stages of development of tobacco and lettuce transformed with the P_{SAG12}-*IPT* gene (Gan and Amasino, 1996; Jordi *et al*., 2000; Garratt *et al*., 2000, 2001a; McCabe *et al*.,

2001), and are consistent with the overproduction of endogenous cytokinins. Cell enlargement observed in P_{SAG12}-*IPT* and P_{CHN}-*IPT* plants could be due to increases in water uptake, resulting from increased osmotic pressure. Such an increase in osmotic pressure would be consistent with sugar (hexose) accumulation characteristic of P_{SAG12}-*IPT* plants (Garratt *et al*., 2000, 2001a; McCabe *et al*., 2001). Tobacco transformed with a copper-inducible *ipt* gene (Cu-*IPT*) exhibited delayed senescence when treated with physiological concentrations of Cu^{2+} (McKenzie *et al*., 1998).

Delayed leaf senescence has also been achieved in transgenic tobacco, using the homeobox gene, *knotted1* (*kn1*), isolated from *A. thaliana*, fused to the senescence-specific promoter, pSAG12 (Ori *et al*., 1999). Normally, the *kn1* gene and its homologues are expressed in shoot meristems. Interestingly, the P_{SAG12}-*kn1* transformed plants exhibited delayed senescence with no significant developmental abnormalities. In addition to the delayed senescence phenotype, there were a number of other characteristics of these plants, which were also observed in P_{SAG12}-*IPT* transformed tobacco plants, the most striking of which was a significant increase in cytokinin concentrations in the leaves. It was proposed that *kn1* may act as a transcription factor, mediating the accumulation of cytokinin (Ori *et al*., 1999). Similarly, in the lettuce cv. Luxor, expression of PetE-*KNAT1*, an *Arabidopsis kn1*-like homologue under the control of the pea plastocyanin promoter PetE, also resulted in a delay in leaf senescence (Frugis *et al*., 2001).

During the onset of leaf senescence and fruit ripening, plasma membranes as well as the membranes of the endoplasmic reticulum, lose their selective permeability and fluidity (Hong *et al*., 2000), such changes being known to initiate programmed cell death (Thompson *et al*., 2000). This loss of selective permeability has been attributed to molecular perturbations in the lipid bilayers, resulting from the increase in the ratio of non-esterified to esterified fatty acids in the membranes. The de-esterification of these fatty acids is caused by the action of senescence-induced lipase (lipolytic acyl hydrolase) (Thompson *et al*., 2000). Furthermore, de-esterification of polyunsaturated fatty acids acts as a substrate for the action of lipoxygenase, which results in lipid peroxidation and, hence, progressive membrane rigidity and loss of functional integrity (Asada and Takahashi, 1987).

Transgenic plants of *A. thaliana* have been generated in which the expression of senescence-induced lipase has been down-regulated through the constitutive expression of the full length gene in its antisense orientation, under the regulation of a 35S promoter (Thompson *et al*., 2000). The resulting plants exhibited delayed leaf senescence, demonstrating that manipulation of lipase expression could also be an effective strategy for extending shelf-life.

In flowers, the antisense inhibition of the 1-aminocyclopropane oxidase gene in the carnation cvs. Red Sim and White Sim delayed petal senescence in transgenic plants (Savin *et al*., 1994), potentially extending vase-life. This inhibition of senescence corresponded to a significant reduction in endogenous 1-aminocyclopropane oxidase and ACC synthase mRNAs. Similarly, antisense

inhibition of the 1-aminocyclopropane oxidase gene in tomato has been demonstrated to delay the onset and rate of fruit ripening (John *et al*., 1995; Bolitho *et al*., 1997). More recently, a rab11/YPT3 homologue from tomato, encoding a guanacine tryphosphate (GTPase), believed to be involved in the control of protein trafficking within cells, has been down-regulated in transgenic tomato, using antisense inhibition (Lu *et al*., 2001). Fruit from plants expressing the antisense gene had normal pigmentation, but failed to develop a soft texture.

The manipulation of antioxidant biosynthesis in lettuce has been achieved using a construct consisting of chimeric genes encoding elements of the ascorbate–glutathione pathway (Garratt *et al*., 2001b). Overexpression of these transgenes enhanced the oxyradical scavenging potential and antioxidant content of transgenic plants. Homozygous plants exhibited up to a six-fold increase in foliar reduced glutathione compared to their azygous controls. Foliar hydrogen peroxide was up to three-fold lower in the upper leaves and up to two-fold lower in the middle and lower leaves of homozygous plants, compared to controls. Lipid peroxidation was also significantly decreased, indicating that membrane integrity was maintained. Furthermore, leaf discs excised from transgenic plants and floated on water for 7 days to induce senescence, expressed foliar hydrogen peroxide concentrations which were 40% lower than those concentrations detected in leaf discs excised from azygous (control) plants. The chlorophyll content of intact 60-day-old transgenic plants was significantly ($P < 0.05$) higher in the upper and lower leaves (>40% and 20%, respectively). As well as improving crop performance during growth, the stimulation of antioxidant capacity, which delayed peroxidation, enhanced the post-harvest performance of the transgenic lettuce plants, with an extension of shelf-life, together with an improvement in appearance and nutritional content.

In addition to delaying visible signs of senescence, attempts have been made to reduce or to delay the generation of off-flavours associated with the storage of food products. This is being achieved by the inactivation or inhibition of the enzymes responsible for producing such undesirable products, or by developing transgenic plants deficient in the undesirable enzyme(s). For example, improvement in the flavour, stability and hence shelf-life of preparations of soybean (*Glycine max*), specifically soy flour and soy milk, has been achieved by the removal of the enzyme lipoxygenase-2 (LOX-2) (Davies *et al*., 1987).

In the case of tomato (*Lycopersicon esculentum*) and tobacco, expression of the yeast Δ-9 desaturase transgene increased the concentration of most monounsaturated fatty acids in both these plants. Additionally, this decreased the concentration of saturated fatty acids in tomato (Polashock *et al*., 1992; Wang *et al*., 1996), leading to changes in the flavour profile of fruits of the transgenic plants. However, whilst this demonstrates the ability to alter flavour profiles by the genetic manipulation of fatty acids, this approach has not, as yet, been applied directly to extending shelf-life. In contrast, significant increases have been observed in the shelf-life of fruits of transgenic plants of tomato with antisense suppressed polygalacturonase activity (Sozzi-Quiroga and Fraschina, 1997). As well as being less susceptible to damage and infection, the transgenic tomato

fruits exhibited retarded over-ripening, but maintained normal development during pre-senescence. Sensory, physicochemical and biochemical monitoring indicated that standard preference ratings, as used by retail outlets, for these transgenic fruits were significantly superior compared to those of non-transformed plants, particularly in terms of fruit colour and flavour (Sozzi-Quiroga and Fraschina, 1997).

13.11 Assessments of plant quality

Assessment of the shelf-life qualities of transgenic leafy vegetables, such as lettuce, has usually involved experimentation with excised leaf disks under controlled laboratory conditions (Wingler *et al*., 1998; Garratt *et al*., 2001a; McCabe *et al*., 2001), for monitoring the retention of chlorophyll and protein. Whilst this approach usually gives an excellent indication of delayed senescence in any specific material, it is important to recognise that any dramatic differences in chlorophyll and protein retentions observed between leaf disks of transgenic and control plants may not always be perceived as easily in this system as at the whole plant level. Thus, it is important to perform comparative studies using material treated, presented and stored under conditions which are as close as possible to normal commercial transport and supermarket storage practices for fresh produce.

In addition to measurements of chlorophyll and protein retentions, parameters such as the evolution of hydrogen peroxide, lipid peroxidation rates and antioxidant activity (Garratt *et al*., 2001b,c), transpiration rates (Wang *et al*., 1997a,b) and biomass production (fresh weight) should also be evaluated, together with assessments of volatile and non-volatile contents of material during storage. Normally, taste assessments are performed on material undergoing shelf-life assessments. However, in general, such an approach is not currently feasible when dealing with transgenic materials. Consequently, assessments of the volatile constituents of the headspace above transgenic material are normally used as indicators of any changes in flavour profile (Roberts and Taylor, 2001).

13.12 Future trends

Approaches to achieving extended shelf-life have centred upon the manipulation of a few key regulatory pathways, such as cytokinin biosynthesis. However, it is clear that if tight control of senescence is to be achieved, without any associated detrimental effects on growth and fertility, the manipulation of elements downstream of cytokinin biosynthesis and other controlling factors needs to be addressed (McCabe *et al*., 2001). Indeed, in order to produce functional 'stay-green' leaves, modification will probably be required of several regulatory pathways (Wingler *et al*., 1998). Similarly, it has been proposed that studies of the differences in the expression of possible candidate genes in P_{SAG12}-*IPT* transformed and non-transgenic plants of lettuce or, indeed, in other species, may

reveal alternative pathways for genetic manipulation in order to achieve more efficient strategies for delaying senescence (McCabe *et al*., 2001). To date, there is little evidence of attempts to delay senescence being applied to root crops, with the exception of the generation of potato transformed with the *ipt* gene (Machá-ková *et al*., 1997). Certainly, the effects of altered carbohydrate partitioning in vegetable crops, such as potato and carrot, could prove interesting.

Rapid advancements in genomics and the application of microarray technology should facilitate the evolution and refinement of new approaches to manipulating and extending shelf-life. Thus, by monitoring differential gene expression during senescence or fruit ripening, new targets may be identified for genetic manipulation in the context of extending shelf-life. In *A. thaliana*, the steady-state mRNA levels of over 800 genes have been studied simultaneously using high-density arrays (Desprez *et al*., 1998). The number of genes that can be assessed using this technology has increased substantially, with arrays containing 7000–10 000 non-redundant expressed sequence tags (ESTs) representing about 7500 genes, being made available through the *Arabidopsis* Functional Genomics Consortium, involving Michigan State University, The University of Wisconsin, Yale University and the Carnegie Institute of Washington at Stanford University. This figure is expected to increase towards the goal of 20 000 genes in the near future. Undoubtedly, such an approach will prove extremely useful in guiding reverse-genetics technology to identify the key genes of relevance in extending the shelf-life of a range of vegetable crop species.

13.13 Sources of further information and advice

The bibliography associated with this chapter provides a prime source of information, since it refers the reader to publications containing original experimental data. In addition, the cited review papers provide background information and overviews of this rapidly expanding topic. Additional advice relating to experimental procedures can be obtained by contacting the authors of the publications; their institution and e-mail addresses, fax and telephone numbers are normally indicated on their published papers. Internet sites worth visiting include those listed below:

Center for Plant Environmental Stress Physiology, Purdue University, USA
http://newcrop.hort.purdue.edu/cfpesp/cf00002.html
Edinburgh Data and Information Access
http://dina.ed.ac.uk/index.shtml
Federation of European Societies of Plant Physiology
http://www.fespp.org
GARNET, the Genomic *Arabidopsis* Resource Network; a platform for *Arabidopsis* international research and for research on other plant species.
www.york.ac.uk/res/garnet/garnet.htm
Plant Stress Resource Page
http://www.plantstress.com/

13.14 References

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14

Minimal processing of fresh fruits and vegetables

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14.1 Introduction

Minimal processing of raw fruits and vegetables has two purposes (Huxsoll and Bolin, 1989):

- keeping the produce fresh, without losing its nutritional quality
- ensuring a product shelf-life sufficient to make distribution feasible within a region of consumption

The microbiological, sensory and nutritional shelf-life of minimally processed vegetables or fruits should be at least 4–7 days, but preferably up to 21 days depending on the market (Ahvenainen, 2000; Wiley, 1994; Ahvenainen and Hurme, 1994). Commercial requirements for the manufacture of ready-to-use prepeeled, sliced, grated or shredded fruit and vegetables are summarised in Table 14.1.

The aim of this chapter is:

- to assess quality and safety aspects of minimally processed fruits and vegetables
- to describe the key steps in the food chain, beginning with raw material and processing and ending with packaging, which affect the quality and shelf-life of minimally processed fruits and vegetables.

14.2 Quality changes in minimally processed fruit and vegetables

As a result of peeling, grating and shredding, produce will change from a relatively stable commodity with a shelf-life of several weeks or months to a

Table 14.1 Requirements for the commercial manufacture of ready-to-use fruit and vegetables (pre-peeled and/or grated or shredded)

Working principle	Demands for processing	Customers	Shelf-life (days) at 5°C	Examples of suitable fruit and vegetables
Preparation today, consumption tomorrow	• Standard kitchen hygiene and tools • No heavy washings for peeled and shredded produce; potato is an exception • Packages can be returnable containers	Catering industry, restaurants, schools, industry	1–2	Most fruits and vegetables
Preparation today, the customer uses the product within 3–4 days	• Disinfection • Washing of peeled and shredded produce at least with water • Permeable packages; potato is an exception	Catering industry, restaurants, schools, industry	3–5	Carrot, cabbage, iceberg lettuce, potato, beetroot, acid fruits, berries
Products are also intended for retailing	• Good disinfection • Chlorine or acid washing for peeled and shredded produce • Permeable packages; potato is an exception • Additives	In addition to the customers listed above, retail shops can also be customers	5–7*	Carrot, Chinese cabbage, red cabbage, potato, beetroot, acid fruits, berries

* If longer shelf-life up to 14 days is needed, the storage temperature must be 1–2°C.

perishable one that has only a very short shelf-life, as short as 1–3 days at chilled temperatures. During peeling and grating operations, many cells are broken and intracellular products, such as oxidising enzymes, are released. Minimally processed produce deteriorates owing to physiological ageing, biochemical changes and microbial spoilage, which may result in degradation of the colour, texture and flavour (Varoquaux and Wiley, 1994; Kabir, 1994).

14.2.1 Physiological and biochemical changes

The most important enzyme in minimally processed fruits and vegetables is polyphenol oxidase which causes browning (Laurila *et al*., 1998b; Varoquaux and Wiley, 1994; Wiley, 1994). Another important enzyme is lipooxidase which catalyses peroxidation causing the formation of numerous bad-smelling aldehydes and ketones. Ethylene production can also increase and because ethylene contributes to the neosynthesis of enzymes involved in fruit maturation, it may play a part in physiological disorders of sliced fruits, such as softening (Varoquaux and Wiley, 1994).

With processing, the respiration activity of produce will increase by between 20% to as much as 700% or more depending on the produce, cutting grade and temperature (Varoquaux and Wiley, 1994; Mattila *et al*., 1995a). If packaging conditions are anaerobic, this leads to anaerobic respiration causing the formation of ethanol, ketones and aldehydes (Powrie and Skura, 1991).

14.2.2 Microbiological changes

During peeling, cutting and shredding, the surface of the produce is exposed to the air and to contamination with bacteria, yeasts and moulds. In minimally processed vegetables, most of which fall into the low acid range category (pH 5.8–6.0), high humidity and the large number of cut surfaces can provide ideal conditions for the growth of microorganisms (Willocx *et al*., 1994).

The populations of bacteria found on fruits and vegetables vary widely. The predominant microflora of fresh leafy vegetables are *Pseudomonas* and *Erwinia* spp., with an initial count of about 10^5 cfu g^{-1}, although low numbers of moulds and yeasts are also present. During cold storage of minimally processed leafy vegetables, pectinolytic strains of *Pseudomonas* are responsible for bacterial soft rot (Varoquaux and Wiley, 1994; Willocx *et al*., 1994). An increase in storage temperature and carbon dioxide concentration in the package will shift the microflora towards lactic acid bacteria (Garg *et al*., 1990; Marchetti *et al*., 1992; Markholm, 1992; Brackett, 1994; Hurme *et al*., 1994; Ahvenainen *et al*., 1994; Manzano *et al*., 1995).

The high initial load of microbes makes it difficult to establish the cell number threshold beyond which the product can be considered spoiled. Many studies show that a simple correlation does not exist between spoilage chemical markers such as pH, lactic acid, acetic acid, carbon dioxide, sensory quality and total microbial cell load (Marchetti *et al*., 1992; Hurme *et al*., 1994; Ahvenainen *et al*., 1994; Manzano *et al*., 1995). In fact, different minimally processed fruit and vegetable products seem to possess different spoilage patterns in relation to the characteristics of the raw materials (Huxsoll and Bolin, 1989; Marchetti *et al*., 1992).

Because minimally processed fresh fruits and vegetables are not heat treated, regardless of additives or packaging, they must be handled and stored at refrigerated temperatures, at 5°C or under in order to achieve a sufficient shelf-life and microbiological safety. Some pathogens such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella* spp. and *Aeromonas hydrophila* may still survive

and even profilerate at low temperatures (Brackett, 1994; Riquelme *et al.*, 1994). On the other hand, minimally processed fruits are relatively safe when compared to other foods, as they are generally acidic enough to prevent growth of pathogens. The normal spoilage organisms in refrigerated produce are also usually psychrotrophic and, therefore, have a competitive advantage over most pathogens.

14.2.3 Nutritional changes

Little is known about nutritive value, that is vitamin, sugar, amino acid, fat and fibre content of minimally processed produce. Washing does not decrease the vitamin content (vitamin C and carotenes) of grated carrot, shredded Chinese cabbage or peeled potatoes significantly (Hägg *et al*., 1996).

14.3 Improving quality

If products are prepared today and consumed tomorrow, very simple and inexpensive processing methods can be used. Most fruits and vegetables are suitable for this kind of preparation. Such products may also be suitable for catering, where they will undergo further processing. If, however, products need a shelf-life of several days, or up to one week and more, as is the case with the products intended for retailing, then more advanced processing methods and treatments are needed using the hurdle concept (Wiley, 1994; Ahvenainen and Hurme, 1994; Leistner and Gorris, 1995). The key steps are summarised in Table 14.2. Preservation is based on the synergies between individual steps such as these. These steps must also take place within a safe processing environment. Hygienic processing within a framework of good manufacturing practices and effective HACCP management is of utmost importance in preventing microbiological and other risks (Huxsoll and Bolin, 1989; Wiley, 1994; Ahvenainen and Hurme, 1994; Ahvenainen *et al*., 1994; Zomorodi, 1990). Some of the key hazards and their methods of control within a hazard analysis critical control point (HACCP) framework are summarised in Table 14.3.

14.4 Raw materials

It is self evident that vegetables or fruits intended for prepeeling and cutting must be easily washable, peelable and their quality must be first class. The correct and proper storage of vegetables and careful trimming before processing are vital for the production of prepared vegetables of good quality (Wiley, 1994; Ahvenainen and Hurme, 1994; Kabir, 1994). The study of various cultivar varieties of eight different vegetables showed that not all varieties of the specified vegetable can be used for the manufacture of prepared vegetables. The correct choice of variety is particularly important for carrot, potato, swede and onion. For example, with carrot and swede, the variety which gives the most juicy grated product cannot

Table 14.2 Key requirements in the minimal processing of fruits and vegetables

* Raw material of good quality (correct cv. variety, correct cultivation, harvesting and storage conditions)
* Strict hygiene and good manufacturing practises, HACCP
* Low temperatures during working
* Careful cleaning and/or washing before and after peeling
* Water of good quality (sensory, microbiology, pH) used in washing
* Mild additives in washing for disinfection or browning prevention
* Gentle spin drying after washing
* Gentle peeling
* Gentle cutting/slicing/shredding
* Correct packaging materials and packaging methods
* Correct temperature and humidity during distribution and retailing

Table 14.3 Hazards, critical control points, preventative and control procedures in processing and packaging of ready-to-use fruits and vegetables

Critical operational step	Hazards	Critical control point(s)	Preventative and control measures
Growing	Contamination with faecal pathogens	Cultivation techniques	– Use synthetic fertiliser* – Inspect the sources of irrigation water*
	Insects and fungal invasions		– Use pesticides
Harvesting	Microbial spoilage and insect invasion	Assesment of produce maturity	– Harvest prior to peak maturity – Minimise mechanical injuries
		Handling practices Temperature control	– Harvest in the morning or at night
	Cross-contamination	Sanitation	– Employ pickers trained in elementary hygiene
Transporting	Microbial growth	Time/temperature	– Keep the temperature low – Avoid long distance transport – Maintain uniform cooling in transport containers – Avoid damage, do not overload the containers
	Cross-contamination	Loading practices Produce Containers	– Separate sound and injured produce in the field – Use well washed/disinfected metal or plastic containers
Washing	Contamination from water	Water	– Use potable water, test routinely for the presence of coliform bacteria
		Washing practices	– Control microbial contamination by chlorination and antimicrobial dipping – Do not overload the washing tanks/change the water periodically
		Dewatering	– Remove excess water

Table 14.3 *Cont.*

Critical operational step	Hazards	Critical control point(s)	Preventative and control measures
Sorting	Cross-contamination	Sorter	– Employ sorters who have experience on the inspection of produce
		Lighting	– Provide adequate lighting
		Conveyer	– Clean and disinfect periodically
Packaging	Microbial growth	Packaging film	– Choose the permeability of film correctly – Analyse gas composition routinely by using simple techniques – Use fungicide impregnated film
		Relative humidity and temperature control	– Dewater the drenched produce carefully – Use films which have antifogging properties – Check product/storage temperature at regular intervals
Storage/ Distribution	Growth and spread of micro-organisms	Temperature control	– Maintain the refrigeration of produce in the range of 0–5°C – Prevent moisture condensation by proper temperature control
		Light	– Take the effect of light into consideration**
		Consumer practice	– Provide labelling with instructions for storage conditions

* For the produce grown close to ground and consumed raw.
**Light may affect the gas composition in the packaging by inducing photosynthesis in green vegetables.
(Source: Gorris, 1996)

be used in the production of grated products which should have a shelf-life of several days (Ahvenainen *et al*., 1994). Another example is potato, where poor colour and flavour become problems if the variety is wrong (Laurila *et al*., 1998a; Mattila *et al*., 1995b). Furthermore, the results showed that climatic conditions, soil conditions, agricultural practices, for example, fertilisation and harvesting conditions, can also significantly affect the behaviour of vegetables, particularly that of potatoes, in minimal processing (Ahvenainen *et al*., 1998).

14.5 Peeling, cutting and shredding

Some vegetables or fruits, such as potatoes, carrots or apples, need peeling. There are several peeling methods available, but on an industrial scale the peeling is

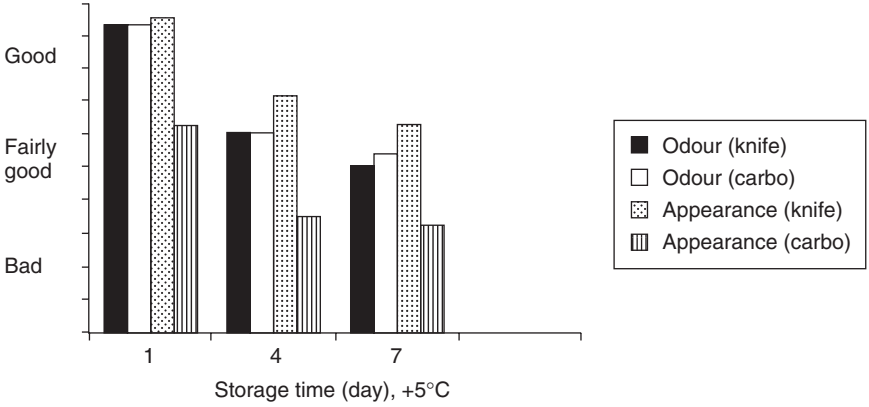

Fig. 14.1 Effect of peeling method and storage time on the odour and appearance of potato packed in a gas mixture of 20% CO_2 and 80% N_2 and stored at 5°C.

normally accomplished mechanically (e.g. rotating carborundum drums), chemically or in high-pressure steam peelers (Wiley, 1994). However, results have shown that peeling should be as gentle as possible. The ideal method would be hand peeling with a sharp knife. The relative effects of carborundum and knife peeling are shown in Fig. 14.1. Carborundum-peeled potatoes must be treated with a browning inhibitor, whereas water washing is enough for hand-peeled potatoes. If mechanical peeling is used, it should resemble knife peeling. Carborundum, steam peeling or caustic acid disturb the cell walls of a vegetable enhancing the possibility of microbial growth and enzymatic changes. Carborundum and knife peeling can be combined with a first stage of rough peeling and then a second stage of finer knife peeling. Enzymatic peeling can be successful, for example in the case of oranges (Pretel *et al*., 1998).

Many studies show that the cutting and shredding must be performed with knives or blades as sharp as possible and made from stainless steel. Carrots cut with a razor blade were more acceptable from a microbiological and sensory point of view than carrots cut with commercial slicing machines. It is clear that slicing with blunt knives impairs quality retention because of the increased breaking of cells and release of tissue fluid. A slicing machine must be installed solidly, because vibrating equipment may possibly impair the quality of sliced surfaces. Mats and blades used in slicing should also be disinfected, for example, with a 1% hypochlorite solution.

14.6 Cleaning, washing and drying

Incoming vegetables or fruits, which are covered with soil, mud and sand, should be carefully cleaned before processing. A second wash must usually be done after peeling and/or cutting (Wiley, 1994; Ahvenainen and Hurme, 1994). For example,

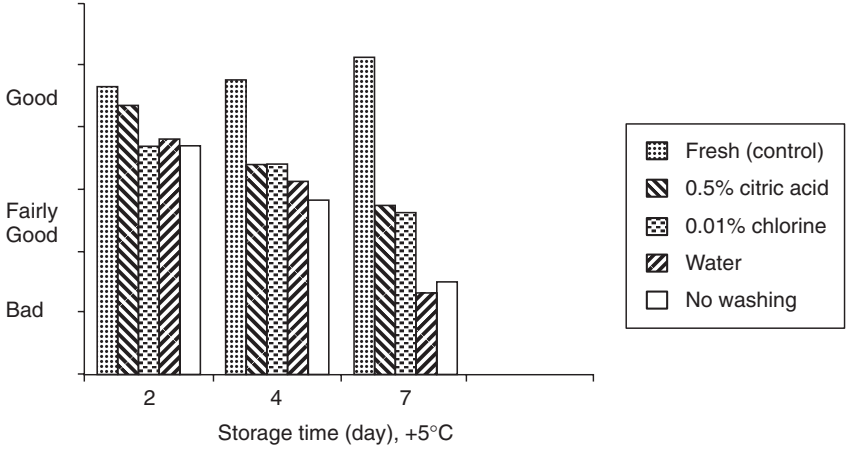

Fig. 14.2 Effect of washing solution and storage time on the odour of grated carrots packed in air and stored at 5°C.

Chinese cabbage and white cabbage must be washed after shredding, whereas carrot must be washed before grating (Hurme *et al*., 1994; Ahvenainen *et al*., 1994). Washing after peeling and cutting removes microbes and tissue fluid, thus reducing microbial growth and enzymatic oxidation during storage. Washing in flowing or air-bubbling water is preferable to dipping into still water (Ohta and Sugawara, 1987). The microbiological quality of the washing water used must be good and its temperature low, preferably below 5°C. The recommended amount of water used is 5–10 kg^{-1} of product before peeling/cutting (Huxsoll and Bolin, 1989) and 31 kg^{-1} after peeling/cutting (Hurme *et al*., 1994; Ahvenainen *et al*., 1994).

Preservatives can be used in washing water to reduce microbial numbers and to retard enzymatic activity, thereby improving the shelf-life. 100–200 mg of chlorine or citric acid per litre is effective in washing water before or after peeling and/or cutting to extend shelf-life (Wiley, 1994; Kabir, 1994; Hurme *et al*., 1994; Ahvenainen *et al*., 1994; O'Beirne, 1995). The relative effects of differing washing solutions are shown in Fig. 14.2. However, when chlorine is used, vegetable material should be rinsed. Rinsing reduces the chlorine concentration to the level of that in drinking water and means that sensory quality is not compromised (Hurme *et al*., 1994). The effectiveness of chlorine can be enhanced by using a combination of low pH, high temperature, pure water and correct contact time (Wiley, 1994; Kabir, 1994). It seems that chlorine compounds reduce counts of aerobic microbes at least in some leafy vegetables such as lettuce (Wiley, 1994; Garg *et al*., 1990), but not necessarily in root vegetables or cabbages (Garg *et al*., 1990; Ahvenainen *et al*., 1994). Chlorine compounds are of limited effectiveness in suppressing growth of *Listeria monocytogenes* in lettuce and cabbage (Skyttä *et al*., 1996; Francis and O'Beirne, 1997). In addition, the use of some

preservatives (e.g. chlorine compounds) is not necessarily allowed in all countries. Alternatives to chlorine include chlorine dioxide, peracetic acid ozone, trisodium phosphate and hydrogen peroxide (Sapers and Simmons, 1998). Hydrogen peroxide vapour treatment, for example, appears to reduce microbial counts on freshly cut cucumber, bell peppers and zucchini, extending shelf-life without leaving significant residues or compromising product quality. However, more research is still required to validate these treatments.

Washing water should be removed gently from the product (Wiley, 1994). A centrifuge seems to be the best method. The centrifugation time and rate should be chosen carefully (Zomorodi, 1990; Bolin and Huxsoll, 1991) so that the process removes free water but does not damage vegetable cells.

14.7 Browning inhibition

A key quality problem for fruits and vegetables such as peeled and sliced apple and potato is enzymatic browning. Washing with water is not effective in preventing discoloration (Wiley, 1994; Mattila *et al*., 1995b). Traditionally, sulphites have been used to prevent browning. However, the use of sulphites has some disadvantages, in particular dangerous side effects for asthmatics. For this reason, the FDA (Food and Drug Administration) in the USA partly restricted the use of sulphites (Anon., 1991). At the same time, interest in substitutes for sulphites is increasing. Enzymatic browning requires four different components: oxygen, an enzyme, copper and a substrate. In order to prevent browning, at least one component must be removed from the system. In theory, 2,5-diphenyloxazole polyphenoloxidase (PPO)-catalysed browning of vegetables and fruits can be prevented by such factors as (Whitaker and Lee, 1995):

- heat or reaction inactivation of the enzyme
- exclusion or removal of one or both of the substrates (oxygen and phenols)
- lowering the pH to 2 or more units below the optimum
- adding compounds that inhibit PPO or prevent melanin formation.

Many inhibitors of PPO are known, but only a few have been considered as potential alternatives to sulphites (Vámos-Vigyázó, 1981). The most attractive way to inhibit browning would be by ‘natural’ methods, such as the combination of certain salad ingredients with each other. Pineapple juice appears to be a good potential alternative to sulphites for the prevention of browning in fresh apple rings (Lozano-de-González *et al*., 1993; Meza *et al*., 1995). Washing in glycine betaine solution has been found to be effective in retaining sensory quality, particularly for prepacked shredded lettuce (Hurme *et al*., 1999).

Probably the most often studied alternative to sulphite is ascorbic acid. This compound is a highly effective inhibitor of enzymatic browning, primarily because of its ability to reduce quinones back to phenolic compounds before they can undergo further reaction to form pigments. However, ascorbic acid eventually oxidises to dehydroascorbic acid (DHAA), allowing quinones to accumulate

and undergo browning, and is best used in combination with other substances such as citric acid. Dipping in hot ascorbic acid/citric acid solutions improved the shelf-life of prepeeled potatoes to about two weeks. However, high concentrations of ascorbic acid (0.75%) have produced an unpleasant taste in fruits (Luo and Barbosa-Cánovas, 1995). Ascorbic acid derivatives have been used as browning inhibitors alone or in combination with other inhibitors for potatoes and apples (Sapers *et al*., 1989; Sapers and Miller, 1992, 1993; Monsalve-Gonzalez *et al*., 1993). Erythorbic acid, an isomer of ascorbic acid, has been used as an inhibitor of enzymatic browning in combination with ascorbic acid or citric acid for potato slices (Dennis, 1993) and for whole abrasion-peeled potatoes (Santerre *et al*., 1991).

Citric acid acts as a chelating agent and acidulant, both of which characteristics inhibit PPO. Promising results have been obtained using citric acid and the combinations citric–ascorbic acid and benzoic–sorbic acid as dipping treatments for minimally processed potatoes (Mattila *et al*., 1995b). 4-Hexylresorcinol is a good inhibitor of enzymatic browning for apples, potatoes and iceberg lettuce (Monsalve-Gonzalez *et al*., 1993; Whitaker and Lee, 1995; Luo and Barbosa-Cánovas, 1995; Castañer *et al*., 1996). It interacts with PPO and renders it incapable of catalysing the enzymatic reaction. 4-Hexylresorcinol has several advantages over the use of sulphites in foods, including (McEvily *et al*., 1992):

- its specific mode of inhibitory action
- the lower levels required for effectiveness
- its inability to bleach preformed pigments
- chemical stability.

Ethylenediamine tetraacetic acid (EDTA), a complexing agent, has been used with potatoes (Cherry and Singh, 1990; Dennis, 1993) and iceberg lettuce (Castañer *et al*., 1996) in combinations with other browning inhibitors. Sporix™, a chelating agent described by its supplier as an acidic polyphosphate, has been found to be an effective browning inhibitor in several fruits and vegetables (Gardner *et al*., 1991; Sapers *et al*., 1989). Sulphhydryl-containing amino acids like cysteine prevent brown pigment formation by reacting with quinone intermediates to form stable colourless compounds (Dudley and Hotchkiss, 1989). Cysteine has been used as a browning inhibitor for potatoes, apples and iceberg lettuce (Molnar-Perl and Friedman, 1990; Castañer *et al*., 1996) and it has also been used as an ingredient in a commercial browning inhibitor (Cherry and Singh, 1990).

Protease enzymes have been found to be effective browning inhibitors for apples and potatoes (Taoukis *et al*., 1989; Labuza *et al*., 1992; Luo, 1992). It is believed that an effective protease acts to hydrolyse and therefore inactivate the enzyme or enzymes responsible for enzymatic browning. Of the proteolytic enzymes tested so far, three plant proteases (ficin from figs, papain from papaya and bromelain from pineapple) in particular have proved to be effective. All the three proteases are sulphhydryl enzymes of broad specificity. According

to Taoukis *et al*. (1989), ficin was as effective as sulphite for potatoes at 4°C, but slightly less effective than sulphite at 24°C. Papain was somewhat effective for potatoes at 4°C. Papain treatment can prevent enzymatic browning of apples about as well as sulphite treatment at both temperatures (4°C and 24°C).

Because there is no one substitute for sulphites in preventing browning, alternatives are usually ascorbic acid-based combinations. A typical combination may include:

- a chemical reductant (e.g. ascorbic acid)
- an acidulant (e.g. citric acid)
- a chelating agent (e.g. EDTA).

In using such combinations, or developing new ones, it is important to take an integrated approach by choosing proper raw materials, peeling method, processing and packaging conditions (Laurila *et al*., 1998b).

14.8 Biocontrol agents

As well as enzymatic browning, a key issue with minimally processed foods is microbiological safety. An emerging technology in controlling pathogen growth is the use of biocontrol technology such as lactic acid bacteria (LAB) which compete with, and thus inhibit, pathogen growth (Breidt and Fleming, 1997). LAB can produce both metabolites, such as lactic and acetic acids, which lower pH, or bacteriocins. Although they are not sufficient in isolation, bacteriocins such as nisin can contribute to dealing with certain cold-tolerant Gram-positive bacteria (Bennik, 1997; Torriani *et al*., 1997). Studies of the use of lactic acid bacteria have suggested using them in combination with other preservation techniques (Breidt and Fleming, 1997) such as:

- reduction of the total microflora in the product by such procedures as washing using sanitisers, heat treatment or irradiation
- addition of a bacteriocin-producing biocontrol culture to achieve a target initial bacterial count (cfu ml^{-1})
- storage of the product under refrigerated conditions.

Product shelf-life would then be determined by the growth of the biocontrol culture. If the product suffered temperature abuse during storage or distribution, for example, the biocontrol culture would grow more rapidly, thus preventing pathogen growth. Such cultures will be a fruitful source of further research.

14.9 Packaging

A key operation in producing minimally processed fruits and vegetables is packaging. The most studied packaging method for prepared raw fruits and vegetables is modified atmosphere packaging (MAP). The basic principle in MAP is that a modified atmosphere can be created passively by using suitable permeable

packaging materials, or actively by using a specified gas mixture together with permeable packaging materials. The aim of both is to create an optimal gas balance inside the package, where the respiration activity of a product is as low as possible whilst ensuring that oxygen (O_2) concentration and carbon dioxide (CO_2) levels are not detrimental to the product. In general, the aim is to have a gas composition where there is 2–5% CO_2, 2–5% O_2 and the rest nitrogen (Kader *et al*., 1989; Day, 1994).

High oxygen MAP treatment has been found to be particularly effective at inhibiting enzymatic browning, preventing anaerobic fermentation reactions and inhibiting aerobic and anaerobic microbial growth (Day, 1997). High oxygen levels may cause substrate inhibition of PPO, or the high levels of colourless quinones subsequently formed may cause feedback production of PPO. Carbon monoxide (CO) gas atmosphere has also been found to inhibit mushroom PPO reversibly. Use of this compound in a MAP system would, however, require measures to ensure the safety of packing plant workers.

Achieving the right gas mixture is one of the most difficult tasks in manufacturing raw ready-to-use or ready-to-eat fruit and vegetable products. The main problem has been the lack of sufficiently permeable packaging materials (Day, 1994). Most films do not result in optimal oxygen and carbon dioxide atmospheres, especially when the produce has high respiration. However, one solution is to make microholes of defined sizes and a defined quantity in the material in order to avoid anaerobiosis (Exama *et al*., 1993). This procedure significantly improves, for example, the shelf-life of grated carrots (Ahvenainen *et al*., 1994). Other solutions are to combine ethylene vinyl acetate with oriented polypropylene and low density polyethylene or to combine ceramic material with polyethylene. Both composite materials have significantly higher gas permeability than polyethylene or the oriented polypropylene that is much used in the packaging of salads, even though gas permeablity should ideally be higher. These materials have good heat sealing properties and they are also available commercially (Ahvenainen and Hurme, 1994). The shelf-life of shredded cabbages and grated carrots packed in these materials is 7–8 days at 5°C and therefore 2–3 days longer than in the oriented polypropylene which is generally used in the vegetable industry (Hurme *et al*., 1994; Ahvenainen *et al*., 1994). A new breathable film has been patented, which has a three-layer structure consisting of a two-ply blown coextrusion about 25 µm thick with an outer layer of K-Resin KR10 and an inner metallocene polyethylene layer. It is claimed that this film gives 16 days' shelf-life at 1–2°C for fresh salads washed in chlorine solution (Anon., 1996). Examples of suitable packaging materials for vegetables are shown in Table 14.4 (Ahvenainen *et al*., 1994).

In dealing with fresh respiring products, it is advantageous to have film permeability alteration to match product respiration rate to avoid the anaerobic conditions favoured by some pathogens. In practice, this can be achieved by linking permeability to temperature change. Whilst the permeation rates of most packaging films are only modestly affected by changes in temperature, newer films have been developed with a temperature 'switch' point at which the film's permeation changes rapidly. This technology uses long-chain fatty alcohol-based

Table 14.4 Packaging materials for vegetables (Ahvenainen *et al*., 1994)

Vegetable	Packaging material and thickness
Peeled potato, both whole and sliced	PE-LD, 50 μm (also PA/PE, 70–100 μm or comparable)
Grated carrot	PP-O, 40 μm, microholed PP-O, PE/EVA/PP-O, 30–40 μm
Sliced swede	PE-LD, 50 μm
Grated swede	PE/EVA/PP-O, 40 μm
Sliced beetroot	PE-LD, 50 μm (also PA/PE, 70–100 μm or comparable)
Grated beetroot	PP-O, 40 μm microholed PP-O, PE/EVA/PP-O, 30–40 μm
Shredded Chinese cabbage	PP-O, 40 μm, PE/EVA/PP-O, 30–40 μm
Shredded white cabbage	PP-O, 40 μm, PE/EVA/PP-O, 30–40 μm
Shredded onion	PP-O, 40 μm (also PA/PE, 70–100 μm or comparable)
Shredded leek	PE-LD, 50 μm, PP-O 40 μm (also PA/PE, 70–100 μm or comparable)

polymeric chains. Under a given temperature these remain within a crystalline state. Once the temperature is exceeded, the side chains melt to a gas-permeable amorphous state (Anon., 1992; Anon., 1998). An alternative technology is to use a film with two differing layers, or two identical layers of differing thicknesses, both with minute cuts. As the temperature increases, the layers expand at differing temperatures causing the holes to enlarge, increasing the film's permeability (Anon., 1994). Safety valve systems have also been proposed to prevent excessive oxygen depletion and carbon dioxide accumulation when a temporary temperature increase occurs (Exama *et al*., 1993).

One interesting MAP method is moderate vacuum packaging (MVP) (Gorris *et al*., 1994). In this system, respiring produce is packed in a rigid airtight container under 40 kPa of atmospheric pressure and stored at refrigerated temperature (4–7°C). The initial gas composition is that of normal air (21% O_2, 0.04% CO_2 and 78% N_2) but at a reduced partial gas pressure. The lower oxygen content stabilises the produce quality by slowing down the metabolism of the produce and the growth of spoilage microorganisms. Gorris *et al*. (1994) have compared the storage of several whole and lightly processed fruits and vegetables under ambient conditions to MVP, and found that MVP improved the microbial quality of red bell pepper, chicory endive, sliced apple and sliced tomato, the sensory quality of apricot and cucumber and the microbial and sensory quality of mung bean sprouts and a mixture of cut vegetables. Gorris *et al.* (1994) also conducted pathogen challenge tests with *Listeria monocytogenes*, *Yersinia enterocolitica*, *Salmonella typhimurium* and *Bacillus cereus* on mung bean sprouts at 7°C. All of the pathogens lost viability quickly during the course of storage.

One of the greatest challenges is designing MAP for 'ready-to-eat' products such as prepared, mixed salads, where respiration rates of each component differ. Experiments have been undertaken on combinations such as carrot, cucumber, garlic and green pepper, using a pouch form package made of low-density polyethylene film. These have demonstrated some improvement in product quality compared with other types of MAP package (Lee *et al*., 1996).

14.10 Edible coatings

One possible 'packaging' method for extending the post-harvest storage of minimally processed fruit and vegetables is the use of edible coatings. These are thin layers of material that can be eaten by the consumer as part of the whole food product. Coatings have the potential to reduce moisture loss, restrict oxygen entrance, lower respiration, retard ethylene production, seal in flavour volatiles and carry additives (such as antioxidants) that retard discoloration and microbial growth (Baldwin *et al*., 1995).

14.11 Storage conditions

Chilling is an important preservative hurdle, as is the control of humidity. Storage at 10°C or above allows most bacterial pathogens to grow rapidly on fresh cut vegetables. Storage temperature is also important when MAP or vacuum packaging is used. Toxin production by *Clostridium botulinum*, or growth of other pathogens such as *Listeria monocytogenes*, is possible at temperatures above 3°C because of increased oxygen consumption in the package (Francis and O'Beirne, 1997). Processing, transport, display and intermediate storage should all be at the same low temperature (preferably 2–4°C) for produce not vulnerable to chilling injury. Changes in temperature should be avoided. Higher temperatures speed up spoilage and facilitate pathogen growth. Fluctuating temperatures cause in-pack condensation which also accelerates spoilage.

Temperature abuse is a widespread problem in the distribution chain, whether in storage, transportation, retail display and consumer handling. Where this is a significant problem, it may be necessary to restrict shelf-life, for example to 5–7 days at a temperature of 5–7°C, when psychrotrophic pathogens have insufficient time to multiply and produce toxin. If the shelf-life of vacuum or MAP products is greater than 10 days, and there is a risk that the storage temperature will be over 3°C, products should meet one or more of the following controlling factors:

- a minimum heat treatment such as 90°C for 10 min
- a pH of 5 or less throughout the food
- a salt level of 3.5% (aqueous) throughout the food
- a_w, water activity value of 0.97 or less throughout the food.
- any combination of heat and preservative factors which has been shown to prevent growth of toxin production by *C. botulinum.*

Practically, if the aim is to keep minimally processed produce in fresh-like state, the last mentioned factors, and mainly various preservative factors, are the only possibilities to increase shelf-life and assure microbiological safety of MA-or vacuum-packed fresh produce (FAIR, 1999).

14.12 Processing guidelines for particular vegetables

Processing and packaging guidelines for prepeeled and sliced potato (Table 14.5), prepeeled, sliced and grated carrot (Tables 14.6 and 14.7), shredded Chinese cabbage and white cabbage (Table 14.8), cut onion (Table 14.9) and leek (Table 14.10) are given below.

Table 14.5 Processing guidelines for prepeeled and sliced potato (Ahvenainen *et al*., 1994)

Processing temperature	4–5°C
Raw material	Suitable variety or raw material lot should be selected using a rapid storage test on prepared produce at room temperature. Attention must be focused on browning susceptibility.
Pretreatment	Careful washing with good quality water before peeling. Damaged and contaminated parts, as well as spoiled potatoes must be removed.
Peeling	1) One stage peeling: knife machine.
	2) Two stage peeling: slight carborundum first, and then knife peeling.
Washing	Washing immediately after peeling. The temperature and amount of washing water should be 4–5°C and 31 kg^{-1} potato. Washing time is 1 min. Microbiological quality of washing water must be excellent.
	In washing water, in particular for sliced potato, it is preferable to use citric acid with ascorbic acid (max. concentration of both 0.5%) possibly combined with calcium chloride, sodium benzoate or 4-hexyl resorcinol to prevent browning.
Slicing	Slicing should be done with sharp knives immediately after washing.
Straining off	Loose water should be strained off in a colander.
Packaging	Packaging immediately after washing in vacuum or in a gas mixture of 20% CO_2 + 80% N_2. The headspace volume of a package is 2 l per 1 kg potato.
	Suitable oxygen permeability of packaging materials is 70 $cm^3\,m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0% (80 µm nylon–polyethylene).
Storage	4–5°C, preferably in dark.
Other remarks	Good Manufacturing Practices must be followed (hygiene, low temperatures and disinfection).
Shelf-life	The shelf-life of prepeeled whole potato is 7–8 days at 5°C. Owing to browning, sliced potato has very poor stability, the shelf-life is only 3–4 days at 5°C.

Table 14.6 Processing guidelines for prepeeled and sliced carrot

Processing temperature	4–5°C
Raw material	Suitable variety or raw material lot should be selected using a rapid storage test on prepared produce at room temperature. Attention must be focused on respiration activity and whitening of surfaces.
Pretreatment	Careful washing with good quality water before peeling. Damaged and contaminated parts, as well as spoiled carrots must be removed.
Peeling and slicing	1) One stage peeling: knife machine. 2) Two stage peeling: slight carborundum first, and then knife peeling. Slicing should be done with sharp knives immediately after washing Optimal size for slices is 5 mm.
Washing	Washing immediately after slicing. The temperature and amount of washing water should be 0–5°C and 3 l per kg carrot. Washing time is 1min. Microbiological quality of washing water must be excellent. In washing water, no additives are needed.
Straining off	Loose water should be strained off in a colander.
Packaging	Packaging immediately after washing in air. Suitable oxygen permeability of packaging materials is 2900 $cm^3\ m^{-2}$ per 24 h, 101.3 kPa, 23°C RH 0% (e.g. 50 µm LD polyethylene or corresponding material), but also material with oxygen permeablity about 70 $cm^3\ m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0% (e.g. 80 µm nylon–polyethylene).
Storage	4–5°C, preferably in the dark.
Other remarks	Good Manufacturing Practices must be followed (hygiene, low temperatures and disinfection).
Shelf-life	Sliced carrot is quite preservable. The shelf-life is at least 7–8 days at 5°C.

Table 14.7 Processing guidelines for grated carrot (Ahvenainen *et al*., 1994)

Processing temperature	0–5°C
Raw material	Suitable variety or raw material lot should be selected using a rapid storage test on prepared produce at room temperature.
Pretreatment	Carrots must be washed carefully before peeling. Stems, damaged and contaminated parts, as well as spoiled carrots must be removed.
Peeling	Peeling with knife or carborundum machine.
Washing	Immediately after peeling. The temperature and amount of washing water: 0–5°C and 3 l per kg carrot, respectively. Washing time is 1 min. Microbiological quality of washing water must be excellent. It is preferable to use active chloride 0.01% or 0.5% citric acid in washing water.
Grating	The shelf-life of grated carrot is the shorter the finer the shredding grade. The optimum grate degree is 3–5 mm.
Centrifugation	Immediately after grating. Grated carrot may be lightly sprayed with water before centrifugation. The centrifugation rate and time must be selected, so that centrifugation only removes loose water, but does not break vegetable cells.
Packaging	Immediately after centrifugation. Proper packaging gas is normal air, and the headspace volume of a package 2 l per kg grated carrot. Suitable oxygen permeability of packaging materials is between 1200 (e.g. oriented polypropylene) and 5800, preferably 5200–5800 (e.g. polyethylene–ethylene vinyl acetate-oriented polypropylene) $cm^3\ m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0%. Perforation (one microhole/150 cm^3) of packaging material is advantageous. The diameter of microhole 0.4 mm.
Storage	0–5°C, preferably in dark.
Other remarks	Good Manufacturing Practices must be followed (hygiene, low temperatures and disinfection).
Shelf-life	7–8 days at 5°C.

Table 14.8 Processing guidelines for shredded Chinese cabbage and white cabbage

Processing temperature	0–5°C
Raw material	Suitable variety or raw material lot should be selected using a rapid storage test on prepared produce at room temperature.
Pretreatment	Outer contaminated leaves and damaged parts, as well as stem and spoiled cabbage must be removed.
Shredding	The shelf-life of shredded cabbage is the shorter the finer the shredding grade. The optimum shredding degree is about 5 mm.
Washing of shredded cabbage	Immediately after shredding. The temperature and amount of washing water: 0–5°C and 3 l per kg cabbage, respectively. The washing time is 1 min. Microbiological quality of the washing water must be excellent. Washing should be done in two stages: 1) Washing with water containing active chlorine 0.01% or 0.5% citric acid. 2) Washing with plain water (rinsing).
Centrifugation	Immediately after washing. The centrifugation rate and time must be selected so that centrifugation only removes loose water, but does not break vegetable cells.
Packaging	Immediately after centrifugation. Proper packaging gas is normal air, and the headspace volume of a package 2 l per kg cabbage. Suitable oxygen permeability of packaging material is between 1200 (e.g. oriented polypropylene) and 5800, preferably 5200–5800 (e.g. polyethylene–ethylene vinyl acetate-oriented polypropylene) $cm^3\ m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0%. For white cabbage, perforations (one microhole/150 cm^3) can be used. The diameter of the microhole is 0.4 mm.
Storage	0–5°C, preferably in the dark.
Other remarks	Good Manufacturing Practices must be followed (hygiene, low temperatures and disinfection).
Shelf-life	7 days for Chinese cabbage and 3–4 days for white cabbage at 5°C.

Table 14.9 Processing guidelines for cut onion

Processing temperature	0–5°C
Raw material	Suitable variety or raw material lot should be selected using a rapid storage test on prepared produce at room temperature.
Pretreatment	Stems, damaged and contaminated parts, as well as spoiled onions must be removed.
Peeling	Peeling with knife or with pressurised air (dry onions).
Washing	Mild washing immediately after peeling. The temperature of washing water should be 0–5°C. Microbiological quality of washing water must be excellent. It is preferable to use active chlorine 0.01% in washing water.
Cutting	The cutting should be done with sharp knives immediately after washing. The shelf-life of cut onion is shorter, the smaller the pieces.
Washing and centrifugation	No washing or centrifugation for cut onion.
Packaging	Immediately after cutting. Proper packaging gas is normal air or gas mixture 5% O_2 + 5–20% CO_2 + 75–90% N_2, and the headspace volume of a package 2 l per kg onion. Suitable oxygen permeability of packaging materials is between 1200 (e.g. oriented polypropylene) and 2900 (50 µm LD polyethylene) $cm^3\ m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0%. If cutting grade is small (i.e. big cuts), quite impermeable materials can also be used, e.g. 80 µm nylon–polyethylene, the permeability of which is 70 $cm^3\ m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0%.
Storage	0–5°C, preferably in the dark.
Other remarks	Good Manufacturing Practices must be followed (hygiene, low temperatures and disinfection).
Shelf-life	Cut onion has very poor stability, the shelf-life is only 3 days at 5°C.

Table 14.10 Processing guidelines for cut leek

Processing temperature	0–5°
Raw material	Suitable variety or raw material lot should be selected using a rapid storage test on prepared produce at room temperature.
Pretreatment	Stems, damaged and contaminated parts, as well as spoiled leeks must be removed. Careful washing with water.
Cutting	The cutting should be done with sharp knives immediately after washing.
	The shelf-life of cut leek is shorter, the smaller the pieces.
Washing	Careful washing immediately after cuttting. The temperature of washing water should be 0–5°C and washing time 1 min. Microbiological quality of washing water must be excellent.
	It is preferable to use active chlorine 0.01% in washing water.
Centrifugation	Careful centrifugation after washing is needed.
Packaging	Immediately after centrifugation. Proper packaging gas is normal air. The headspace volume of a package 2 l per kg leek.
	Suitable oxygen permeability of packaging materials is between 1200 (e.g. oriented polypropylene) and 2900 (50 µm LD polyethylene) $cm^3 m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0%.
	If cutting grade is small (i.e. big cuts), quite impermeable materials can also be used, e.g. 80 µm nylon–polyethylene, the permeability of which is 70 $cm^3 m^{-2}$ per 24 h, 101.3 kPa, 23°C, RH 0%.
	If packaging material is too permeable, the odour of leek can migrate from the package to other products.
Storage	0–5°C, preferably in the dark.
Other remarks	Good Manufacturing Practices must be followed (hygiene, low temperatures and disinfection).
Shelf-life	Cut leek has very poor stability, the shelf-life is only 3–4 days at 5°C.

14.13 Future trends

Much research is still to be done in order to develop minimally processed fruit and vegetable products with high sensory quality, microbiological safety and nutritional value. It is possible to reach 7–8 days' shelf-life at refrigerated temperatures (5°C), but for some products 2–3 weeks' shelf-life may be necessary. More information about the growth of pathogenic bacteria or nutritional changes in minimally processed fruits and vegetables with long shelf-life is needed.

A characteristic feature of minimal processing is the need for an integrated approach, where raw material, handling, processing, packaging and distribution must each be properly managed to make shelf-life extension possible. Hurdle technology using natural preservatives, for example, inhibitors produced by lactic acid bacteria, and the matching of correct processing methods and ingredients to

each other, needs to be developed further in the minimal processing of fresh produce. It is probable that in the future fruits and vegetables intended for minimal processing will be cultivated under specified controlled conditions, and that plant geneticists will develop selected and created cultivars or hybrids adapted to the specific requirements of minimal processing (Varoquaux and Wiley, 1994; Martinez and Whitaker, 1995). Unit operations such as peeling and shredding need further development to make them more gentle. There is no sense in disturbing the quality of produce by rough treatment during processing and then trying to limit the damage by subsequent use of preservatives. Active packaging systems and edible films, as well as more permeable plastic films which better match with the respiration of fruits and vegetables, are particularly active areas for development.

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15

New modified atmosphere packaging (MAP) techniques for fresh prepared fruit and vegetables

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15.1 Introduction

During recent years there has been an explosive growth in the market for fresh prepared fruit and vegetable (i.e. produce) products. The main driving force for this market growth is the increasing consumer demand for fresh, healthy, convenient and additive-free prepared product items. However, fresh prepared produce items are highly perishable and prone to the major spoilage mechanisms of enzymatic discoloration, moisture loss and microbial growth. Good manufacturing and handling practices along with the appropriate use of modified atmosphere packaging (MAP) are relatively effective at inhibiting these spoilage mechanisms, thereby extending shelf-life. Shelf-life extension also results in the commercial benefits of less wastage in manufacturing and retail display, long distribution channels, improved product image and the ability to sell convenient, added-value, fresh prepared produce items to the consumer with reasonable remaining chilled storage life.

The application of novel high oxygen (O_2) MAP is a new approach for the retailing of fresh prepared produce items and is capable of overcoming the many inherent shortcomings of current industry-standard air packaging or low O_2 MAP. The results of an extensive European Commission and industry funded project have shown that high O_2 MAP is particularly effective at inhibiting enzymic discolorations, preventing anaerobic fermentation reactions and moisture losses and inhibiting aerobic and anaerobic microbial growth.

This chapter highlights how extended shelf-life can be achieved by using high O_2 MAP. Practical guidance on issues such as safety, optimal high O_2 mixtures,

produce volume/gas volume ratios, packaging materials and chilled storage temperatures will be outlined so as to facilitate the commercial exploitation of this new technology. Brief reference in this chapter has been made with respect to novel argon (Ar) and nitrous oxide (N_2O) MAP, but in light of the variable results obtained for these novel MAP treatments, the majority of the text concentrates on the applications of novel high O_2 MAP in conjuction with appropriate non-sulphite dipping treatments.

15.2 Establishing an equilibrium modified atmosphere (EMA)

Unlike other chilled perishable foods that are modified atmosphere (MA) packed, fresh produce continues to respire after harvesting and any subsequent packaging must take into account this respiratory activity. The depletion of O_2 and enrichment of carbon dioxide (CO_2) are natural consequences of the progress of respiration when fresh produce is stored in hermetically sealed packs. Such modification of the atmosphere results in a respiratory rate decrease with a consequent extension of shelf-life (Kader *et al*., 1989). MAs can passively evolve within hermetically air-sealed packs as a consequence of produce respiration. If the respiratory characteristics of a produce item are properly matched to film permeability values, then a beneficial equilibrium MA (EMA) can be passively established. However, in the MAP of fresh produce, there is a limited ability to regulate passively established MAs within hermetically air-sealed packs. There are many circumstances when it is desirable to establish the atmosphere rapidly within produce packs. By replacing the pack atmosphere with a desired mixture of O_2, CO_2 and nitrogen (N_2), a beneficial EMA may be established more rapidly than a passively generated EMA. For example, the use of flushing packs with N_2 or a mixture of 5–10% O_2, 5–10% CO_2 and 80–90% N_2 is commercial practice for inhibiting undesirable browning and pinking on prepared leafy green salad vegetables (Day, 1998).

The key to successful retail MAP of fresh prepared produce at the time of writing is to use packaging film of the correct permeability so as to establish optimal EMAs of typically 3–10% O_2 and 3–10% CO_2. The EMAs attained are influenced by produce respiration rate (which itself is affected by temperature, produce type, variety, size, maturity and severity of preparation); packaging film permeability; pack volume, surface area and fill weight, and degree of illumination. Consequently, establishment of an optimum EMA for individual produce items is very complex. Furthermore, in many commercial situations, produce is sealed in packaging film of insufficient permeability, resulting in development of undesirable anaerobic conditions (e.g. <2% O_2 and >20% CO_2). Microperforated films, which have very high gas transmission rates have been developed and are now commercially used for maintaining aerobic EMAs (e.g. 5–15% O_2 and 5–15% CO_2) for highly respiring prepared produce items such as broccoli and cauliflower florets, baton carrots, beansprouts, mushrooms and spinach. However,

microperforated films are relatively expensive, permit moisture and odour losses, and may allow for the ingress of microorganisms into sealed packs during wet handling situations (Day, 1998).

15.3 Use of high O_2 MAP

Information gathered by the author during 1993–1994 revealed that a few prepared produce companies had been experimenting with high O_2 (e.g. 70–100%) MAP and had achieved some surprisingly beneficial results. High O_2 MAP of prepared produce was not exploited commercially during that period, probably because of the inconsistent results obtained, a lack of understanding of the basic biological mechanisms involved and concerns about possible safety implications. Intrigued by the concept of high O_2 MAP, the Campden and Chorleywood Research Association (CCFRA) carried out limited experimental trials on prepared iceberg lettuce and tropical fruits in early 1995. The results of these trials confirmed that high O_2 MAP could overcome the many disadvantages of low O_2 MAP. High O_2 MAP was found to be particularly effective in inhibiting enzymic discolorations, preventing anaerobic fermentation reactions and inhibiting microbial growth. In addition, the high O_2 MAP of prepared produce items within inexpensive hermetically sealed plastic films was found to be very effective in preventing undesirable moisture and odour losses and ingress of microorganisms during wet handling situations (Day, 1998).

The experimental finding that high O_2 MAP is capable of inhibiting aerobic and anaerobic microbial growth can be explained by the growth profiles of aerobes and anaerobes (Fig. 15.1). It is hypothesised that active oxygen radical species damage vital cellular macromolecules and thereby inhibit microbial growth when oxidative stresses overwhelm cellular protection systems (Gonzalez Roncero and

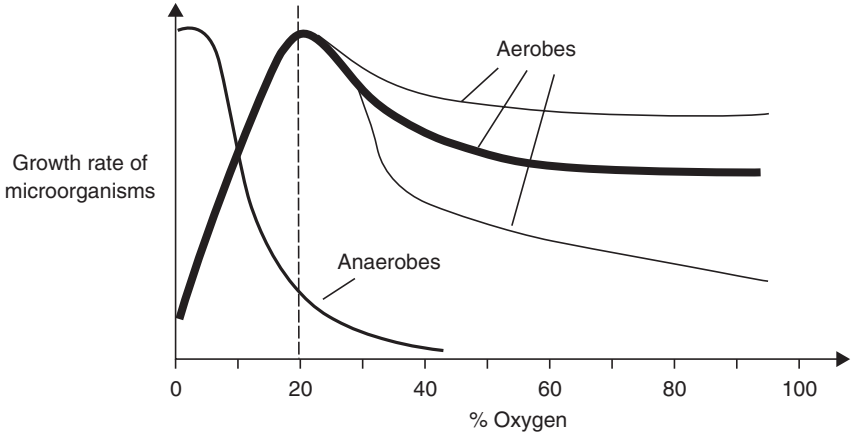

Fig. 15.1 Hypothesised inhibition of microbial growth by high O_2 MAP.

Fig. 15.2 Hypothesised inhibition of enzymic discoloration by high O_2 MAP.

Day, 1998; Amanatidou, 2001). Also intuitively, high O_2 MAP inhibits undesirable anaerobic fermentation reactions (Day, 1998).

Polyphenol oxidase (PPO) is the enzyme primarily responsible for initiating discoloration on the cut surfaces of prepared produce. PPO catalyses the oxidation of natural phenolic substances to colourless quinones which subsequently polymerise to coloured melanin-type compounds. It is hypothesised that high O_2 (and/or high Ar) levels may cause substrate inhibition of PPO or alternatively, high levels of colourless quinones subsequently formed (Fig. 15.2) may cause feedback product inhibition of PPO.

15.4 Argon and nitrous oxide MAP

Argon (Ar) and nitrous oxide (N_2O) are classified as miscellaneous additives and are permitted gases for food use in the European Union (EU). Air Liquide S.A. (Paris, France) has stimulated commercial interest in the potential MAP applications of using Ar and, to a lesser extent, N_2O. Air Liquide's broad range of patents claim that in comparison with N_2, Ar can more effectively inhibit enzymic activities, microbial growth and degradative chemical reactions in selected perishable foods (Brody and Thaler, 1996; Spencer, 1999). More specifically, an Air Liquide patent for fresh produce applications claims that Ar and N_2O are capable of extending shelf-life by inhibiting fungal growth, reducing ethylene emissions and slowing down sensory quality deterioration (Fath and Soudain, 1992). Of particular relevance is the claim that Ar can reduce the respiration rates of fresh produce and hence have a direct effect on extension of shelf-life (Spencer, 1999).

Although Ar is chemically inert, Air Liquide's research has indicated that it may have biochemical effects, probably owing to its similar atomic size to molecular O_2 and its higher solubility in water and density compared with N_2 and O_2. Hence, Ar is probably more effective at displacing O_2 from cellular sites and enzymic O_2 receptors with the consequence that oxidative deterioration reactions are likely to be inhibited. In addition, Ar and N_2O are thought to sensitise microorganisms to antimicrobial agents. This possible sensitisation is not well understood but may involve alteration of the membrane fluidity of microbial cell walls with a subsequent influence on cell function and performance (Thom and

Marquis, 1984). Clearly, more independent research is needed to understand better the potential beneficial effects of Ar and N_2O (Day, 1998).

15.5 Non-sulphite dipping

Enzymic discoloration of fresh prepared produce is one of the major causes of quality loss and spoilage during post-harvest handling, processing and storage (Sapers, 1993; Laurila *et al*., 1998). PPO (EC 1.10.3.1) is the enzyme primarily responsible for the discoloration of fresh prepared potatoes, apples, carrots, parsnips, swede, pears, mushrooms, bananas, peaches, grapes and lettuce, and this discoloration is often the shelf-life limiting quality attribute for these items (Duncan, 1999). PPO activity also results in detrimental changes to the texture and flavour of fresh prepared produce and losses of nutritional quality (Whitaker, 1996).

Given the deleterious effects of PPO activity upon the sensory and nutritional quality of fresh prepared produce, it is not surprising that considerable research has been devoted to inhibit the activity of this enzyme (Duncan, 1999). Sulphites have long been used as food additives to inhibit enzymic and non-enzymic discolorations, to control the growth of microorganisms and to act as bleaching agents and antioxidants (Sapers, 1993; Laurila *et al*., 1998). The most frequently used sulphiting agents for fresh prepared produce are sodium and potassium bisulphites and metabisulphites. Sulphites act as PPO inhibitors and antimicrobial agents and are most effective in acidic conditions (e.g. pH 3–5). For low-acid (e.g. pH 5–8) fresh prepared produce items such as mushrooms, bananas, potatoes and lettuce, sulphites have the tendency to accelerate bacterial decay by adversely affecting cell wall or membrane integrity which may stimulate the growth of certain spoilage bacteria (Duncan, 1999). Also, there are several negative attributes associated with sulphite use which has led to decreased consumer acceptance. In particular, sulphites can induce severe allergic reactions or even anaphylactic shock in a proportion of the asthmatic population (Sapers, 1993). Consequently, the adverse health effects of sulphite consumption have resulted in stricter regulatory restrictions and consumer labelling requirements (Anon., 1991).

The increased regulatory restrictions on the use of sulphites have created an urgent need for safe, practical and functional alternatives which are economically viable (Ahvenainen, 1996). Proprietary chemical non-sulphite formulations (containing, for example, mixtures of ascorbic acid or erythorbic acid or their sodium salt in combination with citric acid, malic acid, tartaric acid, succinic acid, calcium chloride, sodium chloride, 4-hexylresorcinol, sodium acid pyrophosphate and/or cysteine hydrochloride) are commercially available but further research is required to optimise appropriate formulations and dipping protocols for fresh prepared produce items. New opportunities exist for the use of approved starch and pectin-based edible coatings and safe biological agents such as enzymes and PPO inhibitors produced by lactic acid bacteria (Ahvenainen, 1996; Laurila *et al*., 1998).

It should be appreciated that different produce cultivars show large differences in their tendency to discolour after tissue wounding upon preparation. Such differences can be exploited by selecting raw material cultivars that have a low tendency to discolour after preparation so that treatments to inhibit enzymic discoloration can be minimised (Sapers, 1993). In addition, research has demonstrated that combining chemical non-sulphite dipping treatments with optimal MAP yields extended shelf-life and quality benefits greater than those achieved with either dipping or MAP alone (Duncan, 1999). Such combination treatments are likely to be the focus of future research aimed at minimising enzymic discolorations and maximising the maintenance of fresh prepared produce quality.

15.6 Testing the effectiveness of novel MAP techniques

Two industrially funded research clubs were set up at CCFRA to investigate in detail the interesting effects of novel MAP on fresh prepared produce. A High O_2 MAP Club ran from April 1995 to September 1997 and as a follow-up, a Novel Gases MAP Club ran from January 1998 to December 1999. These clubs were supported by a total of nine prepared produce suppliers, five gas companies, four packaging film suppliers, three retailers, two suppliers of non-sulphite dips, two manufacturers of MAP machinery and two gas instrument companies.

In addition, further investigations were carried out during a three year EU FAIR funded project, which started in September 1996. The overall objective of this project was to develop safe commercial applications of novel MAP for extending the quality shelf-life of a wide range of fresh prepared produce items. Other aims included investigations of the effects of novel MAP on non-sulphite dipped prepared produce, labile nutritional components and microbial and biochemical spoilage mechanisms. The major focus of this research was on high O_2 MAP, followed by Ar MAP, and to a minor extent, N_2O MAP.

In summary, the following major results and achievements were made during the course of CCFRA's Club and EU funded novel MAP research:

- High O_2 compatible MAP machines were used safely and successfully during the course of the project's experimental trial work. A non-confidential guidelines document on the safe use of high O_2 MAP was published (BCGA, 1998).
- Substantial evidence was gathered to demonstrate that undesirable sulphite dips could be replaced by several functional non-sulphite alternatives for inhibiting enzymic discoloration of prepared potatoes, apples and bananas. Several non-sulphite dipping variables (i.e. dip formulations and concentrations, dip temperatures and dip times) were optimised and suitable dipping protocols were recommended.
- Enzymic discolorations of prepared non-sulphite dipped potatoes and apples were generally more effectively inhibited by anaerobic (<2% O_2) MAP combinations of N_2, Ar and CO_2, compared with high O_2 MAP. However, high O_2 MAP was found to have certain odour and textural benefits for prepared

potatoes and apples. Also, high O_2 MA packed non-sulphite dipped prepared potatoes and bananas were found to have longer achievable shelf-lives, in comparison with equivalent low O_2 (8%) MA packed control samples.

- For most prepared produce items, under defined storage and packaging conditions, high O_2 MAP was found to have beneficial effects on sensory quality in comparison with industry-standard air packing and low O_2 MAP. High O_2 MAP was found to be effective for extending the achievable shelf-lives of prepared iceberg lettuce, sliced mushrooms, broccoli florets, cos lettuce, baby-leaf spinach, radichio lettuce, lollo rossa lettuce, flat-leaf parsley, cubed swede, coriander, raspberries, strawberries, grapes and oranges (Tables 15.1 and 15.2).
- Ar-containing and N_2O-containing MAP treatments were found to have negligible, variable or only minor beneficial effects on the sensory quality of several prepared produce items, in comparison with equivalent N_2-containing MAP treatments.
- High O_2 MAs were found to inhibit the growth of several generic groups of bacteria, yeasts and moulds, as well as a range of specific food pathogenic and spoilage microorganisms, namely *Aeromonas hydrophila*, *Salmonella enteritidis*, *Pseudomonas putida*, *Rhizopus stolonifer*, *Botrytis cinerea*, *Penicillium roqueforti*, *Penicillium digitatum* and *Aspergillus niger*. High O_2 MAs alone were not found to inhibit or stimulate the growth of *Pseudomonas fragi*, *Bacillus cereus*, *Lactobacillus sake*, *Yersinia enterocolitica* and *Listeria monocytogenes*, but the addition of 10–30% CO_2 inhibited the growth of all these bacteria (e.g. Fig. 15.3 and 15.4).
- Ar-containing and N_2O-containing MAs were found to have negligible antimicrobial effects on a range of microorganisms, when compared with equivalent N_2-containing MAs.
- Respiration rates of selected prepared produce items were not found to be significantly affected by high O_2 or high Ar MAs, but were substantially reduced by the addition of 10% CO_2.

Table 15.1 Overall achievable shelf-life obtained from fresh prepared iceberg lettuce trial

MAP treatments	Storage days at 8°C to drop to quality grade C			Shelf-life limiting quality attribute(s)	Overall achievable shelf-life
	Appearance	Odour	Texture		
5% O_2/95% N_2	4	7	4	Appearance/texture	4 days
5% O_2/10% CO_2/85% N_2	7	7	8	Appearance/odour	7 days
80% O_2/20% N_2	11	11	11	Appearance/odour/texture	11 days

Table 15.2 Overall achievable shelf-life obtained from several fresh prepared produce trials

Prepared produce items	Overall achievable shelf-life (days) at 8°C	
	Industry standard air/low O_2 MAP	High O_2 MAP
Iceberg lettuce	2–4	4–11
Dipped sliced bananas	2	4
Broccoli florets	2	9
Cos lettuce	3	7
Strawberries	1–2	4
Baby leaf spinach	7	9
Lollo rossa lettuce	4	7
Radicchio lettuce	3	4
Flat leaf parsley	4	9
Coriander	4	7
Cubed swede	3	10
Raspberries	5–7	9
Little gem lettuce	4–8	6–8
Dipped potatoes	2–3	3–6
Baton carrots	3–4	4
Sliced mushrooms	2	6

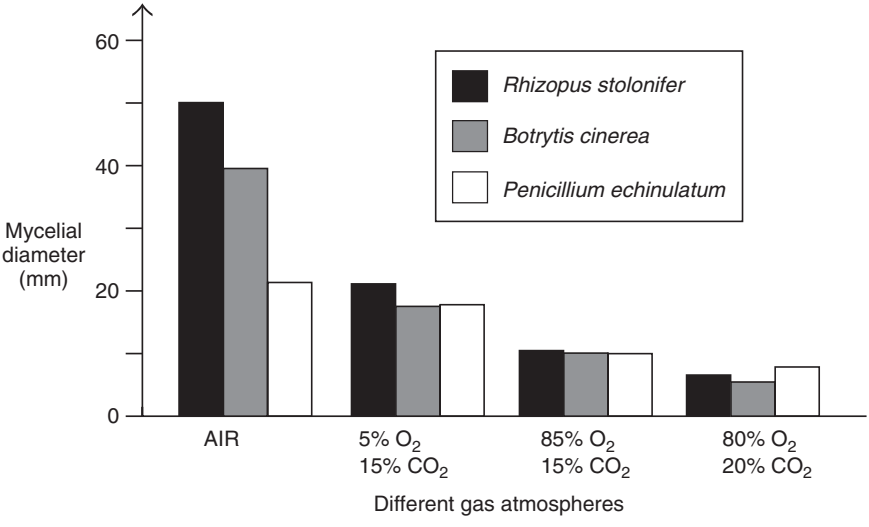

Fig. 15.3 Inhibition of fungal growth by different MAs.

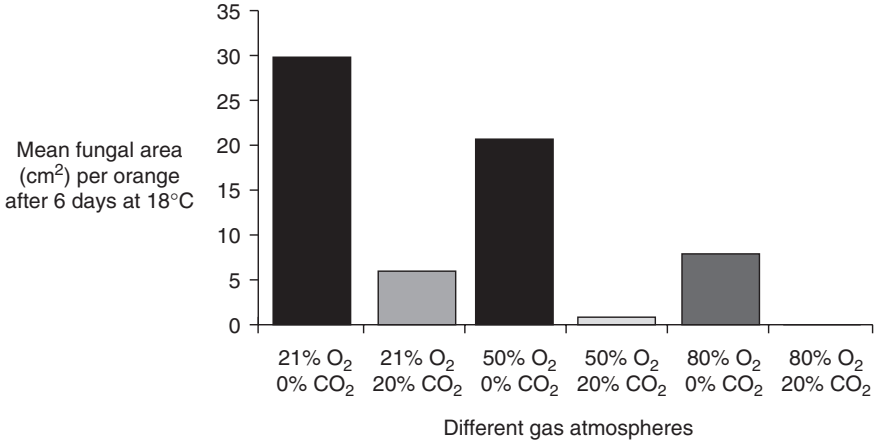

Fig. 15.4 Inhibition of fungal growth on *Penicillium digitatum* infected oranges under different MAs.

- High O_2 and high Ar MAP did not prevent the enzymic browning of non-sulphite dipped apple slices, but no further browning took place after pack opening.
- Ar-containing MAs were found to inhibit the activity of mushroom polyphenol oxidase (PPO), when compared with equivalent N_2-containing MAs. In contrast, no significant inhibition of mushroom PPO activity was found under 80% O_2/20% N_2 when compared with 20% O_2/80% N_2. However, the incorporation of 20% CO_2 into high O_2 MAs may inhibit mushroom PPO as well as the activity of other prepared produce PPOs (Sapers, 1993).
- High O_2 MAP increased membrane damage of apple slices, whereas high Ar MAP decreased membrane damage. However, apple slices stored under O_2-free MAs suffered the most membrane damage, which affected tissue integrity, cell leakage and texture. By comparison, high O_2 and high Ar MAP were not found to affect the cell permeability, tissue exudate or pH of prepared carrots adversely.
- High O_2 and high Ar MAP were found to have beneficial effects on ascorbic acid retention, indicators of lipid oxidation and inhibition of enzymic browning on prepared lettuce.
- High O_2 MAs increased the peroxidase activity of *Botrytis cinerea*, but the addition of 10% CO_2 substantially reduced this activity.
- In comparison with air packing and low O_2 MAP, high O_2 MAP was not found to decrease single antioxidant (ascorbic acid, β-carotene and lutein) levels preferentially in prepared lettuce but did induce the loss of certain phenolic compounds, even though desirable total antioxidant capacity (TRAP) values after chilled storage were increased.
- Extracts from high O_2 MA packed prepared lettuce and onions did not have any cytotoxic effects on human colon cells.

- Ingestion of fresh lettuce resulted in an increase in human plasma TRAP values through the absorption of phenolic compounds and single antioxidant molecules. This increase in human plasma TRAP values was significantly higher than after ingestion of lettuce that had been chilled (5°C) and stored for three days.
- Ingestion of chilled stored lettuce packed under air and high O_2 MAs resulted in measurable increases in human plasma TRAP values, whereas virtually no increases in TRAP values were measured after ingestion of equivalent lettuce packed under low O_2 MAs.
- A guidelines document was compiled which outlines good manufacturing and handling practices for fresh prepared produce using high O_2 MAP and non-sulphite dipping treatments (Day, 2001a).

15.7 Guidelines for the use of high O_2 MAP

It should be appreciated that the potential applications of high O_2 MAP technology are a recent innovation and new knowledge will evolve in the future. Hence, the following guidance provided only reflects the current status of available knowledge and experience of high O_2 MAP for fresh prepared produce. Potential applications of high O_2 MAP to chilled combination food items (e.g. chilled ready meals, pizzas, kebabs, etc.) have been the subject of recent research (Day, 2001b), but are outside the scope of this chapter.

15.7.1 Safety

A specific guidelines document has been published and is publicly available (BCGA, 1998). This document contains clear and concise advice and recommendations on how to control the hazards of utilising O_2-rich gas mixtures for the MAP of food.

Food companies and related industries (e.g. gas companies and MAP machinery manufacturers) are strongly encouraged to purchase this safety guidelines document and to follow the advice and recommendations given closely before undertaking any precommercial trials using high O_2 MAP. Further advice and help on the safety aspects of high O_2 MAP can be sought from qualified gas safety engineers and the BCGA.

15.7.2 Optimal gas levels

Based on CCFRA's practical experimental trials, the recommended optimal headspace gas levels immediately after fresh prepared produce package sealing are: 80–95% O_2/5–20% N_2.

After package sealing, headspace O_2 levels will decline whereas CO_2 levels will increase during chilled storage, owing to the intrinsic respiratory nature of fresh prepared produce. As previously explained, the levels of O_2 and CO_2

established within hermetically sealed packs of produce during chilled storage are influenced by numerous variables; the intrinsic produce respiration rate (which itself is affected by temperature, atmospheric composition, produce type, variety, cultivar and maturity, and severity of preparation), packaging film permeability, pack volume, surface area and fill weight, produce volume/gas volume ratio and degree of illumination (Kader *et al*., 1989; Day, 1994; O'Beirne, 1999).

To maximise the benefits of high O_2 MAP, it is desirable to maintain headspace levels of $O_2 > 40\%$ and CO_2 in the range of 10–25% during the chilled shelf-life of the product. This can be achieved by lowering the temperature of storage, by selecting produce having a lower intrinsic respiration rate, by minimising cut surface tissue damage, by reducing the produce volume/gas volume ratio by either decreasing the pack fill weight or increasing the pack headspace volume, by using a packaging film which can maintain high levels of O_2 whilst selectively allowing excess CO_2 to escape, or by incorporating an innovative active packaging sachet that can adsorb excess CO_2 and emit an equal volume of O_2 (McGrath, 2000).

Also, in order to maintain levels of $O_2 > 40\%$ and CO_2 in the range 10–25% during the chilled shelf-life of the product, it is desirable to introduce the highest level of O_2 (balance, N_2) possible just prior to fresh prepared produce package sealing. Generally, it is not necessary to introduce any CO_2 in the initial gas mixture since levels of CO_2 will build up rapidly within sealed packages during chilled storage. However, for fresh prepared produce items that have low intrinsic respiration rates, that are packaged in a format with a low produce volume/gas volume ratio, that are stored at low chilled temperatures or that have an O_2 emitter/CO_2 adsorber sachet incorporated into the sealed package, then the incorporation of 5–10% CO_2 into the initial gas mixture maybe desirable. Based on the results of controlled atmosphere storage experiments, the most effective high O_2 gas mixtures were found to be 80–85% O_2/15–20% CO_2, which had the most noticeable sensory quality and antimicrobial benefits on a range of fresh prepared produce items (Day, 2001a).

The type of MAP machinery used will greatly influence the maximum achievable O_2 level that can be introduced just prior to fresh prepared produce package sealing. Most light prepared salad items are commercially MA packed on vertical form-fill-seal (VFFS) and horizontal form-fill-seal (HFFS) machines (Hartley, 2000). These machines use a gas flushing or air dilution technique to introduce gas in MA pillow packs just prior to sealing. Since these machines do not use an evacuation step, then about 80% O_2 would be the highest practical level that could be achieved within sealed fresh prepared produce packs by initially flushing with 100% O_2. Higher levels of in-pack O_2 could be achieved by substantially increasing the flow rate of O_2 through the gas flushing lance of these machines, but this is not recommended for economic and safety reasons (BCGA, 1998).

In contrast to VFFS and HFFS machines, thermoform-fill-seal (TFFS), preformed tray and lidding film (PTLF), vacuum chamber (VC) and snorkel type (ST) machines use a compensated vacuum technique to evacuate air and then introduce gas into tray and lidding film and/or flexible MA packs (BCGA, 1998).

Since these machines use an evacuation step prior to gas (i.e. 100% O_2) introduction, much higher levels of headspace O_2 (85–95%) can be achieved within such sealed fresh prepared produce packs. Also, all compensated vacuum machines (except VC machines) are intrinsically safer for high O_2 MAP applications, compared with gas flushing VFFS and HFFS machines, since O_2 is introduced directly into the MA packs after air evacuation and prior to sealing, and consequently O_2 levels in the air surrounding these machines are not enriched (BCGA, 1998).

15.7.3 Produce volume/gas volume ratio

In order to maintain headspace O_2 levels > 40% and CO_2 levels in the range 10–25% during the chilled shelf-life of the product, it is desirable to minimise the produce volume/gas volume ratio of fresh prepared produce MA packs. This can be achieved by either decreasing the pack fill weight or increasing the pack headspace volume. Decreasing the pack fill weight of fresh prepared produce will have the effect of reducing the overall respiratory load or activity within MA packs and hence the rate of O_2 depletion will be reduced. Increasing the pack headspace volume will have the effect of increasing the reservoir of O_2 for respiratory purposes and hence the rate of O_2 depletion will also be reduced. Consequently, low produce volume/gas volume ratios are conducive to maintaining headspace O_2 levels > 40% and CO_2 levels in the range 10–25%.

The important influence of the produce volume/gas volume ratio, in addition to the intrinsic produce respiration rate and packaging film permeability, is well illustrated by the results from CCFRA's bulk iceberg lettuce trial (Day, 2001a). Depletion of O_2 and elevation of CO_2 levels within the high O_2 MA bulk packs of this trial were very rapid because these packs contained 2 kg of fresh prepared iceberg lettuce as opposed to only 200 g for retail MA packs. Consequently, the produce volume/gas volume ratio and overall respiratory load were much higher in these MA bulk packs compared with MA retail packs. Also, the iceberg lettuce used for this bulk pack trial was shredded (10 mm cut) and hence had a much higher intrinsic respiration rate compared with retail salad cut (40–70 mm) iceberg lettuce. In addition, the thicker (60 µm compared with 30 µm for retail) and less permeable bulk oriented polypropylene (OPP)/low density polyethylene (LDPE) bags exacerbated the depletion of O_2 and elevation of CO_2. Hence, it was not surprising that the achievable shelf-life at 8°C for high O_2 MA bulk packed fresh shredded iceberg lettuce was found to be only 2 days, even though the shelf-life of equivalent low O_2 MA bulk packed iceberg lettuce was even shorter (Day, 2001a).

It should be appreciated that there are practical and commercial limits to the reduction of produce volume/gas volume ratios for fresh prepared produce MA packs. Obviously, retail consumers will not readily accept MA packs of fresh prepared produce which appear to be underfilled with too much headspace gas. Therefore, it is recommended that potential users of high O_2 MAP technology should carry out precommercial trials with fresh prepared produce packs having different but practical produce volume/gas volume ratios.

15.7.4 Packaging materials

Based on the results of CCFRA's practical experimental trials, the recommended packaging material for high O_2 MA retail packs of fresh prepared produce is: 30 µm oriented polypropylene (OPP) with antimist coating.

It should be noted that initial experimental trials carried out at CCFRA on high O_2 MAP of fresh prepared produce used an O_2 barrier film, that is, 30 µm polyvinylidene chloride (PVDC) coated OPP with antimist coating, because it was considered at the time to be important to maintain the highest levels of O_2 within high O_2 MA packs. However, extensive experimental trials on high O_2 MAP of fresh prepared iceberg lettuce using 30 µm PVDC coated OPP film clearly demonstrated that excess and potentially damaging levels of CO_2 (30–40%) could be generated within such O_2 barrier film packs, particularly at higher chilled storage temperatures (i.e. 6–8°C). Consequently, 30 µm OPP film was used for subsequent high O_2 MAP experimental trials, instead of 30 µm PVDC coated OPP film, and for the majority of fresh prepared produce items, was found to have sufficient O_2 barrier properties to maintain high in-pack O_2 levels (>40%) but also to be sufficiently permeable to ensure that in-pack CO_2 levels did not rise above 25% after 7–10 days storage at 5–8°C (Day, 2001a).

It should be appreciated that other packaging materials, apart from 30 µm OPP, may be suitable for high O_2 MAP of fresh prepared produce (Air Products, 1995; Day and Wiktorowicz, 1999). For example, laminations or extrusions of OPP with low density polyethylene (LDPE), ethylene vinyl acetate (EVA) or polyvinyl chloride (PVC), or other medium to very high O_2 permeability films may be more suitable for high O_2 MAP of fresh prepared produce items that have a higher respiration rate than iceberg lettuce. Also, the produce volume/gas volume ratio of different retail MA pack formats (e.g. pillow packs or tray and lidding film systems), the intrinsic fresh prepared produce respiration rate and chilled temperature of storage will influence the selection of the most suitable packaging film for high O_2 MAP applications (Day, 2001a).

It is recommended that potential users of high O_2 MAP for fresh prepared produce should initially carry out precommercial shelf-life trials using 30 µm OPP with antimist coating as the packaging film for flexible pillow packs or as a tray lidding film. Regular gas analyses of the in-pack atmospheres during chilled storage will reveal whether the packaging film is not permeable enough (resulting in build-up of excess levels of CO_2 to >25%) or too permeable (resulting in depletion of O_2 to <40% and slow build-up of CO_2 to <10%). If the in-pack O_2 levels fall < 40% and CO_2 levels lie outside the range 10–25% by the end of the chilled shelf-life of the product, then adjustments to the produce volume/gas volume ratio, chilled temperature of storage, pack format and/or permeability of the package film will need to be made and further shelf-life trials carried out.

It should also be noted that O_2 barrier films could be used for high O_2 (or low O_2) MAP of fresh prepared produce items if an O_2 emitter/CO_2 adsorber sachet is incorporated into sealed packages. Appropriate transparent O_2 barrier films

(with antimist coatings) include PVDC coated OPP, and coextrusions or laminations containing ethylene vinyl acetate (EVOH), polyester (PET), polyamide (nylon) and/or PVDC (Air Products, 1995; Day and Wiktorowicz, 1999).

Whatever packaging material is used for high O_2 MAP applications, all of them must comply with statutory legal requirements. In the UK, these requirements include the Materials and Articles in Contact with Food Regulations 1987, Plastic Materials and Articles in Contact with Food Regulations 1998, Producer Responsibility Obligations (Packaging Waste) Regulations 1997 and Packaging (Essential Requirements) Regulations 1998.

All packaging materials should be purchased to an agreed specification which includes details of technical properties and performance. Quality assurance on all incoming packaging materials should be subject to an agreement between the packaging supplier and user. Each delivery or batch should be given a reference code to identify it in storage and use, and the documentation should allow any batch of packaged product to be correlated with deliveries of respective packaging materials. All packaging materials should be stored off the floor in separate and dry areas of the factory and should be inspected at regular intervals to ensure that they remain in acceptable condition. Authorised procedures and documentation should be established and followed for the issue of packaging materials from store (Day, 1992). Further advice on the technical requirements, properties, performance and handling of packaging materials should be sought from reliable suppliers.

15.7.5 Temperature control

The importance of proper temperature control to retard the quality deterioration and assure the microbial safety of fresh prepared produce cannot be overemphasised. For high O_2 MA packed fresh prepared produce, it is recommended that the temperature be maintained below 8°C, ideally in the range 0–3°C, throughout the entire chill chain.

The important influences of storage temperature and packaging film permeability on the quality of high O_2 MA packed fresh prepared produce can be illustrated by the results from CCFRA's fresh prepared iceberg lettuce trials (Day, 2001a). The results from these trials clearly demonstrated that temperature and packaging film permeability are critical factors in determining the development of O_2 and CO_2 levels within high O_2 MA packs, during chilled storage. Higher temperatures of storage correlate to high respiratory rates and hence greater depletion of O_2 and elevation of CO_2 within sealed high O_2 MA barrier (i.e. 30 µm PVCD coated OPP) pillow packs of fresh prepared iceberg lettuce. The most beneficial sensory effects of high O_2 MAP were obtained when the temperature of storage was 3–5°C, the O_2 levels dropped from 70 to 55% and the CO_2 levels reached only 15% after 10 days' storage. In contrast, largely negative sensory effects were obtained when an elevated chill temperature of storage regime (8°C) was employed. Under this elevated chilled temperature of storage

regime, O_2 levels dropped from 80% to 35–40%, whereas CO_2 levels reached 35–40% after 10 days' storage. These high levels of generated CO_2 within the high O_2 MA barrier pillow packs of fresh prepared iceberg lettuce were responsible for the undesirable 'CO_2 damage' discoloration observed. Later high O_2 MAP experimental trials used more permeable OPP film whereby high O_2 (>40%) levels were generally maintained and CO_2 levels did not rise above 25% after 7–10 days' storage at 5°C and 8°C. Under these high O_2 MAP conditions, beneficial sensory effects were observed for the majority of the fresh prepared produce items studied, in comparison with industry-standard air and/or low O_2 MAP (Day, 2001a).

15.7.6 Fresh prepared produce applications

High O_2 MAP has been found to have beneficial effects on the sensory quality of the vast majority of the fresh prepared produce items studied. Under defined storage and packaging conditions and in comparison with industry-standard air packing and/or low O_2 MAP, high O_2 MAP was found to be effective for extending the achievable shelf-lives of retail packs of fresh prepared iceberg lettuce, sliced mushrooms, potatoes, sliced bananas, little gem lettuce, cos lettuce, baby-leaf spinach, radichio lettuce, lollo rossa lettuce, flat-leaf parsley, cubed swede, coriander, raspberries and strawberries. In addition, the results from trials carried out prior to September 1997, showed beneficial sensory effects of high O_2 MAP for fresh prepared tomato slices, baton carrots, pineapple cubes, broccoli florets, honeydew melon cubes, sliced mixed peppers and sliced leeks. Also, high O_2 controlled atmospheres were found to extend the shelf-life of table grapes and oranges (Day, 2001a).

It should be noted that in comparison with industry-standard air and/or low O_2 MAP, high O_2 MAP was not found to have beneficial effects on the sensory quality of retail packs of fresh prepared apple slices, curly parsley, red oak leaf lettuce and galia melon cubes, and bulk packs of shredded iceberg lettuce. However, it is probable that beneficial effects of high O_2 MAP on the above fresh prepared produce items would have been achieved if the chilled storage temperature, high O_2 gas level, packaging film permeability, produce volume/gas volume ratio and/or preparation procedures had been optimised adequately.

Consequently, it is recommended that potential users of high O_2 MAP for specific fresh prepared produce items or combinations carry out precommercial optimisation trials by utilising the advice given previously.

15.8 Guidelines for non-sulphite dipping

As previously explained in section 15.5, the increased regulatory restrictions on the use of sulphites have created an urgent need for safe, practical and functional alternatives to inhibit enzymic discoloration of fresh prepared produce (Anon., 1991; Ahvenainen, 1996; Laurila *et al*., 1998; Duncan, 1999). Numerous chemi-

cal non-sulphite dip formulations (typically containing mixtures of ascorbic acid, citric acid, malic acid and/or sodium chloride) are commercially available or can be prepared in-house. Whichever non-sulphite dip formulation is used, successful application depends on several important factors which need to be optimised, as described in the rest of this section.

15.8.1 Produce raw materials

Fresh prepared produce manufacturers must ensure that produce raw materials taken into their premises are safe, of the desired quality, and stored and handled appropriately to avoid unnecessary damage and contamination. Specifically, in relation to produce raw materials that are subsequently prepared and non-sulphite dipped, it is an unrealistic assumption to expect chemical non-sulphite dips to overcome quality problems caused by using substandard raw materials which have heavy bruising and/or other major blemishes. Consequently, it is recommended that produce raw materials conform to objective and agreed specifications and are stored and handled as gently as possible so as to minimise bruising. Also, it is recommended that suitable produce raw material cultivars, that have a low tendency to discolour after subsequent preparation, are preferentially selected so that non-sulphite dipping treatments can be minimised (Sapers, 1993). In addition, it is advisable that selected produce raw materials should be of appropriate maturity and firmness so that they can withstand the rigours of subsequent preparation procedures whilst being sufficiently ripe to be of good eating quality.

15.8.2 Predipping preparation treatments

Predipping preparation treatments, such as trimming, peeling, cutting, slicing, washing and decontamination, are no different from those used for fresh produce items that are not subsequently non-sulphite dipped (Day, 2001a).

If chlorine or other oxidising decontamination agents are used, then it is recommended that fresh prepared produce items be subjected to a final rinse in potable water prior to non-sulphite dipping. This final rinse step will help to reduce the levels of residual oxidising agents, which if too high can substantially counteract the antioxidant properties of the constituents of non-sulphite dips. As a guide, 0.5 l of rinse water should be applied for every kilogram of fresh prepared produce.

15.8.3 Dipping procedures

It is recommended that non-sulphite dipping procedures should be applied as soon as possible after the fresh produce has been prepared. Enzymic discolorations usually proceed very rapidly after peeling, cutting and/or slicing, and in the case of certain potato and apple cultivars, discolorations are visible within minutes of preparation. Hence, when immediate immersion in a non-sulphite dip is not

possible, fresh prepared produce items should be immersed temporarily under chilled potable water to inhibit enzymic discoloration. The drawback of temporary water immersion for more than a few minutes is that fresh prepared produce items can absorb extensive amounts of water which can lead to soft waterlogged textures, translucent appearances and faster deterioration rates.

It should be appreciated that the variables of dip concentration, dipping time and temperature need to be optimised for each fresh prepared produce application when using chemical non-sulphite dip formulations and dipping protocols. Nevertheless, the following general guidance should be adhered to:

- The constituents of non-sulphite dip formulations must be safe and approved for food use. In the UK, these constituents must comply with the Miscellaneous Additives in Food Regulations 1995.
- Non-sulphite dipping should not cause any detrimental effects to the flavour, odour, texture and nutritional quality of fresh prepared produce.
- The temperature of the non-sulphite dipping solution should be 0–5°C, because non-sulphite dip constituents are absorbed into plant tissues at a more rapid rate when the dipping solution is at a colder temperature than the temperature of the fresh prepared produce item, prior to dipping.
- Non-sulphite dip solution concentrations are typically in the range of 1–3% w/v. For example, a common non-sulphite dip formulation is diluted in chilled potable water to give a final concentration of 2% w/v ascorbic acid, 1% w/v citric acid and 1% w/v sodium chloride.
- Non-sulphite dipping times are usually in the range of 2–5 min. Very short dipping times (<1 min) are generally not long enough to permit sufficient adsorption of the non-sulphite constituents into cut plant tissues. Conversely, very long dipping times (>5 min) are generally not necessary and can lead to excessive water absorption.
- Non-sulphite dipping solutions should be freshly made up and replaced at appropriate intervals depending on the quantity of fresh prepared produce that needs to be dipped.
- The amount of non-sulphite dipping solution required is usually in the range of 1 l per 10–15 kg of fresh prepared produce to be dipped.

15.8.4 Post-dipping treatments

- A final potable water rinse-off step is typically required after chemical non-sulphite dipping of fresh prepared produce items. This final rinse prevents the persistence of any acidic flavour taints caused by the organic acids used in all non-sulphite dip formulations. Typically, 250–500 ml of chilled potable water per kilogram of fresh prepared produce is used, depending upon variables such as the dip concentration, dipping time and the specific produce application. Another important reason for the final rinse is that the prior non-sulphite dipping treatment can be classified as a processing aid and hence the con-

stituents of non-sulphite dip formulations do not need to be labelled as additives.
- Post non-sulphite dipping and rinsing treatments, such as dewatering, packaging and temperature control, are no different from those used for fresh prepared produce items that are not non-sulphite dipped (Day, 2001a).

15.9 Future trends

Novel MAP (particularly, high O_2) has the potential to maintain the quality and assure the microbial safety of fresh prepared produce. The commercial implementation and success of this new technology may encourage greater consumption of conveniently packed fresh prepared produce and help towards improving the health and well-being of consumers. A publication of practical guidance on high O_2 MAP and non-sulphite dipping has already facilitated commercial exploitation of this new technology (Day, 2001a).

The following future research directions are suggested specifically with regard to the high O_2 MAP of fresh prepared produce:

- Further investigate the potential applications of an innovative dual-action O_2 emitter/CO_2 scavenger active packaging sachet that has been developed by Standa Industrie (Caen, France) and marketed by EMCO Packaging Systems (Worth, Kent, UK). Initial trials carried out by CCFRA and LinPac Plastics Limited (Pontefract, Yorkshire, UK) in association with several soft fruit suppliers have clearly demonstrated the shelf-life extending potential of this active packaging device (McGrath, 2000). This O_2 emitter/CO_2 scavenger sachet enables high O_2 levels to be maintained within high O_2 MA packs of respiring fresh prepared produce whilst simultaneously controlling CO_2 below levels that may cause physiological damage to produce. The inclusion of this sachet within high O_2 MA packs of fresh prepared produce that have a high intrinsic respiration rate and/or produce volume/gas volume ratio will prevent excessive depletion of in-pack O_2 levels and build-up of in-pack CO_2 levels. In addition, this sachet could also be utilised in low O_2 MA packs of fresh prepared produce to prevent the development of undesirable anaerobic conditions during chilled storage.
- Thoroughly investigate the potential synergy between high O_2 MAP and other active packaging devices (e.g. moisture absorbers, ethylene scavengers and antimicrobial films) and suitable edible coatings and films (Day, 1994; Baldwin *et al*., 1995; Nussinovitch and Lurie, 1995; Rooney, 1999). The selection criteria of promising active packaging devices and edible coatings and films should be based on their technical efficacy, cost, regulatory status and consumer acceptability (Day, 2000).
- Carry out further underpinning research investigations on the effects of high O_2 MAP on the various spoilage and pathogenic microorganisms associated with fresh prepared produce items. Also, further research is merited on the

effects of high O_2 MAP on the beneficial nutritional components present in fresh produce and on the complex biochemical reactions and physiological processes that occur during storage.
- Establish optimal high O_2 MAP applications for extending the quality shelf-life and assuring the microbial safety of further fresh prepared produce items and combination food products which consist of respiring produce and non-respiring food items (e.g. ready meals, pizzas, kebabs, etc.). Initial trials carried out by CCFRA have already clearly demonstrated that high O_2 MAP is capable of extending the achievable shelf-life of several chilled ready meals, in comparison with CO_2/N_2 MAP and industry-standard air packing (Day, 2001b).

With respect to more general aspects of fresh prepared produce, the following knowledge gaps and suggested research directions are highlighted, in order to assist researchers in the future:

- Provide packaging film permeability data on commercial laminations and coextrusions at realistic chilled temperatures (0–10°C) and relative humidities (85–95%). At the time of writing, virtually all gas permeability data is quoted for single films at unrealistic storage temperatures and relative humidities (e.g. 23°C and 0% RH).
- Provide extensive respiration rate data on a wide variety of fresh prepared produce items at different chilled temperatures and under various gaseous storage conditions. At the time of writing, most respiration rate data available is for whole produce items stored in air.
- Provide data on the physiological tolerance of fresh prepared produce items to low (and possibly high) O_2 levels and elevated CO_2 levels. Currently, extensive data is available on the tolerance of whole produce items to low O_2 and high CO_2 levels (Kader *et al*., 1989) but there is a dearth of information on the tolerance of fresh prepared produce items to varying gaseous levels.
- Provide information on the residual effects of MAP on individual fresh prepared produce items after subsequent pack opening and storage in air.
- Thoroughly investigate an integrated approach to minimal processing techniques, which covers the entire chain from 'farm to fork', so as to maintain the quality and assure the microbial safety of fresh prepared produce (Ahvenainen, 1996).
- Carry out further investigations on new and innovative natural preservatives, such as those produced by lactic acid bacteria and those derived from herbs and spices (Kets, 1999).
- Devise improved washing and decontamination procedures for fresh prepared produce that are based on safe non-chlorine alternatives.
- Develop peeling and cutting machinery that can process fresh produce more gently and hence extend the quality shelf-life of fresh prepared produce.
- Devote more resources into refrigeration equipment, design and logistics so

that optimal storage temperatures for fresh prepared produce can be maintained throughout the entire chill chain.

15.10 References

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15.11 Acknowledgements

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16

Edible coatings for fruits

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16.1 Introduction: the development of edible coatings

Major losses in quality and quantity of fresh fruits occur between harvest and consumption (Sparks, 1976). Savings obtained through reduction of post-harvest fruit losses are regarded as 'a hidden harvest' (Spurgeon, 1976). Several techniques have been developed which are successful in extending shelf-life, through a better understanding of the respiration process in fresh fruits. Controlled atmosphere storage and modified atmosphere storage have been used for preserving fruits by reducing their quality changes and quantity losses during storage. Edible coatings on fresh fruit can provide an alternative to modified atmosphere storage by reducing quality changes and quantity losses through modification and control of the internal atmosphere of the individual fruits.

16.1.1 A historical view of edible coatings

Wax was the first edible coating used on fruits and vegetables. The Chinese applied wax coatings to oranges and lemons in the twelfth and thirteenth centuries (Hardenburg, 1967). Although the Chinese did not realize that the full function of edible coatings was to slow down respiratory gas exchange, they found that wax coated fruits could be stored longer than non-waxed fruits. In the 1930s hot-melt paraffin waxes became commercially available as edible coatings for fresh fruits such as apples and pears. Erbil and Muftugil (1986) reported that coating peach surfaces with wax emulsions decreased water vapor and oxygen transmission thus, diminishing the respiration rate and increasing shelf-life of the fruit. Nisperos-Carriedo *et al*. (1990) observed that oils or waxes and cellulose had similar effects in preventing spoilage and retaining the fresh-picked quality for tropical fruits.

Several attempts have been made to develop other materials that could be used to coat produce and modify internal gas composition for short-term storage. Zhang and Quantick (1997) suggested that chitin and chitosan (deacetylated chitin) from marine invertebrates could be used to make a transparent film for application as an edible coating on fruits and vegetables. In 1982, Lowings and Cutts (1982) reported an edible coating material that is non-phytotoxic, tasteless, odorless and effective in preserving fruits. This coating material is a mixture of sucrose fatty acid esters (SFAE), sodium carboxymethyl cellulose and mono- and diglycerides. SFAE was originally developed as an emulsifier. However, it has been established that the ripening of fruits can be retarded by a coating of SFAE. SFAE mixtures have been commercially available for coating fruits and vegetables since the 1980s, under the trade names 'TAL Pro-long' and 'Semperfresh' (Banks, 1984; Chu, 1986; Santerre *et al*., 1989). Park *et al*. (1994b,c) applied zein coating to the surface of tomatoes and reported that the film coating delayed color change, weight loss and maintained firmness during storage.

16.1.2 Problems associated with edible coatings

Even though some edible coatings have been successfully applied to fresh produce, other applications adversely affect quality. Modification of the internal atmosphere by the use of edible coatings can increase disorders associated with high carbon dioxide or low oxygen concentration (Ben-Yehoshua, 1969). Smock (1940) indicated that waxing apples and pears inhibited normal ripening rate and if sufficient wax was applied, respiration was greatly inhibited and alcoholic flavors were develop by anaerobic fermentation. Smith and Stow (1984) reported that apples (cv. Cox's Orange Pippin) coated with sucrose fatty acid ester had fewer detrimental changes in terms of fruit firmness, yellowing and weight loss but had increased incidence of core flush. Park *et al*. (1994c) reported that tomatoes coated with 0.6 μm zein film produced alcohol and off-flavors inside the tomatoes which were attributable to an internal gas composition that was too low in oxygen and too high in carbon dioxide. Smith *et al*. (1987) summarized the effects on physiological disorders associated with modification of internal atmosphere by use of coatings, as core flush, flesh breakdown and accumulation of ethanol and alcoholic off-flavors.

Wax and SFAE mixtures are the most widely used edible coatings for fruits and vegetables. But, they are not equally effective for all produce. Another problem is that consumers tend to be wary of waxy coatings. Therefore, development of alternative edible coatings which do not impart a waxy taste are desirable. The effects of edible coatings on internal gas composition and their interactions with quality parameters must be determined for coated fresh produce. For example, color change and firmness are very important quality parameters in fruits. Shewfelt *et al*. (1987) stated that color change, loss of firmness, ethanol fermentation, decay ratio and weight loss of edible-film coated fruits are all important qualities for various products.

16.2 How edible coatings work: controlling internal gas composition

Edible coatings can provide protection for fresh products and can also give the same effect as modified atmosphere storage with respect to modifying internal gas composition. The success of edible coatings for fruits depends mainly on selecting films or coatings which can give a desirable internal gas composition that is appropriate for a specific product.

16.3 Selecting edible coatings

If a coating is too thick, detrimental effects can result because the internal oxygen concentration is below a desirable and beneficial level and there is an associated increased carbon dioxide concentration which is above a critical tolerable level. These conditions lead to anaerobic fermentation. This can be remedied by: (1) developing several edible coatings, (2) controlling wettability of edible coatings, (3) measuring gas permeation properties of selected coatings, (4) measuring diffusion properties of skin and flesh of selected fruits, (5) predicting internal gas compositions for the fruits coated with edible films, and (6) observing coating effects on the quality changes of fruits.

16.4 Gas permeation properties of edible coatings

There are several possible edible coatings for fruits such as cellulose, casein, zein, soy protein and chitosan. These were chosen because they have the desirable characteristics of generally being odorless, tasteless and transparent. It is not easy to measure the gas permeation properties of the coatings after they have been applied to fruits. Therefore, separate flat films are prepared and tested. Two known primary methods of preparation of flat films were described by Kamper and Fennema (1984) and Aydt *et al*. (1991). An OX-TRAN 1000^{TM} (Mocon Modern Control, Minneapolis, MN) was used to measure oxygen permeability (OP), and water vapour permeability (WVP) was measured using a variation of the ASTM Standard Method E 96 (ASTM, 1987), known as the 'cup method'. Carbon dioxide CO_2 permeability was measured using a modified permeability cell designed by Gilbert and Pegaz (1969). WVP and gas permeabilities of the coatings can be calculated as shown in Box 1.

OP, WVP and carbon dioxide permeabilities of edible coatings reported in the literature are presented in Table 16.1 and compared with other conventional plastic films. The oxygen permeabilities of most edible coatings were lower than the conventional plastic films. The oxygen permeability (OP) of sucrose polyester (SPE) coatings was 1–3 times higher than that of polyethylene film and was 4–10 times higher than that of polypropylene film. The OPs of SPE coatings were similar to cellulose film values but were higher than those of edible protein

Box 1 Gas permeability

The permeation process can be described mathematically by Fick's first law. The flux (J) which is proportional to the concentration gradient can be defined in one direction as follows:

$$J = -D(\partial C/\partial X) \quad [16.1]$$

where J is the flux, the net amount of solute that diffuses through unit area per unit time ($g\,m^{-2}\,s^{-1}$ or $ml\,m^{-2}\,s^{-1}$), D is the diffusivity constant ($m^2\,s^{-1}$), C is the concentration gradient of the diffusing substance and X is the thickness of the film (m) (Chang, 1981; Crank, 1975; Jost, 1960; Landrock and Proctor, 1952).

With two assumptions, (1) that the diffusion is in a steady state and (2) that there is a linear gradient through the film, the flux (J) is given by:

$$J = D(C_2 - C_1)/X = Q/(At) \quad [16.2]$$

where Q is the amount of gas diffusing through the film (g or ml), A is the area of the film (m^2) and t is the time (s). After application of Henry's law, the driving force is expressed in terms of the partial pressure differential of gas and a rearrangement of terms yield the following equation in terms of permeability:

$$Q/(At) = DS(p_2 - p_1)/X = P\Delta p/X \quad [16.3]$$

where S is the Henry's law solubility coefficient ($mol\,atm^{-1}$), Δp is partial pressure difference of the gas across the film (Pa) and P is the permeability ((ml or g) $m\,m^{-2}\,s^{-1}\,Pa^{-1}$).

Then, the permeabilities of O_2, CO_2 and H_2O vapor can be calculated from equation [16.4]:

$$P = QX/(At\Delta p) \quad [16.4]$$

coatings such as zein. The OP permeabilities of protein films were lower than those of polyethylene (low density), polyethylene and polyvinyl chloride, and were close to that of polyester film. The OP permeabilities of protein films, corn-zein and wheat were also lower than those of cellulose films, methyl cellulose MC(L) and hydroxypropyl cellulose HPC(L) both with low levels (L) of plasticizer. The addition of lipid (Myvacet 7-00™) into HPC film decreased the OP permeability only slightly.

The CO_2 permeabilities of protein films, corn-zein and wheat were lower than those of plastic films, polyethylene (low density), polyethylene and polyvinyl chloride, with the exception of polyester film which exhibits a greater barrier to CO_2 permeation (Table 16.1). CO_2 permeabilities of cellulose films, MC(L) and HPC(L), were higher than those of plastic films. The addition of lipid (Myvacet

Table 16.1 O_2, CO_2 and H_2O vapor permeabilities of edible coatings

Film	Permeability		
	[b]O_2	[b]CO_2	[c]H_2O Vapor
SPE	2.10 ± 0.0001	–	0.00042 ± 0.04
Chitosan (15 cp)	0.0014	–	0.49
Zein	0.36 ± 0.16	2.67 ± 1.09	0.116 ± 0.019
Wheat gluten	0.20 ± 0.09	2.13 ± 1.43	0.616 ± 0.013
MC (L)	2.17 ± 0.45	69.0 ± 19.33	0.092 ± 0.003
HPC (L)	3.57 ± 0.03	143.9 ± 3.76	0.110 ± 0.004
HPC/lipid	3.44 ± 0.06	81.7 ± 4.58	0.082 ± 0.003
Cozeen	0.89	5.25 ± 26.10	0.407
PE	8.30	26.1	–
PP	0.55 ± 0.005	–	0.00065 ± 0.06
PVC	0.09–17.99	1.35–26.98	0.00071
PET	0.13–0.30	0.67–1.12	–

PE is polyethylene, PP is polypropylene, PVC is polyvinyl chloride, PET is polyester (Aydt *et al.*, 1991; Kamper and Fennema, 1984; Park, 1999; Park and Chinnan, 1995a, 1995b; Park *et al.*, 1993, 1994a,d 1998).

[b] Unit of permeability is in $fl\,m\,m^{-2}\,s^{-1}\,Pa^{-1}$; f is an abbreviation for femto (10^{-15}).

[c] Unit of permeability is $ng\,m\,m^{-2}\,s^{-1}\,Pa^{-1}$; n is an abbreviation for nano (10^{-9}).

7-00™) into HPC film decreased the CO_2 permeability by 43.2%. CO_2/O_2 permeability ratios of edible films were higher than those of plastic films (Kader *et al*., 1989).

SPE coatings provide very high water vapor barriers compared with other edible coatings, as shown in Table 16.1. WVPs of SPE coatings were lower than that of polyethylene film and more than 100 times lower than the values for cellulose and protein films. These high oxygen and water vapor barrier properties will make SPE coatings desirable for fresh produce as a replacement for wax (Risse *et al*. 1987; Segall *et al*. 1974). The WVPs of other edible coating films were much higher than those of plastic films. The WVP of wheat protein film was 0.603–0.630 $ng\,m\,s\,Pa^{-1}$, the highest of all edible films tested. Wheat protein film exhibited high permeability to water vapor probably because wheat protein was dispersed by addition of ammonium hydroxide (6N) as part of the formulation, and also contained a higher concentration of plasticizer, 40% (wt. plasticizer/wt. protein). The addition of lipid (Myvacet 7-00™) into HPC film decreased the water vapor permeability by 24.7%. Plastic is the most widely used food wrap, but water vapor commonly condenses on the inner surface of plastic packaging materials thus creating a potential source of microbial contamination in fresh produce (Ben-Yehoshua, 1985). Thus, a film with greater water vapor permeability is desirable, although a film with extremely high water vapor permeability is also not desirable as it can result in excessive moisture loss from fruits during storage.

16.5 Wettability and coating effectiveness

The effectiveness of edible coatings on fruits and vegetables depends primarily on controlling the wettability of the coating solutions, which affects the coating thickness of the film (Park, 1999). Edible coating formulations must wet and spread on the fruit's surface uniformly and upon drying form a coating that has adequate adhesion, cohesion and durability to function properly (Krochta and Mulder-Johnston 1997). Hershko and Nussinovitch (1998) indicated that suitable hydrocolloid coatings could only be achieved by exploring the wettability of the coating solution further. Coatings on fruits and vegetables that exceed a critical thickness can cause detrimental effects from reduced internal O_2 concentration and increasing CO_2 concentration associated with anaerobic fermentation. Tomatoes coated with 66.04 μm zein film produced alcohol and off-flavors internally (Park *et al*., 1994c).

Choi *et al*. (2001) reported that the contact angle of a chitosan coating solution on the apple skin was 89.0°. The wettabilities of edible coatings can be calculated as shown in Box 2.

Because the coating angle is close to 90°, it implies that chitosan coating solution does not easily coat apple skin that has a wax barrier. The measured contact

Box 2 Wettability

The wettability of a solid by a liquid is determined by the balance between adhesive forces (work of adhesion, W_a) of the liquid on the solid and cohesive forces (work of cohesion, W_c) of the liquid. Adhesive forces cause the liquid to spread over the solid surface while cohesive forces cause it to shrink:

$$W_a = \gamma_{LV} + \gamma_{SV} - \gamma_{SL} \quad W_c = 2\gamma_{LV} \qquad [16.5]$$

The contact angle of a liquid drop on a solid surface is defined by the mechanical equilibrium of the drop under the action of three interfacial tensions: solid–vapor (γ_{SV}), solid–liquid (γ_{SL}) and liquid–vapor (γ_{LV}). This equilibrium relation is known as Young's equation (Rulon and Robert, 1993):

$$\cos\theta = (\gamma_{SV} - \gamma_{SL})/\gamma_{LV} \qquad [16.6]$$

When a solid comes into contact with a liquid in the presence of vapor, the liquid will adhere well on the solid surface if the total free energy required for the creation of the new interface decreases. The physical significance of this energy change is the work needed to separate the solid and liquid from the solid/liquid interface. The equilibrium spreading coefficient (W_s) is defined by equation [16.7] (Rulon and Robert, 1993) and it can only be negative or zero:

$$W_s = W_a - W_c = \gamma_{SV} - \gamma_{LV} - \gamma_{SL} \qquad [16.7]$$

angles on watermelon, melon, tomato and mandarin were also from 87.5° to 90.0° (Table 16.2). The critical surface tension (γ_C) of apple skin was 18.7 dyne cm^{-1} ($R^2 = 0.99$). Hershko and Nussinovitch (1998) observed that the critical surface tension of garlic skin was 18.3 dyne cm^{-1}. Hagenmaier and Baker (1993) found that the critical surface tension was about 23 dyne cm^{-1} for grapefruit (Table 16.2). These results indicate that the skin of most fruit covered with a layer of wax has a low surface energy. The layer with low surface energy interacts with liquids primarily through dispersion (van der Waals) forces (Rulon and Robert, 1993). The contact angle of the chitosan coating solution on the pear skin was 8.5° (Table 16.2), an unusually low value. It may have been caused by the surface characteristics of pears which have a coarser surface than that of other fruits. Park and

Table 16.2 Surface tension, contact angle and critical surface tension of fruits and vegetables

Coating emulsion	Surface tension (dyne cm^{-1})	Fruits	Critical surface tension (dyne cm^{-1})	Contact angle (degrees)	Spreading coefficient G (dyne cm^{-1})
Chitosan emulsion					
Chitosan[a] Without emulsifier[a]	61.5	Apple	18.7	88.9	–66.8
		Tomato	–	90.0	–
		Melon	–	88.0	–
		Tangerine	–	87.5	–
		Pear	–	8.5	–
Other emulsions					
Alginate (2.0%)[b], without emulsifier	51.5	Garlic[a]	18.3	81	–43.0
Alginate (2.0%)[b], added β-sitosterol (2000 ppm)	31.1	Garlic[a]	18.3	51	–11.2
Shellac[c]	33.4	Grapefruit[b]	23.0	53	
Polyethylene wax[c]	33.4	Grapefruit[b]	23.0	56	
Carnauba wax[c]	28.8	Orange[b]	20.0	46	
Resin[c]	35.6	Orange[b]	20.0	46	

[a] Choi *et al.* (2001).
[b] Hershko and Nussinovitch (1998).
[c] Hagenmaier and Baker (1993).

others (1996) reported that pear surface was more evenly coated by corn-zein and Semperfresh™ solutions.

16.6 Determining diffusivities of fruits

Knowledge of the diffusivities of gases in bulky plant organs is essential in understanding physiological changes, gas exchanges and internal gas composition. The internal gas composition of fruits is determined by the diffusivities of skin, flesh and stem (Burg and Burg, 1965; Cameron and Yang, 1982). Burg and Burg (1965) designed a system to determine gas resistance factors which can be used to estimate gas diffusivities in bulky plant organs using the ratio of internal concentration to the ratio of the production of carbon dioxide and ethylene in the steady state. The diffusivities of gases in bulky plant tissue can be calculated as shown in Box 3.

There have been several reports on determining the diffusivities of bulky plant organs. Burg and Burg (1965) defined a resistance factor (R) which could be estimated for bulky plant organs, in banana and tomato, as the ratio of internal concentration to the ratio of production of carbon dioxide and ethylene in the steady state. They estimated that more than 60% of gas exchange takes place through the stem scar in tomatoes. But this resistant factor is only an empirical value without conventional dimensions and is not constant with changes in the surface to volume ratio. Cameron and Yang (1982) measured the efflux of a metabolic inert gas, ethane, which is neither produced nor metabolized to a significant degree by the tissue. It was shown that over 97% of gas exchange in tomato fruits occurs through the stem scar. However, the measurement of ethane efflux introduces several uncertainties because they did not measure the diffusivities of exocarp, pericarp and stem scar separately.

Solomos (1987), in a review of the principles of gas exchange in bulky plant organs, considered stationary states for CO_2 diffusion through spherical- and cylindrical-shaped plant organs and determined the diffusivities of flesh and skin of apple in the peeled and intact fruit. The effect of the stem in gas transfer was not considered in determining the apparent diffusivities of apple.

Wax undoubtedly serves as a gas barrier to oxygen, carbon dioxide and water vapor and other metabolic gases and also provides protective functions (for example, mechanical damage, fungal and insect attack). Therefore, it can be assumed that the primary factor which regulates the internal concentration of gases is the skin in bulky plant organs. In apple the resistance of apple skin to gas diffusion was 10- to 20-fold greater than that of the flesh, depending on the cultivar (Solomos, 1987). Chinnan and Park (1995) built such a system from Plexiglass (diffusion cell, Fig. 16.1) and used it to determine the gas diffusivities of skin, pericarp and stem scar of tomatoes (see Fig. 16.2).

The gas diffusivities of exocarp plus pericarp, pericarp and stem scar increased as the tomatoes developed from the green stage to the red stage. The oxygen and carbon dioxide diffusivities of the stem scar increased 1.2–1.3 times as the

Box 3 Diffusivity

Gas exchange in bulky plant tissue can be approximated by Fick's first law. The flux of a gas in Fick's law is dependent on the gradient of concentration and diffusivities of plant organs. However, to determine the gradient of gases, Fick's second law can be employed (Chang, 1981; Gerard, 1931; Hill, 1928; Ricciardi, 1977; Solomos, 1987, 1989). If diffusion is one-dimensional and the diffusion coefficient is constant, the rate of transfer through unit area becomes:

$$\partial C/\partial t = D\partial C/\partial X \qquad [16.8]$$

In the non-steady state, all the solutions can be obtained either by the method of separation of variables and Fourier series or by the Laplace transformation (Carslaw and Jaeger, 1959; Crank, 1975; Doty, 1946; Edwards and Penny, 1985; Jost, 1960; Tuwiner, 1962).

If surface concentrations are constant, the following boundary and initial conditions may apply:

$C = C_1,\ x = 0,\ t \geq 0$
$C = 0,\ x = L,\ t \geq 0$
$C = 0,\ 0 < x < L,\ t = 0$

The solution in the form of a trigonometrical series is:

$$C(x,t) = C_1(1 - x/L) - 2/\pi \sum_{n=1}^{\infty} C_1/n \sin(nx/L)\exp(-Dn^2\pi^2 t/L^2) \qquad [16.9]$$

As t approaches infinity the terms involving the exponential vanish and we simply have the linear concentration distribution. The rate at which the gas emerges from unit area of the surface $x = L$ of the test sample is given by $-D(\partial C/\partial X)_{x=l}$, which is easily deduced from equation [16.9]. By integrating with respect to t, we obtain the total amount of diffusing substance Q_t which has passed through the membrane in time t as follows:

$$Q_t/LC_1 = Dt/L^2 - 1/6 - 2\pi \sum_{n=1}^{\infty} (-1)\exp(-Dn^2\pi^2 t/L^2) \qquad [16.10]$$

As t approaches infinity, equation [16.10] approaches the line:

$$Q_t = DC_1/L(t - L^2/6D) \qquad [16.11]$$

This has a intercept L on the t-axis given by:

$$L_t = L^2/6D \qquad [16.12]$$

The intercept L_t is referred to as the 'time lag'. Thus, the measured values of concentration of the diffusion constant can be determined from the linear portion of the plot (Floros and Chinnan, 1989).

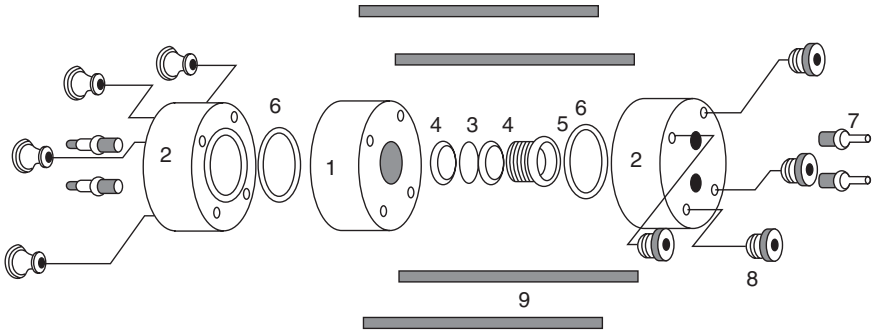

Fig. 16.1 Diffusion cell is constructed from Plexiglass™ to determine diffusivities. The cell is composed of three main parts: the sample holder, the supplying chamber and the sampling chamber. The face of each part is tooled for an O-ring which provides a tight connection. Chinnan and Park (1995) modified and reconstructed the apparatus for this gas diffusion study. (1) Sample holder, (2) gas chamber, (3) sample, (4) sample retainers, (5) threaded bush, (6) sealing O-ring, (7) tubing adapters, (8) thumb nuts, (9) thread rods.

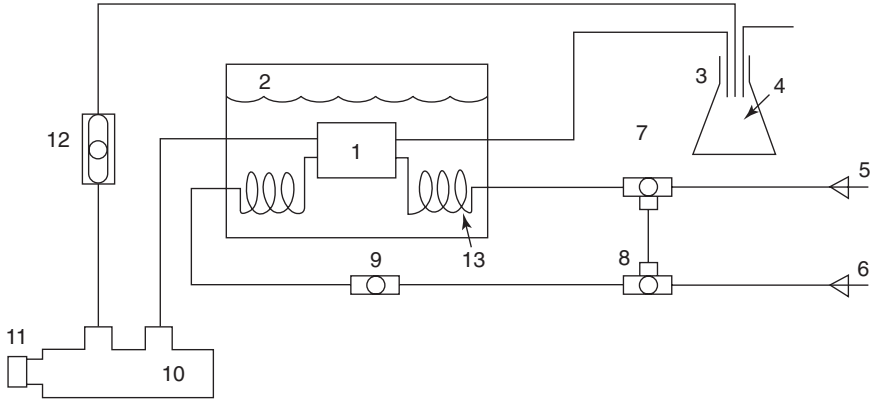

Fig. 16.2 Diffusivity can be measured by the following procedures (Chinnan and Park, 1995). Each of the cored and sliced samples prepared for the study is placed in the diffusion cell and a premixed gas (9.9% O_2, 10.1% CO_2, 80.0% N_2) is introduced to the supplying chamber. The amount of CO_2 and O_2 diffusing through the sample in time t into the sampling chamber can be measured by gas chromatography. The sampling interval is 5 min, and the total sampling period is 2 h. The diffusion cell is immersed in a water bath maintained at 21°C. All equipment for determining gas diffusivities is placed in a heat insulated chamber and the temperatures at several places inside the chamber are monitored. (1) Diffusion cell, (2) water bath, (3) flask, (4) mineral oil, (5) test gas inlet, (6) nitrogen inlet, (7) three-way valve, (8) three-way connector, (9) two-way valve, (10) sampling chamber, (11) silicone septum, (12) gas flowmeter, (13) brass tubing.

tomatoes developed from green to red. The extent of increase in gas diffusivities for exocarp plus pericarp and pericarp were greater than that of the stem scar during the ripening process. Progressive loss of firmness during the ripening process is the result of a gradual transformation of protopectin into pectin which is degraded by the enzyme polygalacturonase in the cell wall (Hobson and Davies, 1971). This enzymatic degradation of pectin can probably be attributed to greater diffusion of gases in the bulky organs of tomato.

16.7 Measuring internal gas composition of fruits

A cylindrical plug of tissue was removed from individual fruits (oranges, apples, tomato, cantaloupe, water melon and pineapple) using a rubber stopper corer. A glass tube was sealed around the hole to the surface of the produce sample. In order to measure internal gas composition, gas in the glass tube was allowed to equilibrate with internal gases (Banks and Kays, 1988; Park *et al*., 1994c). Then a gas sample was taken from the glass tube with a syringe injected through the sealing stopper. By immersing both produce sample and attached glass tube in water atmospheric contamination at the point of syringe insertion can be prevented. Gas samples will be analyzed by gas chromatography. Required equilibrium times (when gas composition of the inside of the glass tube is constant) need to be determined by periodically monitoring gas changes inside the glass tube. Equilibrium time can be expected to vary with variety, ripeness, temperature and harvesting season for various fruits but two hours is usually enough time.

16.8 Future trends

Using gas permeation data on edible coatings, diffusivity data for the skin and flesh of fruits and mathematical models, the internal gas composition can be predicted for selected fruits. Predictions of internal gas compositions with and without coatings will enable better matches to be made between individual fruits and individual edible coatings. The mathematical model could be verified by comparing predicted and measured internal gas composition for various coating materials and thicknesses on selected fruits. Also optimum edible coating thickness can be calculated for each produce–coating combination.

16.8.1 Predicting internal gas composition

Gas diffusion models will be determined according to physical shape and composition of individual fruits. For example, if one-dimensional steady state diffusion with a constant diffusion coefficient is assumed, the gas diffusion model for a hollow sphere can be used to predict the internal oxygen composition of some fruits such as apples and cantaloupes as follows. In one-dimensional diffusion with a constant diffusion coefficient, the rate of gas transfer in the sphere is

Box 4 Optimal edible coating thickness

The hollow sphere model can also be used to determine the optimal edible coating thickness in some fruits such as apple and cantaloupe melons. In edible film-coated apple and cantaloupe, the flux of oxygen passing through the spherical fruit wall from the center to the interface between the film coating and the fruit surface should equal the flux of oxygen passing through the edible coating from the interface between the film coating and the fruit surface to the atmosphere, and should equal the rate of oxygen consumption of the edible film-coated apple and cantaloupe in the steady state (Carslaw and Jaeger, 1959; Chang, 1981; Crank, 1975; Doty, 1946; Jost, 1960; Solomos, 1987):

$$\begin{aligned} Q_t &= 4\pi D_{\text{app}} ab(C_2 - C_1)/(b-a) \\ &= 4\pi D_{\text{cz}}((C_2 - C_{\text{x}})/X)b^2 = R_c(\text{O}_2)W \end{aligned} \quad [16.17]$$

where $R_c(O_2)$ is the oxygen consumption rate of coated fruits, D_{cz} is the diffusivity of edible coatings and X is the thickness of the edible coating. C_x is oxygen concentration at the surface between the edible coating and the surface of fruits.

The optimal coating thickness which will create a desirable range of internal oxygen concentrations (C_1) in apples, (i.e. 2–3%) and cantaloupe melons (3–5%) can be calculated from equation [16.18]:

$$X = 4\pi D_{\text{cz}}(C_2 - C_{\text{x}})b^2/(R_{\text{c}}(\text{O}_2)W) \quad [16.18]$$

where $b + X$ becomes b when X is very small. C_x is determined from equation [16.17] with $C_2 = C_x$.

(Carslaw and Jaeger, 1959; Chang, 1981; Crank, 1975, Doty, 1946; Jost, 1960; Solomos, 1987):

$$\partial C/\partial t = D(\partial^2 C/\partial r^2 + (2/r)(\partial C/\partial r)) \quad [16.13]$$

on substituting $u = \text{Cr}$ in the equation [16.13], we have: $\partial u/\partial t = D\ (\partial^2 C/\partial r^2)$. In the steady state, the differential equation for this case is:

$$\text{d}(r^2\ \text{d}C/\text{d}r)/\text{d}r = 0 \quad [16.14]$$

In a hollow sphere where $a \le r \le b$, if gas concentrations are kept constant at the surfaces so that they are equivalent to C_1 at $r = a$ and C_2 at $r = b$, then $C = [aC_1(b - r) + bC_2(r - a)]/r(b - a)$. By integrating with respect to time t over the surface area, the total amount of diffusing gas Q_t passing through the wall can be determined by (Carslaw and Jaeger, 1959; Crank, 1975; Solomos, 1987):

$$Q_t = 4\pi D_{\text{app}} ab(C_2 - C_1)/(b-a) \quad [16.15]$$

where D_{app} is apparent diffusivity of the hollow sphere and a and b are constants for individual fruits.

However, in the steady state the flux of oxygen passing through the spherical fruit wall should equal the rate of gas consumption, thus:

$$Q_t = 4\pi D_{app} ab(C_2 - C_1)/(b - a) = R(O_2)W \quad [16.16]$$

where $R(O_2)$ is respiration rate of oxygen per fruit and W is weight of the fruit.

The internal oxygen composition, C_1, can be predicted using equation [16.16]. The correlation factors can be calculated from actual measurement of internal gas composition. Also, the predicted internal gas composition of edible film-coated fruits and vegetables can be verified by measuring internal gas composition. Optimum edible coating thickness can be calculated for each produce–coating combination as shown in Box 4.

16.8.2 Measurement of quality and shelf-life change

Quality criteria for edible film-coated fruits must be determined carefully and the quality parameters must be monitored throughout the storage period. For example, the color change and firmness are very important quality parameters in some fruits. The color change, loss of firmness, ethanol fermentation, decay ratio and weight loss of edible film-coated fruits need to be monitored (Shewfelt *et al*., 1987). The color change is monitored by the change in hue angle. An Instron universal test machine can be used to measure firmness by a non-destructive method (Bourne, 1982). Sensory evaluation and consumer acceptability tests need to be examined during storage.

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17

High pressure processing of fruit and vegetables

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17.1 Introduction

By subjecting foods to high pressure (HP), microorganisms and enzymes can be inactivated without the degradation of flavour and nutrients associated with traditional thermal processing. The technology was first commercialised in Japan in the early 1990s for the pasteurisation of acid foods for chilled storage. In spite of massive research efforts, particularly in Europe and the USA, commercial development outside Japan has been slow so far, mainly because of the very high investment and processing costs of HP processing as well as regulatory problems in regions such as Europe. Recent examples of HP processing of fruit products are shown in Table 17.1.

The HP process is non-thermal in principle, even if the pressure increase itself causes a small rise in temperature. HP affects all reactions and structural changes where a change in volume is involved, as in the gelation of proteins or starch. The mechanism behind the killing of microorganisms is a combination of such reactions, the breakdown of non-covalent bonds and the puncturing or permeabilisation of the cell membrane. Vegetative cells are inactivated at about 300 MPa at ambient temperature, while spore inactivation requires much higher pressures (600 MPa or more) in combination with a temperature rise to 60–70°C. Certain enzymes are inactivated at 300 MPa, while others are very difficult to inactivate at all within the pressure range which is available today. Moisture level is extremely important in this context, little effect being noticeable below 40% moisture content.

Table 17.1 Recent examples of HP processing of fruit products (adapted from Cheftel, 1997)

Company	Product	Pressure/temperature/time combination	Role of HP
Japan			
Meidi-ya	Fruit-based products (pH < 4.5); jams (apple, kiwi, strawberry); jellies; purees; yoghurts; sauces	400 MPa, 10–30 min, 20°C	Pasteurisation, improved gelation, faster sugar penetration; limiting residual pectinmethylesterase activity
Pokka Corp. (ceased in 1998)	Grapefruit juice	200 MPa, 10–15 min, 5°C	Reduced bitterness
Wakayama Food Ind.	Mandarin juice (winter season only) (only about 20% of HP juice in final juice mix)	300–400 MPa, 2–3 min, 20°C	Reduced odour of dimethyl sulphide; reduced thermal degradation of methyl methionine sulphoxide; replace first thermal pasteurisation (after juice extraction) and final pasteurisation before packing: 90°C, 3 min
Nisshin fine foods	Sugar-impregnated tropical fruits (kept at −18°C without freezing) for sorbet and ice cream	50–200 MPa	Faster sugar penetration and water removal
QP corp	Ice-nucleating bacteria (for fruit juice and milk)	/	Inactivation of *Xanthomonas*, no loss of ice nucleating properties
Ehime Co.	Japanese mandarin juice	/	Cold pasteurisation
Takansi	Fruit juice	/	Cold pasteurisation
Pon (test market in 2000)	Orange juice	/	/
Europe			
Pampryl (France)	Fruit juice (orange, grapefruit, citrus, mixed fruit juice)	400 MPa, room temperature	Inactivation of microflora (up to 10^6 cfu g^{-1}), partial inactivation of pectinmethylesterase
Orchard House Foods Ltd (UK)	Squeezed orange juice	/	Inactivation of microflora and enzyme, keeping natural taste
United States			
Avomex	Avocado paste (guacamole, chipotle sauce, salsa) and pieces	700 MPa, 10–15 min, 20°C	Microorganism inactivation, polyphenoloxidase inactivation, chilled process

Pulsed or oscillating pressurisation is more effective in spore inactivation than continuous pressure. Rapid decompression increases the impact force on the spore coat much more than the preceding compression and makes possible sterilisation at lower pressures than continuous pressure. At low pressures, 50–300 MPa, considerable germination of spores can occur, strongly influenced by temperature and pH, which allows organisms to be killed by moderate pressures. In the case of a large variety of moist products, pressurisation to above 100 MPa in less than 30 s, mainly at a temperature around 90°C with a holding time of only a few minutes, resulted in complete inactivation of even thermoresistant spores. The combination of nisin (a bacteriosin), high pressure and lowered temperature may allow for a considerable reduction in processing time and/or pressure in HP treatment. Microbial kill is completed without the frequently encountered survival of some pathogens (Hauben *et al*., 1997; Garcia-Graells *et al.*, 1998).

17.2 High pressure (HP) technology

The main components of a high pressure system are:

- a pressure vessel and its closure
- a pressure generation system
- a temperature control device
- a materials handling system (Mertens, 1995).

Most pressure vessels are made from a high tensile steel alloy 'monoblocs' (forged from a single piece of material), which can withstand pressures of 400–600 MPa. For higher pressures, prestressed multilayer or wire-wound vessels are used (Mertens, 1995). Vessels are sealed by a threaded steel closure, a closure having an interrupted thread (which can be removed more quickly), or by a sealed frame that is positioned over the vessel. In operation, after all air has been removed, a pressure transmitting medium (either water or oil) is pumped from a reservoir into the pressure vessel using a pressure intensifier until the desired pressure is reached. This is termed 'indirect compression' and requires static pressure seals. Another method, termed 'direct compression' uses a piston to compress the vessel, but this requires dynamic pressure seals between the piston and internal vessel surface, which are subject to wear and are not used in commercial applications.

Temperature control in commercial operations can be achieved by pumping a heating/cooling medium through a jacket that surrounds the pressure vessel. This is satisfactory in most applications as a constant temperature is required, but if it is necessary to change the temperature regularly, the large thermal inertia of the vessel and relatively small heat transfer area make this type of temperature control very slow to respond to changes. In such situations, an internal heat exchanger is fitted.

There are two methods of processing foods in high pressure vessels: in-

container processing and bulk processing. Because foods are reduced in volume at the very high pressures used in processing (for example, water is reduced in volume by about 15% at 600 MPa), there is considerable stress and distortion of the package and the seal when in-container processing is used. Plastic and foil pouches are the best candidates for HP processing, and research is continuing on the optimum design of the package, seal integrity and other suitable packaging materials. Materials handling for in-container processing is achieved using automatic equipment, similar to that used to load/unload batch retorts. Bulk handling is simpler, requiring only pumps, pipes and valves.

HP equipment has long been in use in commercial production of quartz crystals and ceramics. This equipment is also suitable for food processing with some modification. Among the many equipment manufacturers, the following may be mentioned: Mitsubishi Heavy Industries and Kobe Steel Ltd in Japan, Flow International Corporation, GEC Alstom-ACB Pressure Systems, Stansted Fluid Power and Engineered Pressure Systems International, in Europe and the USA.

Pressure chambers for food processing are available of up to 500 l in volume and for pressures up to 800 MPa. For cost reasons, there is a practical limitation at 600 MPa, which will be sufficient for most applications. For technical reasons, all available units are *batch* systems, even if development work is being undertaken to develop truly *continuous* systems. By combining a number of units in a staggered fashion, semi-continuous production can be achieved. The pressurising medium is usually water and foods are packed in flexible packaging with little or no headspace in order to be able to withstand and evenly distribute the pressure. Most systems are vertical, some with an external high pressure intensifier to minimise the number of sensitive high-pressure components in the hydraulic system. The ACB company has developed a semi-continuous *horizontal* pressure vessel with a double set of pistons for loading and unloading in a straight line. Commercial lines are designed to be automated to streamline production and minimise time for loading, pressurisation, holding, depressurising, unloading and drying.

Semi-continuous processing of fruit juices at 4000–6000 l h^{-1}, using pressures of 400–500 MPa for 1–5 min at ambient temperature, is used by one company in Japan, whereas another uses a similar process operating at 120–400 MPa followed by a short heat treatment before the juice is packaged. The process is highly energy efficient although at present the capital costs of equipment remain high. It is possible that such liquid foods could also be used as the pressurising fluid by direct pumping with high pressure pumps. Such systems would reduce the capital cost of a pressure vessel and simplify materials handling. If liquids were also rapidly decompressed through a small orifice, the high velocity and turbulent flow would increase the shearing forces on microorganisms and thus increase their rate of destruction (Earnshaw, 1992). Developments in high pressure processing reported by Knorr (1995a) include combined freeze concentration, pressure freezing and high pressure blanching. Initial results suggest that pressure blanched fruits are dried more rapidly than those treated by conventional hot water blanching.

Examples of semi-continuous systems have been developed by, for example, the companies Alstom and Flow Pressure Systems. In the Flow Pressure semi-continuous system, the liquid to be processed is pumped into one or several so-called isolators (pressure vessels in which a separator partitions the food liquid from the ultra high pressure (UHP) water source). After pressure treatment, the liquid is pumped into a holding tank and aseptic filling station. In the Alstom system, the pressure chamber is filled with the liquid to be treated and compressed directly by a mobile piston (pushed by pressurised water) up to a maximum pressure of 500 MPa. After a predetermined holding time, pressure is released and the liquid pumped by the piston to a holding vessel. Several pressure chambers can be served in parallel by the same main pressure generator so that a continuous downstream flow can be maintained. Since the pressure chamber is completely filled with product, the capacity per cycle is considerably increased compared to the processing of already packaged products in a conventional batch system and cycle time is reduced by about 30%.

Hoogland (2001) has described the development, within a Dutch consortium, of a more cost efficient new generation of HP processing equipment. By using composite materials instead of steel the cost of the pressure vessel is reduced. The use of internal pressure intensifiers, pressurised by external pumps, further reduces cost. With the new system, now at pilot plant stage, cycle times are being reduced to 2–5 min. Another advantage of using composite materials for the pressure chamber is that the chamber wall, which dissipates some of the adiabatic heat generated when pressurising the food load, will not cool the product surface. Since pressure and product temperature have a synergistic inactivation effect, cooling at the chamber wall could compromise the inactivation process. Many efforts are being made to substitute batch processing with a truly continuous HP process. Unilever, for example, have patented a continuous system in which the material to be treated is passed down an open narrow tube in a steady flow. A pressure differential of 100 MPa or more is maintained between the entrance and exit ends of this tube.

Overall estimates by several equipment manufacturers point towards investment costs for a commercial system in the range 0.5–2 million Euro and production costs at 400 MPa of 0.1–0.2 Euro kg^{-1} of processed goods. A high pressure plant for fruit juice pasteurisation is about 20 times the cost of an equivalent heat exchanger system (Manvell, 1996). Actual costs will depend on chamber capacity, fill density, time–pressure–temperature combinations in processing and the degree of utilisation of the line. Investment cost will be about 75% of total production costs.

17.3 Impact of HP on spore-forming bacteria

The formation of 'spores' is a survival strategy of some bacterial genera in extreme stress conditions, especially *Bacillus* and *Clostridium*. Although bacte-

rial spore counts can be lowered by exposure to high pressure, combination with other preservation methods, such as mild temperature elevation, is required for substantial reduction of viable spore counts (Hoover, 1993). Larson *et al.* (1918) observed that pressure treatments up to 1800 MPa at room temperature were not sufficient to obtain commercial sterility of food products. Sojka and Ludwig (1994, 1997) have suggested the use of a two-step process to overcome the problems associated with the extreme pressure resistance of bacterial spores: an initial mild pressure treatment to induce spore germination followed by a treatment at higher pressure and temperature to kill the germinated spores. However, biological diversity in germinability within one spore population and the lack of information on the kinetics of germination seem to limit this approach (Heinz, 1997; Wuytack, 1999).

A combination of pressure with temperatures of 60°C and higher is required for extensive inactivation of spores: the lower the pressure applied, the higher the required temperature to induce a preset extent of inactivation (Sale *et al.*, 1970; Heinz, 1997; Wuytack, 1999). At temperatures below 60°C in combination with a pressure of about 400 MPa, maximal three log-cycle reductions were obtained for *Clostridium sporogenes* and *Bacillus coagulans* spores (Roberts and Hoover, 1996; Mills *et al.*, 1998). To achieve sterility with minimal impact on nutritional value, flavour, texture and colour, high pressure processing using multiple high pressure pulses and achieving an end temperature above 105°C under pressure for short time has also been proposed (Meyer *et al.*, 2000; Krebbers *et al.*, 2001). The major benefit of high pressure treatment for food preservation is the reduction in the thermal resistance of spores. This synergistic effect seems to be somewhat impaired at high temperatures.

17.4 Impact of HP on vegetative bacteria

At moderate pressure, growth and reproduction rate of vegetative bacteria are retarded while at higher pressures inactivation occurs. Although pressure stability is largely dependent on the type of microorganism, the species and the medium conditions, pressures between 200 and 600 MPa at room temperature are usually sufficient to cause a substantial reduction in viable vegetative cells. Vegetative forms of prokaryotes such as yeasts and moulds are most pressure sensitive and inactivated by pressures between 200 and 300 MPa. Gram-negative bacteria can be inactivated by pressures of about 300 MPa and are, in their turn, less pressure stable than gram-positive bacteria, for which pressures higher than 400 MPa are required for inactivation. However, numerous exceptions to these general statements can be found. Some very pressure-resistant strains of *E. coli* O157:H7 were found by Benito *et al.* (1999), for example. In addition, in contrast to laboratory conditions, microorganisms are often more stable in actual food products. In general, the protective effect of real food products has been attributed to the presence of proteins and sugars. On the other hand, synergistic effects between

pressure and acidification or addition of antimicrobial substances can be exploited to lower the pressure resistance of microorganisms (Hauben *et al.*, 1997; Garcia-Graells *et al.*, 1998).

17.5 Impact of HP on enzymatic activity

Some key enzymes in fruit and vegetable processing include:

- polyphenoloxidase (PPO) which is responsible for enzymatic browning
- lipoxygenase (LOX) which induces changes in flavour, colour and nutritional value
- pectinmethylesterase (PME) which is responsible for cloud destabilisation and consistency changes
- peroxidase (POD) which gives rise to unfavourable flavours.

PPO is not very heat resistant (Lourenço *et al.*, 1990; Yemenicioglu *et al.*, 1997; Weemaes *et al.*, 1998a). Upon pressurisation, in contrast, PPO may display, depending on its source, either enhancement of catalytic activity or inactivation. Pressures needed to induce substantial inactivation of PPO vary between 200 and 1000 MPa, depending on the enzyme origin and microenvironmental conditions such as medium composition or pH (Weemaes, 1998). Whilst PPO in some fruits such as apple and grape is sensitive to pressure, PPO in others, such as pear and plum, is resistant to pressure inactivation. Low pressure may protect PPO from thermal inactivation and enhance catalytic activity, for example in the case of apple, pear, potato and strawberry (Weemaes *et al*., 1998b).

For LOX, thermal stability at atmospheric pressure largely varies with the enzyme source and medium (Indrawati, 2000). Detailed studies of pressure inactivation have been performed for tomato, soybean, green bean and pea LOX. Threshold pressures for inactivation in a narrow range between 400 and 600 MPa have been reported (Heinisch *et al.*, 1995; Ludikhuyze *et al.*, 1998a; Tangwonchai *et al.*, 1999; Indrawati *et al.*, 1999; Indrawati, 2000). For soybean, green bean and pea LOX, complete kinetic characterisation of the inactivation kinetics has been accomplished in a pressure–temperature domain from 0.1 to 650 MPa and from −10 to 80°C. For green bean and peas it was noted that pressure stability of LOX decreased with increasing system complexity, in other words inactivation occurred faster *in situ* (in the intact vegetable) compared to in crude extract (Indrawati, 2000). For soybean LOX on the other hand, higher pressure stability was observed in milk compared to in a buffer solution (Seyderhelm *et al.*, 1996). In a similar way to avocado PPO and orange PME, an antagonistic effect between low pressure and high temperature was noted for pea LOX. In the case of soybean and green bean LOX, an antagonistic effect between temperature lower than 30°C and pressure higher than 500 MPa has been observed (Ludikhuyze *et al.*, 1998b; Indrawati *et al.*, 1999).

PME from different fruits has been reported to be quite thermoresistant: temperatures between 80 and 95°C are required to induce significant inactivation

and even then PME remains active (Van den Broeck, 2000). This resistance was ascribed to the presence of heat labile and heat stable PME isozymes (Versteeg *et al.*, 1980; Wicker and Temelli, 1988; Van den Broeck *et al.*, 2000b). Pressure stability has mainly been investigated for orange PME and to a lesser degree for grapefruit, guava and tomato PME. Threshold pressures for inactivation at room temperature of PME from different sources have been reported to vary largely from about 150 to 1200 MPa, depending on the origin and the medium in which the inactivation is carried out (Van den Broeck, 2000). Inactivation occurs faster in acid medium and is protected by an increased amount of soluble solids (Ogawa *et al*., 1990). Most studies report only partial inactivation of PME, which is ascribed to the presence of isozymes with different pressure resistance. Complete kinetic characterisation of inactivation of PME from oranges in a broad pressure (0.1–800 MPa) and temperature (15–65°C) domain revealed a slight antagonistic effect of low pressure and high temperature (Van den Broeck *et al.*, 2000b).

In contrast to thermal resistance, tomato PME was found to be much more pressure resistant than orange PME and an extreme antagonistic effect of high temperature and pressure was noted in this case. At 60°C, a temperature where inactivation at atmospheric pressure occurs, pressure up to 700 MPa completely inhibited inactivation. At higher pressure, inactivation again occurred although the inactivation rate was still slower at 900 MPa compared with atmospheric pressure (Crelier *et al.*, 1995; Van den Broeck *et al.*, 2000a). At atmospheric pressure, optimal activity was found at 55°C. Application of low pressure increased the activity of PME, which became maximal at a pressure of 100–200 MPa in combination with a temperature of 60–65°C (Van den Broeck *et al.*, 2000a).

POD, which is generally considered to be the most heat stable vegetable enzyme, is at least in some cases also extremely pressure resistant. In green beans, a pressure treatment of 900 MPa merely induced slight inactivation at room temperature, while in combination with elevated temperature enhanced the inactivation effect at 600 MPa (Quaglia *et al.*, 1996). Contradictory results were found by Cano *et al.* (1997) who reported POD in strawberry purée and orange juice to be increasingly inactivated at room temperature with pressure up to 300 and 400 MPa, respectively, whereas at higher pressure activity decreased again. At higher temperature (45°C), a decrease in activity was found for all pressures (50–400 MPa).

17.6 HP processing, fruit and vegetable quality

HP processing has a range of effects on:

- texture
- colour
- flavour
- vitamin content.

17.6.1 Texture

In general, pressures up to 350 MPa can be applied to plant systems without any major effect on overall texture and structure (Knorr, 1995b). Several studies revealed that pressure treatment of fruit and vegetables can cause both firming and softening (Basak and Ramaswamy, 1998), the effects being dependent on pressure level and pressurisation time. In general, the softening curves revealed that texture changes caused by pressure occurred in two phases: a sudden loss as a result of the pulse action of pressure followed by further loss of gradual recovery during pressure holding phase. At low pressure (100 MPa), instantaneous pressure softening was caused by compression of cellular structures without disruption, while at higher pressure (>200 MPa) severe texture loss occurs owing to rupture of cellular membranes and consequent loss of turgor pressure. During pressure holding time, the instantaneous texture loss can be gradually recovered and some products become even firmer than their fresh counterparts. In many cases, pressure-treated vegetables do not soften during subsequent cooking, which is attributed to the action of PME that is only partially inactivated by pressure. Simultaneous disruption of cell structures allows interaction of the enzyme with the pectic substance. Hence, the de-esterfied cell wall pectin can crosslink with divalent ions, leading to increased compactness of cellular structure.

17.6.2 Colour

For many fruit and vegetable products such as fruit jam, strawberries, tomato juice, guava purée, avocado purée and banana purée, high pressure treatment was noted largely to preserve fresh colour (Watanabe *et al*., 1991; Poretta *et al.*, 1995; Donsi *et al.*, 1996; Yen and Lin, 1996; Lopez-Malo *et al.*, 1998). The brightness (L-colour value) and redness/greenness (a-colour value) of pressure-treated products were found to be superior compared with their thermally treated counterparts. However, during storage of guava and banana purée, the green colour gradually decreased because of browning as a result of residual PPO activity (Lopez-Malo *et al.*, 1998; Palou *et al.*, 1999a). The longest acceptability storage time was achieved by using high pressure, low pH and refrigerated storage. A detailed kinetic study regarding the combined effect of pressure and temperature on colour of broccoli juice revealed that the chlorophyll content and green colour (a-value) were stable for up to 4 h treatment at 800 MPa and 40°C. Only when high pressure is combined with temperature higher than 50°C, were some colour changes noted. Degradation of chlorophyll content was described by a first order model, with chlorophyll a being less stable than chlorophyll b. On the other hand, loss of green colour was described by a consecutive step model because both conversion of chlorophyll to pheophytin and further conversion to pyropheophytin occurred (Van Loey *et al.*, 1998; Weemaes *et al.*, 1999).

17.6.3 Flavour

For most fruit juices, the potential benefits of using high pressure mainly arise from the fact that fresh flavour can be maintained during pressure treatment.

Many authors reported that trained sensory panels were unable to differentiate between fresh and pressurised juice made from the same raw material (Ogawa *et al.*, 1990; Watanabe *et al.*, 1991; Bignon, 1996). For tomato and onions, however, some flavour defects caused by pressure treatment were perceived: tomato had a rancid taste while onions smelled less intensely and more like fried onions (Butz *et al.*, 1994; Poretta *et al.*, 1995). In the former case, the rancid flavour was attributed to a marked increase in n-hexanal which is largely responsible for fresh tomato flavour in a concentration of 1–2 mg kg^{-1}. Higher concentrations impart the rancid flavour. For onions, pressure treatment was reported to diminish dipropylsulphide, a compound responsible for pungency and the characteristic odour of fresh onions and to increase transpropenyldisulphide and 3,4-dimethylthiophene concentrations leading to a flavour of braised or fried onions.

17.6.4 Vitamin content

Bignon (1996) observed that vitamin A, C, B_1, B_2 and E content of fruit and vegetable products is not significantly affected by pressure treatment in contrast to thermal treatment. Besides, in the case of strawberries and guava purée, the decrease in vitamin C content during storage after pressure treatment (400–600 MPa/15–30 min) was found to be much lower compared to the fresh products (Sancho *et al.*, 1999). A more detailed kinetic study of pressure–temperature stability of ascorbic acid in buffer, orange juice and tomato juice was performed by Van den Broeck *et al.* (1998). They found only significant degradation of ascorbic acid when pressure of about 850 MPa was combined with temperatures between 60 and 80°C, and more in tomato and orange juice than in buffer. As well as vitamins, some minor studies of other health characteristics such as antimutagenicity and toxicity have been performed. Fruit and vegetables such as carrots, cauliflower, kohlrabi, leek and spinach are characterised by strong antimutagenic potencies, which were found to be sensitive to heat but not to pressure. For beet and tomatoes antimutagenic activity was affected, but only under very extreme conditions, that is 600 MPa/50°C or 800 MPa/35°C (Butz *et al.*, 1997).

17.7 Combining HP processing with other preservation techniques: the case of fruit

A characteristic shared by most fruits and low-pH foods is their high acidity. Although most species of bacteria are inhibited by the resulting hydrogen ion concentration, lactic acid bacteria, yeast and moulds are more aciduric and many find these pH values to be tolerable, if not optimum, for growth. It is because of acidity, therefore, that fungi and lactic acid bacteria are the principal spoilage microorganisms of fruit and fruit products. High pressure processing is a potentially useful way of helping to inactivate spoilage bacteria and control enzymatic activity. However, as has been indicated, it cannot be used in isolation.

Pasteurisation or sterilisation of low-acid foods using high pressure, for example, is only feasible when combined with other preservation techniques which enhance inactivation. Factors such as heat, antimicrobials, ultrasound and ionising radiation can potentially be used in combination with high pressure. These approaches will not only help to accelerate the rate of inactivation, but can also be useful in reducing the pressure level and, hence, the cost of the process, while eliminating the commercial problems associated with sublethal injury and survivor tails.

As an example, studies of residual PPO activity in fruit purées after HP treatments suggest that inhibition of undesirable enzymatic reactions, such as browning, requires the combination of pressurisation with one or more additional factors, such as low pH, blanching or refrigeration temperatures to inhibit (or at least reduce significantly) enzyme activity (Lopez-Malo *et al.*, 1998; Palou *et al.*, 2000; Lopez-Malo *et al.*, 2000). Other research suggests that blanching, for example, is important for pressure treatment of fruit and vegetables to minimise enzymatic and oxidative reactions (Hoover, 1993). The effects of blanching and HP treatments on PPO activity of banana purée adjusted to pH 3.4 and water activity, a_w 0.97, showed PPO activity was reduced during steam blanching and further reduced after HP treatment (Palou *et al*., 1999a).

A key role for high pressure processing is in reducing the severity of the processes traditionally used to preserve foods. The use of high pressure in combination with mild heating has considerable potential (Palou *et al.*, 1999b; Lopez-Malo *et al.*, 2000). The antimicrobial effect of high pressure can be increased with heat, low pH, carbon dioxide, organic acids and bacteriocins such as nisin (Palou *et al.*, 1997a, 1997b, 1997c; Papineau *et al.*, 1991; Mallidis and Drizou, 1991). Knorr (1995a, 1995b), Papineau *et al*. (1991) and Popper and Knorr (1990) reported enhanced pressure inactivation of microorganisms when combining pressure treatments with additives such as acetic, benzoic or sorbic acids, sulphites, some polyphenols and chitosan. These combination treatments allow lower processing pressure, temperature and/or time of exposure. It has been suggested that some food preservatives show enhanced activity when subjected to high pressure, though others may be adversely affected (Tauscher, 1995; Palou *et al.*, 1997a). The use of high pressure as one amongst several hurdles provides a way, for example, of reducing the dependence on sulphites as antibrowning and antimicrobial agents. It has also been suggested that the efficiency of high pressure enzyme inactivation be improved by applying pressure cycles. Successive applications of HP treatments resulted in higher inactivation of many enzymes (Hendrickx *et al.*, 1998). Enzyme activity after a multicycle process was lower than that of a single-cycle process of the same total duration (Ludikhuyze *et al.*, 1997).

A number of examples illustrate the potential application of high pressure treatment. Lopez-Malo *et al.* (1999) evaluated the effects of high pressure treatments at 345, 517 or 689 MPa for 10, 20 or 30 min at initial pHs of 3.9, 4.1 or 4.3 on (PPO) activity, colour and microbial inactivation in avocado purée during storage at 5, 15 or 25°C. Standard plate, as well as yeast and mould counts of high pressure-treated purées, were <10 cfu g^{-1} during 100 days of storage at 5, 15

or 25°C. Significantly less ($p \leq 0.05$) residual PPO activity was obtained with increasing pressure and decreasing initial pH. Avocado purée with a residual PPO activity <45% and stored at 5°C maintained an acceptable colour for at least 60 days and achieved a shelf-life of 35 days when stored at 15°C.

Palou *et al.* (2000) have analysed the effects of continuous and oscillatory high pressure treatment on guacamole. Significantly less ($p < 0.05$) residual PPO and LOX activity was obtained by increasing the process time and number of pressurisation–decompression cycles. LOX was inactivated with a 15 min continuous treatment of oscillatory high pressure. The lowest residual PPO activity value (15%) was obtained after four high pressure cycles at 689 MPa with 5 minutes of holding time each. Standard plate as well as yeast and mould counts of high pressure-treated guacamole were <10 cfu g^{-1}. Sensory acceptability and colour of high pressure guacamole were not significantly different ($p > 0.05$) from those of a guacamole control. Browning during storage was related mainly to changes in the hue attributed to a decrease in the green contribution to the colour. A shelf-life of 20 days was achieved at <15°C.

17.8 Future trends

Most review articles have pointed out the potential of high pressure as a significant non-thermal alternative in food processing and preservation which allows better retention of food qualities such as colour, flavour and nutrient value. However, systematic quantitative data on its effectiveness and safety remain limited. However, the use of systematic kinetic studies has resulted in the development of inactivation models for some food spoiling enzymes and microorganisms (Sonoike *et al*., 1992; Hashizume *et al*., 1995; Ludikhuyze *et al*., 1998b; Weemaes, 1998; Van Loey *et al*., 1998; Indrawati, 2000; Reyns *et al*., 2000; Van den Broeck, 2000). As an example, Figure 17.1 shows a theoretical case study which combines data on pressure–temperature kinetics for some food quality-related enzymes (PPO, LOX, PME and ALP), microbial inactivation and chlorophyll degradation. In contrast to the various enzymes, the vegetative organisms follow a similar pattern, suggesting that enzymes are generally more resistant than vegetative microorganisms to pressure–temperature treatments. This model suggests that food quality-related enzymes may be more critical in defining optimal HP treatments. It can also be seen that at pressure–temperature combinations that result in sufficient inactivation of food-spoiling enzymes and microorganisms, total chlorophyll content is only slightly affected. This supports the view that nutritional and sensory quality is only minimally affected by pressure.

This kind of systematic kinetic approach provides a way forward for future research. Indeed, this kind of kinetic information on microbial and enzyme inactivation, together with more quantitative data on the effect of pressure on sensory and nutritional quality, is indispensable for regulatory approval (Food and Drug Administration (FDA) approval in the USA, Novel Food regulations in the EU).

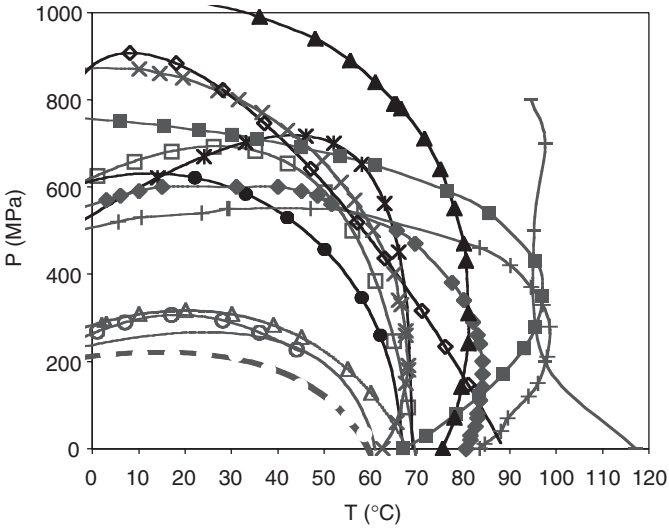

Fig. 17.1 Simulated pressure–temperature combinations resulting in six log-unit reduction of microorganisms, 90% reduction of enzyme activity and 90% of chlorophyll loss after a treatment time of 15 min: PPO (▲); ALP (■); BSAA (◇); pea LOX *in situ* (+); pea LOX in juice (◆); green bean LOX *in situ* (●); green bean LOX in juice (□); soybean LOX (◗); PME (×); total chlorophyll content (—); yeast (△); *Z. bailii* (○); *L. casei* (——); *E. coli* (— — —).

The issue of toxic or allergenic compounds in pressure-treated food products also needs further investigation. Developments in these areas in the future would facilitate a larger scale industrial breakthrough of this new technology.

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18

The use of vacuum technology to improve processed fruit and vegetables

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18.1 Introduction: the role of vacuum technology

It is well established that processing treatments for fruits and vegetables that are designed to preserve them in various forms (fresh, frozen, pasteurised or dried), have an effect on organoleptic qualities such as texture, colour or flavour. In reducing problems caused by deterioration that occur after harvest or during processing, there is a choice between:

- selecting more resistant varieties of raw material,
- adding corrective additives in the final preparation, or
- implementing 'minimal' physical treatments or novel technologies.

One of these new technologies contributing to the preservation of the original properties of fruit or vegetables is 'vacuum technology', which is also called 'vacuum infusion' or 'vacuum impregnation'.

Vacuum technology is considered to be a pretreatment for processed fruit or vegetables leading to improvement in their quality by active incorporation of functional ingredients in the product structure. Passive impregnation by common dipping of the plant products in a solution is usually used in the production of alcohol-based or candied fruits. The penetration of preservatives or humectant agents by soaking can be also required in dried products. However, the processing times of such treatments are long, extending from several hours to several days, and mass transport phenomena are mainly governed by molecular diffusion of the compounds present in aqueous solution. In contrast, vacuum infusion technology is based mainly upon rapid hydrodynamic mass transfer and consists of putting the food product under vacuum before the introduction of an impregnation solution. This allows, within a few seconds, the occluded air initially

contained in the fruit or vegetable pores to be replaced by the impregnation solution, owing to the positive pressure differential which results when atmospheric conditions are restored. This treatment seems to adapt well to porous products and can be applied to whole or cut fruits and vegetables.

Vacuum technology was used for a long time in the treatment of various industrial materials such as wood, metal and so on. In the fruit or vegetables sector, vacuum impregnation was rarely studied in the past. It has received new interest for its potential to improve the organoleptic quality of foods and in the design of minimally processed products.

In the first part of this chapter, modelling of the mass transfer occurring during vacuum treatment and the following modification of the structural and physical properties of products will be described. The second part will highlight different known applications that allow improvement in the quality of stabilised products and/or the extension of their shelf-life.

18.2 Principles: mass transfer and product behaviour

18.2.1 Mass transfer

The most complete description of the mass transfer phenomena occurring during vacuum infusion is generally found in studies dealing with mass exchange in the osmotic dehydration of fruit pieces immersed in concentrated solutions. These two techniques, vacuum application and prolonged immersion of plant products in hypertonic solutions, can be easily coupled (Shi and Fito, 1994; Shi *et al*., 1995). Without encroaching on the specific field of osmotic dehydration, it seems very important to underline the close link which can exist between the two techniques. In these soaking processes, the use of vacuum forces accelerates the penetration of aqueous solution compared with the apparently slow molecular diffusion process that is predominant in the osmotic process.

When a vacuum pulse is applied, trapped gases are expanded and partially removed from the food matrix. After restoring atmospheric pressure, a positive pressure differential results which allows penetration of the liquid into the free voids in the structure until internal and external pressure equilibrium is reached. The time taken to reach a vacuum usually depends on the efficiency of the vacuum system (pump, closed volume of apparatus, etc.) and only lasts at best for a few seconds. In most cases, products have to be maintained under vacuum for a few minutes to ensure good extraction of internal gases, but this step could be unnecessary if degassing is completed during the pressure drop. At the end of the treatment, vacuum release is generally obtained instantly.

Fito and Pastor (1994) and Fito (1994) gave a clear description of the mass transfer phenomena observed in vacuum technology. The mass transfer occurring during the vacuum treatment is referred to as the 'hydrodynamic mechanism' (HDM). Intercellular spaces in plant products are described as a whole by elementary cylindrical pores occupied by an ideal gas undergoing isothermal compression (Fig. 18.1).

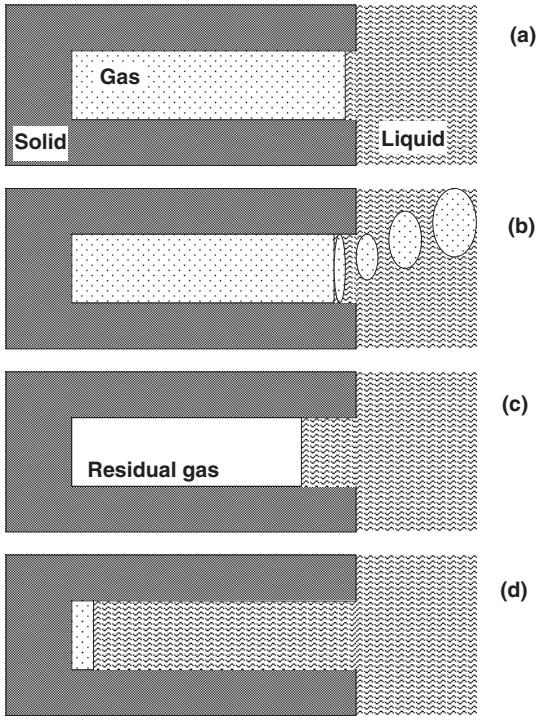

Fig. 18.1 Main stages during vacuum infusion of porous food immersed in a liquid. The situation in an elementary ideal pore (adapted from Fito, 1994); (a) the capillary effect under normal pressure, (b) degassing under vacuum conditions, (c) capillary effect under reduced pressure, (d) HDM at restored normal pressure.

The penetration of solution into the ideal rigid pores breaks down into two stages. First, the pores fill by capillary action in the first part of the treatment that corresponds to atmospheric immersion and vacuum application. Secondly, when restoring normal pressure, the resulting driving force induces liquid flow in the pores. The quantity of external liquid transferred can be almost as great as the available void space in the food structure. The impregnated sample volume fraction (X), usually measured by a gravimetric method, has been modelled on the basis of the HDM and the Hagen Poiseuille equation. X is a function of the product effective product porosity (ε_e) and the apparent compression rate ($r = P_2/P_1$; P_1 is the applied vacuum pressure, P_2 is the restored atmospheric pressure). Thus, Fito and colleagues established that the simple expression of the volume fraction occupied by the liquid in the fruit or vegetable product after vacuum infusion is:

$$X = \varepsilon_e(1 - 1/r) \qquad [18.1]$$

Capillary pressure is not considered in this simplified expression, because it appears to be negligible with respect to the driving force imposed on the system when

the work is carried out at sufficiently low pressure (lower than 600 mbar according to Fito, 1994). Effective porosity is expressed *a priori* as the percentage of sample volume initially occupied by the gases (Calbo and Sommer, 1987), but is defined more precisely as the sample volume fraction available for an HDM mechanism; this parameter is thus determined from an experimental procedure by calculating the slope of the linear function given by adjusting the X versus $1 - 1/r$ curve (Fito, 1994; Del Valle *et al*., 1998). In the case of fruit and vegetables, the porosity values were found to be extremely variable depending on the raw materials, for example average ε_e values are 0.20 for apple and 0.05 for apricot. These variations in porosity can explain the observed variations in weight gain measured in fruit or vegetable pieces after vacuum impregnation step carried out under equivalent experimental conditions (Table 18.1). Moreover, the effective porosity will depends not only on the type of fruit or vegetable, but also on their variety and their maturity (Del Valle *et al*., 1998; Sousa *et al*., 1998).

Table 18.1 Weight gain of various fruits and vegetables after vacuum infusion in water at 20°C (50 mbar, 1 min) and some indicative effective porosity values from different literature sources

Product	Shape (d = diameter, t = thickness)	Weight gain (%)	Effective porosity
Apple, Granny Smith	Slice (d = 3 cm, t = 0.5 cm)	32	0.18–0.25[2–4]
Banana	Slice (d = 2.5 cm, t = 0.5 cm)	17	0.08–0.31[1,4,5]
Cherry	Whole	1	–
Citrus peel	Slice (d = 3 cm)	57	–
Kiwi	Slice (d = 4 cm, t = 0.5 cm)	2	0.005[3]
Mango	Slice (d = 3 cm, t = 0.5 cm)	9	0.03–0.15[1,3]
Orange	Segment	3	–
Pear	Slice (d = 3 cm, t = 0.5 cm)	24	0.14[1]
Pineapple	Slice (d = 3 cm, t = 0.5 cm)	5	0.05[1]
Strawberry	Half	10	0.03–0.11[3,4]
Button mushroom	Slice (t = 0.5 cm)	66	–
Carrot	Slice (d = 2.5 cm, t = 0.5 cm)	6	–
Chicory	Leaf	19	–
Courgette	Slice (d = 4 cm, t = 0.5 cm)	43	–
Eggplant	Slice (d = 5 cm, t = 0.5 cm)	180	–
Onion	Slice (d = 3 cm, t = 0.5 cm)	10	–
Potato	Slice (d = 4 cm, t = 0.5 cm)	3	–
Red pepper	Slice (d = 2 cm, t = 0.5 cm)	13	–
Spinach	Leaf	43	–
Turnip	Slice (d = 4 cm, t = 0.5 cm)	5	–
Basil	Leaf	58	–
Mint	Leaf	50	–

[1]Fito (1994), [2]Del Valle *et al.* (1998), [3]Salvatori *et al.* (1998), [4]Fito *et al.* (1996), [5]Sousa *et al.* (1998).

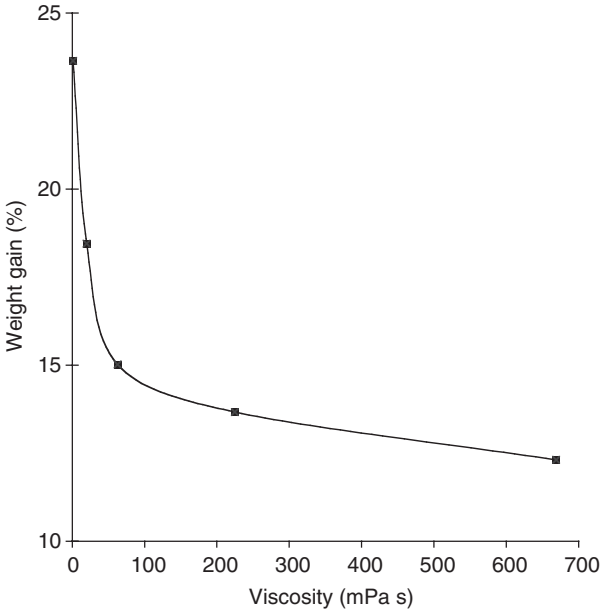

Fig. 18.2 Effect of viscosity on the weight gain of apple slices (diameter 20 mm, thickness 8 mm) after vacuum infusion at 20°C in water and different pectin solutions. Vacuum treatment conditions are 50 mbar for 1 min 15 s.

Equation [18.1], which is derived from capillary flow theory, is not adapted in the case of the infiltration of non-Newtonian liquids or high viscosity solutions. Figure 18.2, reporting results obtained in our laboratory, shows the effect of the solution viscosity – adjusted using low methylated (LM) pectin – on the weight gain of apple slices chosen as a model fruit after vacuum treatment. The decrease in values for weight gain with viscosity indicated that the hydrodynamic mass transfer was limited and could not be predicted from the previous model. Even if applied vacuum pressure represents the main control factor of the process, the composition and the concentration of the aqueous solutions used are potential variables that modify the liquid intake in porous fruits or vegetables. The influence of viscosity (as mentioned above) and the interaction between hypertonic solutions and plant products (osmotic and HDM coupled phenomena are discussed above and in sections 18.3–18.7) appear to be substantial governing factors.

The other variables upon which the vacuum process depends were not studied much systematically, that is temperature of impregnation solution, time to achieve vacuum, time maintained under vacuum, time to restore atmospheric pressure. The time to achieve vacuum and time to restore atmospheric pressure were not noted to have any substantial effect in the literature, whereas the time the vacuum was maintained had no consequence on the HDM transfer beyond a few minutes

(2 min suggested by Fito and Pastor, 1994). The few existing data concerning the effect of temperature showed that just a slight variation in mass transfer rate was induced (Hoover and Miller, 1975). In practice, temperature conditions are limited when nearing the liquid boiling point under vacuum, for example near 46°C for water at 100 mbar. Finally, the temperature effect on liquid viscosity or food matrix plasticity is certainly suggested to play a role in vacuum technology.

18.2.2 Modifications to structural and physical properties

Several authors (Fito *et al*., 1996; Sousa *et al*., 1998; Salvatori *et al*., 1998) reported that the HDM mechanism is accompanied by deformation of the food matrix which influences the final liquid uptake and affects the mechanical properties of the product after treatment. The deformation phenomenon corresponds first to an extension of the internal occluded air volume inside the product when degassing at the time the vacuum is created, and secondly to a partial retraction in pore volume caused by structure relaxation at the time of return to atmospheric pressure. As a function of the viscoelastic properties of the internal structure and the cohesive forces in plant cellular tissue, the deformation–relaxation phenomenon could induce irreversible effects, involving in some cases rigidity loss caused by embrittlement or rupture in the cell wall junctions, possibly accompanied by juice loss. Generally, this phenomenon, correlated with the pressure driving forces and perhaps with the time during which they operate, results in an increase in effective porosity values and enhances the quantity of infused liquid in the product with the detrimental effect of a moderate loss of firmness.

From microscopic observations of kiwi fruit before and after vacuum treatment with glucose solutions, Muntada *et al*. (1998) noticed that the size of the cells in the infused plant tissue and their arrangement were preserved even if ruptures in the cellular walls were observed. This was in agreement with the previous work of Bolin and Huxsoll (1987) on apple, which showed that vacuum impregnation causes the rupture of a non-negligible number of cells. As it will be emphasised hereafter, this food structure damage may be masked by the reinforcement of the cell wall structure by calcium or by strengthening of the wall with gelling agents or other solutes, which could improve texture of the processed products still more. The paradox of the vacuum technique becomes clear when considering both the microscopic observations and the negative effect of the deformation–relaxation phenomenon: the moderate loss of integrity as a consequence of the vacuum treatment can be compensated to a large extent by the active role of the transferred solutes.

Thanks to the incorporation of functional agents with vacuum technology it is possible to modify the physical or physicochemical properties of fruit and vegetables. The work of Martinez-Monzo *et al*. (1998a) seems to be a particularly representative example, providing promising prospects for the development of a pretreatment that will modify the initial composition of porous fruit, making it more resistant to damage caused by the freezing–thawing process. The infusion of cryoprotectants (low molecular weight solutes) or cryostabilisers (high

molecular weight solutes) into apple pieces before freezing did not modify significantly the measured glass transition temperatures, but when concentrated cryoprotectant solutions were used, a notable reduction in freezable water was obtained. The reduction in freezable water content should contribute to decrease the damage produced by ice crystals because of the reduction in their volume fraction. After impregnation with modified grape must as the chosen cryoprotectant, cryo-scanning electron microscopy observations of the cellular structure of apple showed that the formation of ice crystals was similar in intercellular space and inside the vacuole, without detecting any apparent disturbances in the cell (size, shape and intracellular arrangement). With a cryostabiliser like high methylated (HM) pectin, in the first instance the penetration of viscous pectin solution was not complete, leaving empty intercellular spaces. Secondly a difference between the ice crystals in inter- and intracellular spaces respectively was observed. The presence of pectin could nevertheless increase the stability thanks to modification of the ice crystals in the intercellular spaces and reinforcement of the structure by intercellular bridges formed from polysaccharide gel.

In considering the changes in thermal properties (thermal conductivity, thermal diffusivity and specific heat), Martinez-Monzo *et al*. (2000) indicated first that the vacuum impregnation treatment applied to apple could increase (up to 24%) thermal conductivity considerably. This result was mainly justified by replacement of the inner gases by liquid in the fruit pores, which reduces the thermal resistance commonly related to the void fraction. The increase in thermal conductivity is consequently proportional to the fruit porosity, the quantity of transferred solution and the osmotic pressure of the solution. The specific heat was not modified in the case of isotonic solutions whereas thermal diffusivity only increased slightly (2–4% higher). In addition, when the concentration of the impregnating solution increased and became hypertonic, the increase of conductivity and diffusivity values was less significant because the aqueous fraction tended to decrease in the product. For the highest concentrations, this even led to a reduction in thermal diffusivity and specific heat up to values below the initial ones. Finally, Martinez-Monzo *et al*. (2000) established predictive equations for the thermal parameters of infused products. The thermal parameters measured for apple were estimated with reasonable accuracy by the equations. The proposed models could be adjusted to other high moisture foods and could be used to evaluate the potential advantage to the effectiveness of heat transfer when vacuum treatments are applied prior to thermal processing.

18.3 Applications

The use of vacuum technology was proposed as a pretreatment in many processings and product applications: post-harvest storage, frozen fruits or vegetables, blanched, canned and osmo-dehydrated products, and so on. The major role of vacuum technology that has been exploited is the modification of food structure in order to improve the strength and firmness of products after a physical

treatment for preservation and/or during storage. The main applications are described hereafter depending on various usual post-treatments. To give an overview of the technology, published applications are listed in Table 18.2 including the raw material studied, the functional agent(s) used in the impregnation solution, the operating conditions of the vacuum treatment, as well as the stabilising treatment following the vacuum step and the aimed for final application.

18.4 Post-harvest storage

The dipping of whole fruits in aqueous preservative solutions, which is improved by vacuum application, has been used to prolong the post-harvest conservation of many products: apples (Scott and Wills, 1977, 1979; Lidster *et al*., 1986) lemons (Valero *et al*., 1998a, 1998b), avocados (Wills and Sirivatanapa, 1988), mangoes (Tirmazi and Wills, 1981), tomatoes (Wills and Tirmazi, 1979), strawberries (Ponappa *et al*., 1993). The compounds used in the impregnation solution are usually calcium salts (mostly calcium chloride) and many plant hormones (polyamines). Vacuum infusion seems to be used as an alternative to the pressure infiltration process (Poovaiah, 1986; Wang *et al*., 1993). The benefit of calcium application is generally related to the ability of the cation to interact with cell membranes and walls, as well as to its regulatory role at the metabolic level. According to Poovaiah (1986), the beneficial effects of calcium enrichment of whole fruit after harvest have multiple causes. First, calcium plays a special role in maintaining the cell wall structure in fruits and other storage organs by interacting with the pectic acid in the cell walls to form calcium pectate. The presence of impregnated calcium thus allows cell wall rigidity and fruit firmness during storage and/or ripening to be maintained or even increased. Secondly, calcium interacts with the cellular membrane by modifying its structure and exerts a regulating role on the permeability of this membrane and the transport of some substances involved in product ripening and senescence. Thirdly, many enzymatic reactions (e.g. polypeptide phosphorylation by protein kinase) would be calcium dependent.

By this triple action, calcium acts favourably to delay senescence and to control physiological disorders during fruit or vegetables storage. For example, vacuum infiltration of calcium applied to various apple varieties (Gravenstein, Cox's Orange Pippin) and harvests made it possible to decrease to a few per cent (less than 7%) the number of fruits affected by the undesirable 'bitter pit' phenomenon after a three week storage period at 20°C (Scott and Wills, 1977). According to the authors, bitter pit is a physiological disorder of apples, affecting predominantly the calyx end of the fruits, particularly important in certain cultivars grown for export in Australia, New Zealand and South Africa. By comparison, the rate of damaged products found in untreated apples reached 33–52% in some cases. In addition, internal breakdown, as well as 'bitter pit' disorder, observed during a 12–15 week storage period of different apple cultivars at low

Table 18.2 Main applications of vacuum infusion technology in common fruit and vegetable processing

Application	Solution composition	Vacuum treatment*	Quality improvement	References
Post-harvest storage				
Apple	$CaCl_2$ (4–10% w/v)	15–87 kPa, 1–2 min	Reduction of 'bitter pit' and internal breakdown	Scott and Wills (1977, 1979)
	$CaCl_2$ (2–4%)	n.c.	Firmness, ascorbic acid content, senescence delaying	Poovaiah (1986)
	Different flavonoid glycosides and phenolic acids	5.1 kPa, 2.5 min	Firmness	Lidster *et al.* (1986)
Avocado	$CaCl_2$ (1 M)	33–50 kPa, 5 min	Ripening delaying	Wills and Sirivatanapa (1988)
Lemon	Putrescine or $CaCl_2$ (1 mM), gibberellin (10 ppm)	27 kPa, 8 min	Firmness, colour change delaying during ripening	Valero *et al.* (1998a, 1998b)
Mango	$CaCl_2$ (4–8% w/v)	33 kPa	Ripening delaying	Tirmazi and Wills (1981)
Strawberry	$CaCl_2$ and different polyamines (1–100 mM)	17 kPa, 8 min	Firmness	Ponappa *et al.* (1993)
Tomato	$CaCl_2$ (6–20% w/v), other divalent metal ions: Mn, Co, Mg	60–86 kPa, 4.5 min	Ripening delaying	Wills and Tirmazi (1979)
Blanching/canning				
Apricot	$CaCl_2$, MgCl, KCl, potassium citrate or malate	<75 kPa, 1 h	Firmness	French *et al.* (1989)
Button mushroom	Water	0.3 kPa, 5 min	Yield	McArdle *et al.* (1974)
Button mushroom	Xanthan gum (0.5 and 1%)	75 kPa, 30 min	Yield, texture	Gormley and Walshe (1986)
Button mushroom	Egg white proteins (5% w/v)	1.3 kPa, 3 min	Yield	Demeaux *et al.* (1988)
Peach	Citrus pectinmethylesterase extract with $CaCl_2$	85 kPa, 0.5–2 h	Firmness	Javeri *et al.* (1991)
Strawberry	Ca lactate (1–2%)	17 kPa, 8 min	Yield, firmness	Main *et al.* (1986)
Turnip	Water	10 kPa, 15 min	Firmness	Moreira *et al.* (1994)
Freezing				
Apple	Pectin, alginate, gelatine	10 kPa, 0.5 min	Texture	Matringe *et al.* (1999)
Strawberry	Pectin, alginate	13 kPa, 3–5 min	Weight loss and colour during thawing	Barton (1951)
	Ca lactate (1–2%)	17 kPa, 8 min	Firmness	Main *et al.* (1986)
Osmotic dehydration of fruits	Sugars	Continuous or pulsed vacuum	Dehydration rate	(See text)
Citrus fruit peeling				
Orange and grapefruit	Pectinases and cellulases	Up to 8 kPa	Required time for easy peel removal, fruit appearance	Pretel *et al.* (1997), Rouhana and Mannheim (1994), Soffer and Mannheim (1994)
Hydration				
Dry bean	Water	17–20 kPa, 5 min	Appearance after canning	Sastry *et al.* (1985)
Ohmic heating				
Potato	NaCl (3%)	40–45 kPa, 5 min cycles	Electrical conductivity	Wang and Sastry (1993)
Fresh cut product				
Potato and apple	Na ascorbate, Ca ascorbate, $CaCl_2$	17–99 kPa, 0.5–2 min	Browning inhibition	Sapers *et al.* (1990)

*Vacuum absolute pressure values are converted in kPa unit; times correspond to the maintaining times under vacuum.

temperature (between −1°C and 5°C) were also significantly reduced by the vacuum treatment with calcium (Scott and Wills, 1979). Moreover, Poovaiah (1986) found that the firmness of Golden Delicious apples stored for 15 weeks at 0°C was improved after vacuum infusion in a 3–4% calcium chloride solution. At the same time, the ascorbic acid content was enhanced up to twofold, while carbon dioxide production and ethylene evolution appeared to be significantly reduced. The increase in the ascorbic acid content of fruit was also observed by Tirmazi and Wills (1981) on mangoes whereas their ripening was simultaneously delayed by one week at ambient temperature. The ripening of two varieties of avocado (Fuerte and Hass) was delayed from one to four days during storage at 20°C by a vacuum step application in a 4% calcium chloride solution (Wills and Sirivatanapa, 1988). The delay in ripening and senescence would thus make it possible for many products to be maintained for longer in the distribution chain even at ambient temperature, which is of particular interest for most developing countries where little or no refrigeration is used.

The vacuum infiltration of polyamines, which are positively charged molecules, would play the same role as calcium in delaying softening and senescence of plant products owing to their ability to bind the cell wall or to stabilise the membrane, and to their implication in physiological processes. The similar action of these compounds is illustrated particularly well by Ponappa *et al*. (1993) who compared the effect of impregnation with calcium and with different polyamines on the preservation of fresh strawberry slices. Among the polyamines studied in this work, spermidine and spermine had a greater effect than putrescine and appeared to be as effective as calcium in maintaining the firmness of the fruits after four and nine days' storage at 20°C and 1°C, respectively. The vacuum infiltration of putrescine (Valero *et al*., 1998a) or gibberellin (Valero *et al*., 1998b) increased the firmness of whole lemons preserved at 15°C at the same time as it delayed the colour changes in unripe-picked fruits.

Finally, the works of Lidster *et al*. (1986) displayed the potential of post-harvest vacuum infusion in solutions containing flavonoid glycosides (quercetin) and phenolic acid (chlorogenic acid) to suppress fruit softening of Spartan and Golden Delicious apples held at 20°C and 0°C. This effect was mainly explained by the inhibitory properties of these compounds on β-galactosidase.

18.5 Heat treatment: blanching and canning

Heat treatments are responsible for irreversible denaturation of cellular tissue in fruits or vegetables causing softening and juice loss. Vacuum infusion technology was consequently used before heat treatment such as blanching, pasteurising and canning with an aim of limiting thermal damages in the product. It is of particular interest to note the treatment of button mushrooms (McArdle *et al*., 1974; Gormley and Walshe, 1986; Demeaux *et al*., 1988), strawberries (Main *et al*., 1986), apricots (French *et al*., 1989) and turnips (Moreira *et al*., 1994).

McArdle *et al*. (1974) showed that vacuum impregnation of mushroom with

only water before blanching and canning improved the weight yield in the final product. The water retention resulting in this case could be also improved thanks to the preliminary infusion of a hydrocolloid like xanthan gum (Gormley and Walshe, 1986). Xanthan impregnation tended to decrease the shrinkage of mushroom during the blanching/canning cycle and thus to reduce the product weight loss. Moreover, the pretreatment with xanthan led to a more acceptable, less tough texture of canned mushrooms. Demeaux *et al*. (1988) indicated that the use of gelling agents such as egg white proteins are much more effective than xanthan gum which does not gel, in terms of weight loss reduction of canned mushroom.

The firmness of turnip dices stabilised by blanching (97°C, 3 min) and consecutive acidification (220 min dip in acetic acid solution at different constant temperatures: 20, 50, 70 and 90°C) was improved only by preliminary vacuum infusion of water which plays a protective role explained by the change in cell turgor (Moreira *et al*., 1994).

Calcium lactate infusion in fresh whole or sliced strawberries improved their texture and reduced their weight loss measured after canning (Main *et al*., 1986) owing to the presence of calcium which reinforces the cell wall structure by forming pectates (see above). This improvement in texture by calcium infusion was also observed by French *et al*. (1989) on canned apricot – Patterson cultivar fruits – even if the chelator effect of exogenic or endogenous citrate tended to limit calcium effectiveness especially on low maturity fruits because of their stronger acidity.

The vacuum infusion of exogenous pectinmethylesterase (PME) in fruit was found to be effective in increasing firmness in thermally processed foods. PME is a cell wall-bound enzyme in fruits and vegetables, which de-esterifies pectin. In post-harvest ripening of fruits, PME activity precedes depolymerisation by polygalacturonase, resulting in fruit softening. However, the PME is postulated to increase firmness of fruits and vegetables by demethylation of endogenous pectin and subsequent chelation of divalent cations by ionised carboxylic acid groups on adjacent pectic acid chains (Suutarinen *et al*., 1999). In the presence of calcium, the firming effect is proportional to the PME activity preceding the thermal treatment and can be reinforced by vacuum-assisted infusion of exogenous PME. In blanched (95°C, 30 s) or blanched–canned (104°C, 12 min) peaches, vacuum-infused citrus PME and calcium increased the firmness of these thermally processed products up to a value nearly four times that of un-infused controls (Javeri *et al*., 1991).

18.6 Freezing

Freezing/thawing cycles applied to fruits or vegetables cause substantial damage to the cellular structure, that is denaturation of the membranes and rupture of the cell walls, leading to loss of turgor and rigidity. This generally results in a strong juice exudation when defrosting the product. With the aim of limiting these

problems, Barton (1951) showed that fresh fruits mixed with sugar and gelling agents and consequently submitted to a vacuum step, give frozen/defrosted products with better organoleptic quality. In the case of strawberry slices as proposed by this author, the use of pectin and alginate before freezing made it possible to maintain the shape, weight and colour of the fruit to a greater degree than untreated fruit particularly with HM pectin. In addition, Main *et al*. (1986) showed that preliminary calcium impregnation on whole or sliced strawberries only slightly improved the fruit resistance to shear. The low effectiveness of calcium in improving firmness was explained by insufficient demethylation of the endogenous pectins in the fruit for the purpose of pectate formation. When the freezing/defrosting cycle was followed by heat treatment, the effect on texture was stronger owing to increased demethylation activated during temperature rise. Preliminary vacuum impregnation of the fruits in solutions containing gelling agents was proposed by Cierco (1994) as a new method for improvement in the quality of frozen strawberries. Using this process, the author obtained frozen/thawed strawberries that maintained the features and taste of fresh ones even after several years' storage at –20°C. More recently, Matringe *et al*. (1999) showed the possibility of introducing various gelling hydrocolloids (gelatine, pectin, alginate and starch) through the application of vacuum to fresh apple pieces before freezing. If the gelling agent uptake was sufficient, a structuring effect was observed on the defrosted product. An example of this texture modification is presented in Fig. 18.3. The 'cuttability' – defined as the force to cut a one centimetre thick apple cube measured by a texture analyser equipped with a blade – of impregnated samples with gelatine appeared to exhibit similar behaviour to a simple hydrocolloid gel. Indeed, apple dices treated with gelatine before freezing definitely showed higher gel strength (the slope of the curve is steeper). Then, the impregnated sample showed a tendency to be cut like a gel (there is a breaking point before the end of the measurement), which was completely different from the control case for which the gel strength value only corresponded to continuous crushing. Matringe *et al*. (1999) explained this phenomenon by formation of gel-filled intercellular spaces predominating over the softened structure of defrosted apple.

18.7 Osmotic dehydration and other applications

The simultaneous application of vacuum to fruits throughout the entire osmotic dehydration process, or in the first minutes of the treatment or through regular pulsed cycles, was regularly discussed by Fito's group and others. These authors dealt largely with mass transfer kinetics and rates in vacuum osmotic dehydration (Fito, 1994; Fito and Pastor, 1994; Shi and Fito, 1994; Shi *et al*., 1995; Panades-Ambrosio *et al*., 1996; Rastogi and Raghavaro, 1996; Castro *et al*., 1997; Martinez-Monzo *et al*., 1998b), with microstructural modifications (Barat *et al*., 1999, 2000), and with composition and physicochemical changes (Chafer *et al*., 2000; Moreno *et al*., 2000; Chiralt *et al*., 2001).

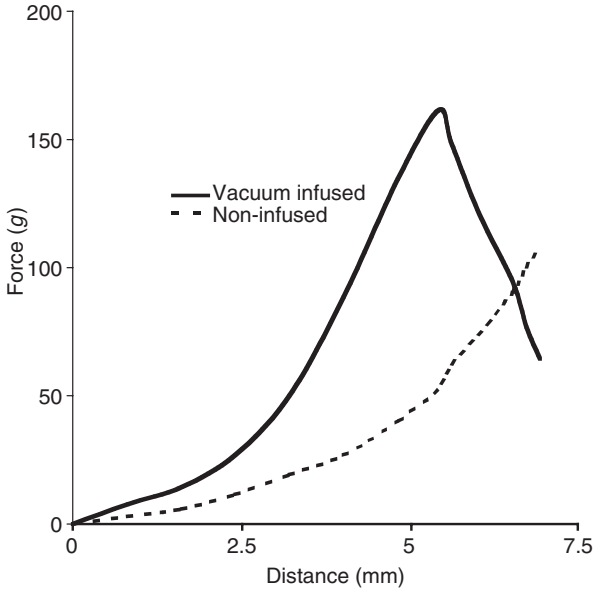

Fig. 18.3 Texture analysis profiles of frozen–defrosted 1 cm^3 apple cubes, vacuum infused with gelatine and non-infused, representing shearing force ('cuttability') versus cutting distance.

It emerges from these various studies that vacuum application during osmotic treatment has all the more effect because the product is porous. The vacuum accelerates solute exchange towards the matrix thanks to a forced and early penetration of the solution; it is above all favourable to water extraction, as water molecules can migrate more easily in the intercellular pores filled with liquid, leading to higher water loss levels. Generally, pulsed vacuum osmotic dehydration is recommended because of its economical advantages and satisfactory mass transfer improvement.

There was no significant difference in the volume change in fruits at the macroscopic level between atmospheric pressure and vacuum osmotic dehydration caused by the dehydration effect, but cell deformation and cell wall shrinkage were not as important in vacuum treatment because of the absence of gas in the food structure.

In the works listed above, the noticeable quality improvements (pH, water activity, stability, colour, texture, etc.) in fruits treated by 'vacuum osmotic dehydration' are mainly explained by the protective effect of infused solutes or by a larger overall reduction in water content in the products.

Other interesting applications offered by vacuum technology have been proposed in the literature:

- vacuum hydration of dry beans (Sastry *et al.*, 1985): vacuum hydration pretreatments greatly decreased the incidence and severity of splitting in the canned product and accelerated water uptake;

- vacuum infiltration of sodium chloride into potato pieces before ohmic heating (Wang and Sastry, 1993): this infiltration is especially effective on particles with a thickness of less than 1 cm, modifying to a significant degree the electrical conductivity of the product;
- designing a bioindicator to check the effectiveness of continuous aseptic heat treatments of particles in food liquid (Sastry *et al*., 1988): the bioindicator is made from mushrooms pieces vacuum impregnated with alginate solution and spores of *B. stearothermophilus*: the bacterial spores are immobilised by formation of the alginate gel after dipping in a calcium bath;
- vacuum application of browning inhibitors to cut apple and potato (Sapers *et al*., 1990): ascorbate- or erythorbate-based inhibitors were used to prolong colour stability or appearance of fresh cut products stored at 4°C.

18.8 Future trends

Three ideas are presented below as applications of great interest or as new research fields:

1 The vacuum infusion of solutes before or during osmotic dehydration is well studied, but no direct approach has been proposed to use vacuum technology before other drying treatments (convective-, vacuum- or freeze-drying). This could improve the quality of dried products through modifications in their chemical composition and their thermophysical properties, while modifying the drying kinetics.
2 The vacuum infusion of enzymes in the structure of fruits and vegetables has been mentioned in connection with designing enzymatically modified food (Baker and Wicker, 1996), but has not been exploited sufficiently. Enzymatic modification of the internal characteristics of intact fruit or vegetables by vacuum infusion leads to an interesting transfer/reaction process in food matrix engineering. The applications of enzyme vacuum infusion appear to be numerous, depending on the specific activity and function of the enzyme: peeling, firming or softening, generating volatile aroma from glycosidic precursors, off-flavours removal, degradation of non-digestible or toxic components, and so on. Some applications, primarily those involving structure modification, have been studied with success and/or reached commercial development. As described in section 18.5, improvement in the firmness of fruits by exogenous pectinmethylesterase was enhanced when the infusion was carried out under vacuum. A more advanced application is the use of infused pectinases and cellulases for improvement in peeling citrus fruits (Rouhana and Mannheim, 1994; Soffer and Mannheim, 1994; Pretel *et al*., 1997).
3 The possible enrichment or formulation of fruit and vegetable pieces with nutritional compounds or other solutes can be considered. As pointed out earlier, the use of vacuum technology on raw materials, was of interest in prolonging the shelf-life or appearance of raw product. With complementary

objectives, this treatment could help to develop new fresh products (fresh cut salads, ready-to-use ingredients for pastries or dishes, dietary fresh-like products, etc.) by incorporating physiologically active components, water activity or pH depressors, antimicrobials, and so on. Fito's group suggested the formulation of functional fresh fruit or vegetable pieces ('functional' here refers to a specific role in nutrition) with different calcium, zinc and iron salts which could represent a percentage of the determined recommended daily intake of these minerals for human consumption (Fito *et al.*, 2001).

Vacuum technology is a promising tool for many commercial processed fruits and vegetables. However, there is no specific regulation concerning these innovative vacuum-infused products and their regulatory status has to be clarified. The FAIR European programme, referenced in the following section 18.9, indicated that infused products could sometimes be considered as novel foods requiring a new commercial appellation. In a general way, these new products will have to undergo tests for harmlessness or stability to receive official acceptance at national or European level.

18.9 Sources of further information and advice

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