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Part I

Introduction
1

Flavour quality of fruit and vegetables: are we on the brink of major advances?

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1.1 Introduction

A consistent theme permeating current publications and discussions about food is that many fruits and vegetables do not appear to have the flavour that people remember from the past. Thus despite the attention paid by purveyors of these products to their appearance, size, shape and freedom from blemishes there is an underlying dissatisfaction with their flavour when consumed. Over the last 50 years there have been many changes in the way fruit and vegetables are grown, stored and distributed. Plant breeders have made considerable advances in producing varieties of fruits and vegetables which have characteristics that are appropriate for the grower, the distributor and the retailer (e.g. increased yields, resistance to pests and diseases, superior appearance and with enhanced keeping qualities) but because of the complexities of flavour evaluation have not always given adequate weight to the requirements of flavour quality. There has been a suggestion that an antagonism exists between attributes such as shelf life and appearance and flavour quality. For example, in Charentais melons extension of shelf life by breeding or genetic engineering has led to a ‘strong reduction in aroma volatiles’. (El-Sharkaway et al., 2005) and in Red Delicious apples aroma production is negatively correlated with the intensity of red skin colouring, a characteristic much favoured by Western American consumers (Fellman et al., 2000). In addition, many of these same agronomic, shelf life/distribution factors have seen the less robust, but often high flavour quality, varieties of many products disappear from the market. As a
result we now have the situation where the storage life of fruit and vegetables can exceed their flavour life.

The increasing urbanisation of our society means that many people never have the opportunity to taste totally fresh produce from their backyard plot or from a local market garden and hence are lacking an adequate reference to judge product quality. In a society where, increasingly, food is being seen as a source of nutraceuticals, the nutritional value of fruit and vegetables is coming under increasing scrutiny. Community concerns about the effects of poor diets on health are being reflected in campaigns to increase the consumption of fruit and vegetables and one of the key factors in achieving this is to provide produce which is of high flavour quality, is readily accepted by the consumer and promotes repeat buying. It has recently been suggested that plant volatiles, i.e. aroma provides sensory clues as to the health and nutritional status of a foodstuff (Goff and Klee, 2006).

Over recent decades our knowledge of the compounds responsible for the flavour characteristics of fruit and vegetables has increased enormously. The use of advanced chemical analytical techniques using both instrumental and human sensory detection methodology has enabled us to identify those components which appear to be the key contributors to the flavour of a particular product. Our understanding of the physiological and biochemical basis of flavour has also advanced rapidly. The factors that contribute to the particular flavour of a product are many and their interaction is highly complex and our understanding of this area is as yet at a comparatively low level. However, over the last decade the emerging tool of molecular genetics has enabled rapid strides in our knowledge of flavour and its formation in fruits and vegetables. The ability to follow the myriad of biochemical changes that occur during fruit and vegetable development from their beginnings to senescence has begun to fundamentally extend our knowledge of these areas and looks set to have profound implications for the way we can control these factors in the future (see Chapters 4, 5, 6, and 13 in this book).

These techniques have enabled rapid advances in our understanding of the role of the key compounds which either control or contribute to the physiological changes that occur during the life of fruit and vegetables. The role of, for example, plant hormones such as ethylene, jasmonic acid and salicylic acid are now being elucidated at a molecular level. The interaction of the primary and secondary metabolic systems is also now beginning to be understood (see Chapter 14) and the biosynthetic pathways for the formation of many of the compounds which confer the unique flavour characteristics of fruits and vegetables are now established. Similarly, the formation of many of the compounds formed either enzymatically or chemically during processing, including cooking, of many plant materials is now established and provides a basis for future research directions (see Chapter 11).

This increasing knowledge holds the promise that we may be able to significantly improve the quality of produce either fresh and/or processed by, for example, more focused conventional plant breeding, genetic engineering or by devising treatments which will enable their quality to be maintained during the
storage and distribution regime which seems an inevitable result of the sometimes incompatible demands of current lifestyles.

1.2 The promise of metabolic engineering

Because of the complexity of the biochemical systems found in plant materials and their interdependence, improvement of food quality by metabolic engineering via insertion of a single or small number of genes seems on the whole likely to be unsuccessful. For example, the formation of volatile aroma compounds during fruit ripening can be viewed as the ultimate biochemical step where the products are lost from the system and hence have no further role as substrates. This loss represents a metabolic endpoint which is linked directly or indirectly to many of the plant’s biochemical pathways. It seems appropriate then that this step is mediated by an enzyme or enzymes which are able to transform a range of substrates, i.e. have low selectivity. It also follows that the composition and quantity of the substrates available to these final step enzymes for transformation will depend on the nature and activity of the underlying biochemical systems which supply them. Leaving aside issues of compartmentation, it seems unlikely that the insertion of a single gene into this complex system will have significant impact on overall aroma production. However as greater knowledge of the biochemical systems in plants and their interactions is developed more effective genetic engineering approaches may become possible (see Chapters 13 and 14). The possibilities of this approach are exemplified by the work of Tikunov et al., 2005 who have applied a new approach in metabolomics using non-targeted data analysis. Their methodology, applied to 94 tomato genotypes, reveals that there are six biochemical systems supplying the majority of the 322 volatile metabolites that they identified in the various tomato genotypes. Their results support the concept of hierarchical modularity which proposes that cellular functionality is organised in a set of functional modules which are then organised into a few large modules which in turn can be grouped into even larger modules. The supply of metabolites from these functional modules to the final volatile metabolite formation process will be presumably subject to a myriad of factors both biochemical and environmental and may go some way to explaining the small but significant differences in composition and concentration of volatiles found in even closely related cultivars. There is much evidence to suggest that, during the highly active period of ripening of climacteric fruit characterized by, for example, aroma production, the supply of substrates limits the rate of volatiles production rather than the activity or selectivity of the enzymes involved in the ultimate or penultimate steps. It has also become apparent that many enzymatically mediated metabolic transformations are brought about by groups of isoenzymes, each member of which may have differing activity and substrate selectivity, and which may be expressed differentially during the development process. Since non-volatiles such as sucrose, glucose, fructose, amino acids, organic acids and a host of other compounds derived from both primary and secondary metabolism may all
play a role in flavour quality, it is apparent that the total system must be understood and taken into account when trying to implement improvements by metabolic engineering. Indeed, examples from the literature suggest that metabolic engineering for flavour quality attributes will not be straightforward and in Chapter 13 the current status of metabolic engineering and its potential has been reviewed (see also Aharoni et al., 2006).

While the potential is clear, the present laboratory work is at the level of proof of principle and has yet to be translated into commercial applications.

1.3 Postharvest treatments, storage and distribution

Despite our best efforts, in many cases, the flavour life of the product has either not been reached or has been exceeded before consumption. The resulting consumer dissatisfaction resulting in a lowering in demand for the product has both commercial and health implications.

Many postharvest procedures are designed to control the onset of ripening or delay the onset of senescence. The objectives are to maintain the product in good condition during the period between harvest and consumption whether this be days or months and also to enable the maintenance of the supply of the product in the market during periods when it is not being produced. The globalisation of the fruit and vegetable market has further increased the demands for longer shelf life of products.

However, many highly perishable fruits have a flavour life which is considerably less than the minimum time dictated by commercial needs. This problem has been overcome to some extent by adopting a regime of harvesting the product before maturity and using various treatments to delay maturing for the required time. This has proved to be successful in the case of, for example, bananas where the knowledge of the action of the plant hormone ethylene has enabled treatment with this compound to induce ripening on demand and hence provide a controlled flow of ripe bananas into the retail chain. However, consumers were able to distinguish between the flavour quality of tree and artificially ripened bananas (Scriven et al., 1989).

On the other hand, the dissatisfaction of consumers with the flavour of apples indicates that even the highly sophisticated storage regimes involving controlled atmosphere environments that are being used are not capable of maintaining the flavour life of the product even if appearance is satisfactory (see Chapters 6 and 8). Additionally it has been shown that for a number of crops, early harvest even if followed by apparently normal ripening behaviour results in deficiencies in the production of key flavour compounds (see Chapter 10).

Low temperature treatment is a staple of most postharvest treatments. However, many fruit and vegetables are susceptible to chilling injury. Inadequate control of temperature during storage and distribution and in the consumers’ environment leads to the formation of flavour defects and influences repeat buying activity. Overall there is still a need to increase the sophistication of current postharvest
treatments and to develop new ones based on our increasing knowledge of the biochemical and physiological characteristics of horticultural produce.

1.4 Plant breeding aspects

If plant breeders are to develop varieties of fruit and vegetables that meet public expectations of shelf life, appearance and flavour, and agriculturists’ expectations of robustness and viability, breeding programmes focused on using appropriate and efficient selection criteria are needed. Molecular markers can now be linked with the genetic basis of complex traits and provide a way forward for the more conventional breeding approaches to produce new products of enhanced flavour quality. Social resistance to genetically engineered foodstuffs in some countries may assist the development of marker selected plant breeding using more conventional techniques. The use of biological techniques such as Quantitative Trait Locus (QTL) (see Chapter 12) will enable a much sharper focus on the specific traits associated with fruit and vegetable flavour quality and on their interaction.

The combination of high resolution rapid throughput chemical analysis, advanced data processing and bioinformatics is known as non-targeted functional genomics (see Chapter 9). Tikunov et al. (2005)’s approach enables the identification of the major biochemical systems operating in a plant organ and will help our understanding of the complexities that will need to be dealt with as we attempt to improve the flavour quality of the fruit. The combination of molecular techniques and metabolite profiling with traditional plant breeding methods is called ‘smart breeding’ (Gur and Zamir, 2004; McCouch, 2004) and promises to deliver in the short- to medium-term improved cultivars of fruit and vegetables which will exhibit significant improvements in the flavour quality of these products.

1.5 Quality assessment

The study of the flavour quality of fruit and vegetables is a complex topic. Organoleptic quality is a very complex characteristic. On the one hand we are dealing with plants which have complex biochemical systems which continue to operate in one way or another during the period from harvest to the mouth of the consumer, a period where it may be subject to a range of environments, treatments and conditions which can have a profound influence on its eating quality. On the other hand the assessors of quality, the consumers, are themselves highly variable with regard to their response to a particular foodstuff either because of social conditioning (see Chapter 5) or because of genetic variations in their sensory systems. Consequently, the continuing development of our knowledge of the physiology of the human sensory system and the use of consumer taste panels as part of the quality assessment process remains a high research priority. However,
the development of rapid, objective and non-invasive quality assessment methods such as spectroscopic or electronic nose techniques for quality evaluation of individual pieces of produce holds the promise of enabling the establishment of quality standards based on internal qualities rather than the current predominantly visual cues (see Chapter 15).

It is arguable that harvest is the operation that most affects the quality of produce since it affects among other things eating and storage quality and ability to reach good quality after storage (see Chapter 10). While there are a range of maturity indices in use, the ability to assess the quality of individual product items either on the plant or immediately after harvest using this technology will lead to much more sophisticated orchard and handling system management practices. The use of standards for fruit and vegetable grading will become more widespread and will also be driven by the globalisation of trade in these products. This is likely to be supported by national and international regulatory regimes. The development of standards is, however, a complex issue and is likely to be a slow and contentious process. These standards will enable produce to be graded into usability classes based on objective measurements and aimed at different market segments. The segmentation of the market based on these grades and with their associated price premiums seems highly likely if the economic gain to growers and suppliers is sufficiently large to offset the costs associated with grading, separating, storing and distributing the products in this manner. The marketing of low pungency onions based on an internationally agreed standard provides an example of this approach (see Chapters 3 and 5).

1.6 The future

How then to answer the question of the title: ‘Are we on the brink of major advances in the flavour quality of fruit and vegetables?’ Flavour is emerging as one of the most important quality attributes of horticultural products which needs to be optimised in breeding, agronomic and post-harvest practices. There is little doubt that our knowledge of the factors contributing to the flavour quality of fruit and vegetables has increased rapidly over the last decade and that this increase will continue in the future. This knowledge base will give rise to programmes involving, for example, directed breeding, metabolic engineering and enhanced quality assessment, storage and distribution systems which will result in higher flavour quality produce becoming available to the consumer. However, the complex nature of flavour and the means of evaluating its quality mean that formidable challenges still need to be addressed, some of which are technical, some cultural and some commercial, before this aspiration can be met. Nevertheless, as the content of the chapters of this book demonstrate, the potential for the development of high flavour quality fruit and vegetables for the global retail market over the coming decades is high. The developing understanding of the factors that contribute to flavour quality provides a basis for shifting the focus of horticultural development from agronomic and commercial concerns to one of flavour quality. This will require extensive cooperation.
between plant and molecular scientists, plant breeders, horticulturists, chemical analysts and data processing experts but signs of this combination are already emerging (see Chapter 4 and Tikunov et al., 2005)

The emerging availability of objective rapid non-invasive quality testing techniques is the key to the use of defined standards for fruit and vegetable grading. Pressure for the introduction of grading systems will be accelerated by the increasing globalisation of trade in these products. Grading on internal quality attributes rather than on external appearance is expected to substantially change the way fresh fruit and vegetables are produced and marketed. In the future, consumers can expect to be faced with choices of different grades of produce where the grades have been determined by objective quality attributes which influence flavour. The inevitable price differential of these grades will amplify the choices. This more segmented and complex marketing approach would seem to require a continuing and comprehensive consumer education programme to overcome inherent consumer conservatism in food purchase practices. It will also require the installation of much more sophisticated handling, distribution and storage systems than are in common use today.

While flavour is recognised as an important factor in consumer acceptance and repeat purchase, it is seldom included in economic analysis of the fruit and vegetable market because of the difficulty in measuring it and matching it to consumer expectations and has largely been ignored in economic analyses of the industry. The increasing consumer demand for quality produce will result in this oversight being addressed and signs of this are already emerging. The ‘political economy’ of flavour, driven by the growing globalisation of the trade in horticultural products, will cross national, international and regional borders and seems likely to be a source of continuing debate over the coming decades (see Chapter 3 this volume).

The growing consumer awareness of the role of food intake in health and well-being is being translated into a demand for quality produce with superior sensory attributes. Our understanding of the factors influencing fruit and vegetable quality, while by no means complete, is now sufficiently advanced that we can look forward in the coming decades to advances in all aspects of fruit and vegetable production and distribution which will make high quality produce available to all consumers.

1.7 References


2

Consumer acceptance of fruit and vegetables: the role of flavour and other quality attributes

B. Brückner, Institute of Vegetable and Ornamental Crops, Germany

2.1 Introduction

Producers and consumers have clear expectations of fresh fruit and vegetables’ appearance, texture smell and taste. These attributes seem to be set by nature. Organic producers believe that fruit and vegetables are at their best when production resembles the natural cycle as closely as possible. Inherently, the quality of the production process is central to this view, and not the consumer preference for attributes in the product.

But individual liking is critical for product acceptance and hence for product quality, making flavour a critical feature. Not only flavour but also compounds such as vitamins and secondary phytochemicals are also given more consideration today, under the aspect of different optima for individual consumers.

Flavour more than any other quality criteria has a double meaning. It is represented by substance content and physical properties, but also relates to consumer perceptions, thus integrating product and user and defining the result of their interaction. In this context, flavour seems to reflect the prototype of the modern concept of quality.
2.2 Concepts of quality

The scope and definition of food quality has changed over recent decades. The original approach focused on inspection and control of the finished, ready-made product. This turned out to be complex and expensive, and attention shifted to earlier stages in the production process, aiming to identify problems earlier and possibly avoid them. During this shift of focus, the quality of the process itself increasingly became the subject of examination and optimization efforts. In the framework of a management operation, processes need to be devised in a way that improves product properties, bringing them closer to fulfilling quality targets. Concurrently, a shift in orientation from the product to the consumer took place, creating an opportunity at the level of horticultural production and post-harvest technology. Efforts could exceed maintaining quality, but also could be aimed at optimizing quality attributes for increased consumer acceptance. This was a totally new perspective, at least in horticulture, but brought with it the challenge of understanding consumer preferences.

Clearly, consumers have an instant, individual impression as to the quality of the foods they consume. Independent of intellectual or educational abilities, this process requires a minimum of attention and involvement. It leads to a spontaneous feeling of pleasure or displeasure. The consumption of food without a perception of liking or not liking is hardly conceivable. Odour or flavour (taste, odour and mouthfeel) have stronger effects on quality impressions than optical or acoustical signals, therefore it recently was suggested (Peri, 2006) that sensory analysis be denominated as the ‘science of the quality perception’. Food quality is often defined in terms of acceptance (very good, fair, bad), whereas measurement of quality uses selected, intrinsic properties, physically belonging to the product.

2.3 Internal versus external quality

Intrinsic properties are often used not only to define products, but also as a tool, a scale to assess the performance of processes, thus indicating whether the processes are able to guarantee an intended limit or exceed it. This method is used to evaluate the success of breeding a new cultivar, or improvements in cultivation technologies, plant nutrition regimes, storage conditions, and handling and processing steps. The ‘quality attribute’ provides information on the process under examination, rather than on the product. Optimization of quality is not intended, but maximizing within given limits.

From the multitude of possible quality criteria, easily measurable attributes were selected (Shewfelt, 2000). In a production environment, criteria that allow fast and inexpensive measurement are favoured (e.g. degrees Brix (°Bx)), whereas, in scientific research, the criteria chosen are often those which require the development and application of sophisticated new technologies). Degrees Brix (symbol °Bx) is a measurement of the mass ratio of dissolved dry matter to water in a liquid, which is approximately equivalent to sugar content in fruit. Usually it
Consumer acceptance of fruit and vegetables is measured with a refractometer. A 5 °Bx solution is 5% (w/w), with 5 grams of sucrose sugar per 100 grams of liquid.) This strategy aims at maximised ‘internal validity’, i.e. accurate measurement of ‘true’ values at high precision, with little variance (van Trijp and Schifferstein, 1995). Any ‘external validity’, whether the chosen criteria are relevant for retail, consumers or market success, is not considered. Because of the concentration on the product and the methods of measurement, differences in individual results, and especially differences resulting from integration of quality attributes, will not necessarily have direct, verifiable consequences for the consumer. No scale is applied to evaluate potential consequences for consumer acceptance.

2.4 Internal validity – intrinsic properties
Definitions in the classical disciplines of quality analysis are focused on internal validity. Analytical chemists determine the concentrations of individual substances, such as pigments, sugars, acids, vitamins, aroma volatiles, bioactive substances and fibres. Biochemists quantify changes in enzyme activity, gene expression, proteins and metabolites, and post-harvest physiologists look at the development of colour, texture, turgor, vitamin content and the formation of off flavours. A literature search at the Institute for Scientific Information in January 2006 on quality attributes of fruit and vegetables found 32 851 scientific articles covering the period from 1945 to 2006 (www.isiknowledge.com, 2006).

The keyword ‘quality’ in combination with individual attributes such as acid, sugar, vitamin and colour, were covered most often. More complex, outward-oriented topics like acceptability, liking, flavour and preference were less frequently subject to scientific examination. There is an interesting trend in the number of publications, when analysed on a time scale. The relation of the number of articles on a particular topic originating from the last ten years to those on the same topic from the 50 years preceding was calculated. The topics with the most increasing frequency were: liking, consumer, flavour, colour, sensory, acceptability and acceptance.

2.5 External validity
One reason for the hesitant scientific adoption of more consumer- and market-oriented topics within quality research is the high degree of complexity and interaction, making such research fall into the competencies of different scientific disciplines. Perception of the technical product specifications is indirect, via intrinsic signals or sensory attributes. In addition, extrinsic and cost cues affect the quality perception process when changing from the objective, product world to the subjective, quality judgement world of the consumer. Product quality analysis at this level may result in high internal validity. External validity is produced only when further aspects of the perception process are included. Perceived signals are
modified according to the situation. Also, earlier quality expectations can influence the process (Deliza and MacFie, 1996). Using those signals, perhaps consciously realized, a purchase decision is made. If it is positive, the food item may be modified by preparation before consumption, and further sensory experiences will contribute to overall acceptance, which again may be more or less conscious.

Besides the product itself, many circumstances influence consumer choice, which raises the question as to whether consumer acceptance is measurable at all, independently from the market situation. To emphasize the importance of external validity, many researchers have included aspects of market success in their quality definitions: ‘performance in the marketplace’ (van Trijp and Schifferstein, 1995), and even more precisely: ‘level of continued purchase or consumption by a specified population’ (Land, 1988). Despite its clear market orientation, this definition contains the essential statement that quality assessment must be performed by the individual consumer: ‘continued purchase or consumption’ can only mean that the consumer had no negative experience. Even more explicitly, the definition of Connor locates the quality decision at the consumer: ‘consumers mostly know and can say, what they like and dislike, even when they find it difficult to say why’ (Conner, 1994). But consumers are not identical, but rather individuals who can be grouped into consumer segments made meaningful according to their preferences (Moskowitz, 2002). The emphasis of a ‘specified population’ in Land’s definition makes it clear that optimization of quality will only be successful if the requirements of different target groups are considered (Greenhoff and MacFie, 1994; Pagliarini et al., 2001, Brückner and Schonhof, 2001).

2.6 Individuality in flavour quality perception

Consumer acceptance depends on the intensity of the attributes that he or she perceives (Lawless and Heymann, 1998). It is a non-linear function, rising with increasing intensity of stimulus and reaching a maximum value. This value marks the optimum of acceptability. With further increasing intensity, acceptability drops again. One specific curve is not valid for all consumers.

Usually the mean values for consumer acceptance are calculated. Often, flat acceptance curves are interpreted as representing almost constant acceptance over a range of attribute intensities. But this may only be true for the averaged results. A flat average curve may also result from one consumer’s curve rising and another consumer’s curve decreasing, depending on the position of the maximum acceptability for the respective consumer. This simplification may lead to conclusions that are justified for none of the consumers, so no general intensity for optimal acceptability can be identified.

Sometimes the differences between individual consumers regarding scale usage (using only part of the range or scoring always at the low or at the high end of the scale regardless of the perceived product differences) are taken into account by calculating a standard deviation of 1 and a mean value over all products of zero.
This standardisation solves problems associated with the technical transfer of the consumers’ flavour perception into a written document, but does not remove the necessity to take the differences in consumer (or consumer segment) preferences themselves into account.

2.7 Integration in flavour quality perception

Quality perception is not analytic (Lawless, 1999; Lawless and Heymann, 1998). Rarely is it possible to trace the complex composition of flavour notes back to their single constituents. A rather simple but important interaction occurs with bitter and sweet tastes (Walters, 1996). In experiments over three years (Schonhof et al., 2004) with different Brassica species (broccoli and cauliflower), it was shown that perceptions of sweet and bitter were the largest differences among the products, even though the sugar contents were actually the same (Brückner et al., 2005). Other investigations showed that sugar concentration alone was not responsible for the impression of sweetness, the sweet taste could only be defined in combination with acid concentration or juiciness (Brückner and Auerswald, 2000). In all these cases, a multivariate interplay was crucial for flavour perception and consumer acceptance.

2.8 The whole is more than the sum of its parts

The synthetic, holistic character of the perception of flavour quality is even more obvious in the example of tomato aroma. None of the many volatile substances identified can be associated with tomato-like impressions. Tomato notes emerge only from mixtures of 10 to 20 separate substances (Buttery et al., 1989; Krumbein and Auerswald, 1998; Mayer et al., 2003). The different volatiles possess fruity notes, like apple, pear, banana, kiwi, cucumber and lemon, or grass, potato, nuts or honey and caramel.

Research has shown that withdrawal without substitution of a single substance from a mixture of three, six or nine components do not necessarily lead to a distinguishable change (Laska and Hudson, 1992). Again, the flavour perception was not analytical, but the mixture was recognized as having its own pattern, not as a bundle of substances, hence comprising different perception when one was lacking. The investigations of Small et al. (1997) showed that olfactory and gustatory processing of single substances in a mixture took place in different regions of the brain to where the mixture of the same substances was processed. This research was made possible using Positron-Emission-Tomography, which demonstrates increased local brain activity by identifying increased blood flow. A new quality, different from the qualities of the components, emerged clearly.
2.9 Authenticity

Another example of a pattern of uniquely combined ingredients is in the notion of ‘authentic’ food products. A certain blend constitutes an original, authentic product, a modification will not. Authentic instead of accidental, the ‘genuine’ instead of the arbitrary, is an important trend in the food sector.

In the area of fruit and vegetables, there are many indigenous, regional varieties or species that are underutilized or forgotten. In some cases there is written, verbal or visual documentation, but descriptions of flavour and culinary quality are extremely scarce. Often, what constitutes the original sensory (and most often analytical) profile is not defined, nor is the difference to similar products. Therefore, an integrated, holistic flavour perception from experienced persons is necessary. Analytical, instrumental data can be used to define the authentic product, but the question of whether to assign a product as ‘original’ has to be answered earlier and by other means.

One example of such a problem arose with ‘Teltow Turnips’, a famous speciality 200 years ago. Teltow turnips were grown south of Berlin. The growing tradition was interrupted during the GDR-era. After the re-unification of Germany and because of increased interest in this regional speciality, a demand for authentic seed emerged. A sensory authenticity test had to be developed. A panel of former growers and experts compiled a catalogue of the most important and characterizing attributes (Brückner et al., 2005b). Eight attributes were selected and unknown material was tested against these attributes to separate original and other material. Only with this sensory information could instrumental analytic values (of sugars and glucosinolate profiles) be assigned to the authentic phenotype.

2.10 Conclusion

Flavour has a central role for fruit and vegetable quality. It triggers consumer acceptance. When flavour expectations are exceeded by experience, this will be communicated and repeated purchase fostered.

With better understanding of flavour metabolism, its genetic control, improved breeding methods, increased knowledge of maturity mechanisms and the effects of pre- and post-harvest horticultural systems as reported in this book, we are on the brink of major advances to improve fruit and vegetable flavour quality. Research efforts will need to be directed towards both the product and its user, the ultimate quality assessor. This requires a readiness to collaborate across disciplines, systems thinking and to integrate a high degree of complexity. But these challenges are not confined to flavour and quality research and, as in other research fields, there are many examples of such integration in this book and elsewhere in the literature.

2.11 References


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3

Economic valuation of fruit and vegetable taste: issues and challenges

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3.1 Introduction

Taste matters if it has an economic value. In case of fruits, vegetables and nuts consumer expectations determine the desired demand and guide the response of suppliers and distributors affecting accessibility of fresh produce at retail outlets. Fresh produce sells at a relatively high margin and generates substantial revenues for supermarket chains. Many production and postharvest practices aim at preventing the potential deterioration of taste, e.g., over-ripeness. A change in taste of a familiar produce item results from plant breeding and the alteration of production practices.

This chapter reviews the role of taste from the economic and marketing perspectives. In its broad interpretation, taste guides an individual’s purchase decisions limited by the available income (Fisher and Shell, 1971). Taste in its narrow interpretation is an attribute or a quality indicator. In this latter meaning, taste cannot be separated from other characteristics of the product. A purchase of a product implies a purchase of a set of product attributes, one of them being taste. On occasion, taste, the attribute, sways the tastes of consumers causing a change in the marketplace. However, the attribute taste induces a change in tastes, interpreted as preferences in economics, through interaction with other attributes, for example, nutrient content or handling properties such as convenience.

Taste is an internal attribute long recognized as essential for consumer acceptance and repeated purchase (Shewfelt, 1990), but is seldom included in economic analysis. Reasons for this encompass the difficulty to measure taste objectively in a non-destructive way and communicate this information to buyers prior to
purchase. Over time, consumers have an opportunity to substitute produce having inconsistent internal quality embedded in taste, for example in stone fruit (Byrne, 2005), with produce, which taste variability cannot be discerned and satisfies the majority of consumers.

The discussion in this chapter focuses on the role of taste as an attribute and makes a practical, but plausible, assumption that the majority of consumers of fresh produce do not distinguish between taste and flavor. Consequently, taste and flavor are used interchangeably although flavor is a relatively complex concept involving a combination of taste and aroma. Although four conventionally distinguished tastes: sweetness, bitterness, sourness and saltiness shape individuals’ taste preferences and influence food selection and purchase, fresh fruit and vegetable consumption is typically associated with sweet and bitter taste. Sour taste, also described by consumers as tartness or astringency, is relevant in fruit, vegetable and raw nut consumption. Consideration of the taste known as umami is not considered in this chapter because it is not associated with eating fresh produce or major types of nuts.

Tastes differ; differences result from taste perceptions (Drewnowski and Rock, 1995; Drewnowski et al., 2001), discernible taste inconsistency of individual fruits or vegetables resulting from the maturity stage, or postharvest handling, e.g., chilling injury. Although taste influences consumption, the consumption of fresh fruits and vegetables or nuts is strongly determined by the budget constraint faced by a household and shaped by culture (Mennella et al., 2005).

Taste expectations are directly influencing purchase of fresh produce and reflect taste perceptions. Taste perceptions are inherently subjective, creating both opportunities and barriers in fresh produce marketing. In the case of fresh fruit and vegetables, taste perceptions are complex because people eat produce at different stages of maturity, of distinct varieties, originating from different geographical areas and raised using alternative production methods (e.g., organic, IPM, conventional) under variable natural resource conditions (e.g., soil, climate).

The produce market faces major structural adjustment in the future because the fresh produce taste will be increasingly affected by genetic manipulation on one hand, and production practices which reduce or eliminate the use of synthetic chemicals on the other. Taste expectations will be subject to change and with it consumer purchase behavior. Consumers will respond by altering their purchase patterns to emerging choices and will affect competitive conditions within the produce industry, reshaping production location and distribution. In many parts of the world, changes in fresh produce consumption will accelerate, driven by health concerns and disease prevention with unclear effects of the importance of taste in food consumption (Verbke, 2006).

3.2 Taste and economics

Taste is relevant in economics if it is a predictor of human behavior. Economics modeling formulates and tests the causal relationships between taste and market
price as the outcome of interaction of supply and demand. Taste of fruits, vegetables or nuts influences the market price discovery process through buyer preferences. Preferences influence demand and by changing it have an effect on the price. However, this approach seldom explicitly accounts for taste as an attribute of a product. More likely, taste and its variations are perceived as quality changes and are measured indirectly.

3.2.1 Taste and price
Economists distinguish two types of effects of taste on price. The structural effect takes place when taste changes the demand. For example, the demand for watermelon led to breeding a seedless variety. A segment of consumers who prefer seedless watermelons seeks and buys such fruits creating a new market for watermelons with this particular attribute. The price of seedless watermelon is discovered by forces of supply and demand acting on this market. Although there is a possibility of substitution between seedless and traditional varieties, the traditional variety is not a perfect substitute and some consumers may consider buying a different fruit rather than purchase the variety with seeds. That is, the consumers show strong ‘taste’ for the seedless variety of watermelons, although the taste of the fruits of seedless and traditional varieties is largely perceived as ‘the same’. The taste in the context is a reflection of consumer preferences and associated with the satisfaction (utility) derived from the consumption of seedless watermelon.

The above example may lead to a discussion of the difference between a change in tastes (i.e., preferences) and the change in taste, where taste is interpreted as a change in quality. Taste as a change in quality is how the authors of many applied economic studies interpret the perceived or measurable changes in taste of fruits or vegetables. Griliches (1971a,b) offers an in-depth discussion of economic aspects of change in taste and change in quality.

If taste is considered an important attribute in price determination, applied economic studies use the hedonic pricing technique. This approach assumes that price is a summation of values a buyer associates with the amount of an attribute contained in a unit of a marketed product. Griliches (1971a) proposed hedonic price indexes to account for an incremental change in quality and its effect on price, while Rosen (1974) showed a way to apply hedonic pricing to the valuation of quality attributes to manufactured goods. In the case of fresh produce, Waugh (1928) studied vegetable prices at a Boston wholesale market with respect to quality preceding later works by Griliches and Rosen. The hedonic pricing technique found numerous applications in food price studies, including the seminal work by Ladd (Ladd and Martin, 1976; Ladd and Suvannunt, 1976). However, taste was used only occasionally and, most often, in wine pricing studies.

The purchase of a bundle of attributes implies a purchase of some amount of taste in each unit of a product. It is assumed in the hedonic pricing equation that the included attributes determine the price. Once the equation is estimated, a marginal (i.e., for each added unit of taste) monetary value associated with taste attribute can
be calculated. The calculated amount is the implied price paid for taste by buyers and has practical implications. It signals to suppliers the magnitude of benefits they can expect from delivering an additional unit of taste embedded in the food item. In empirical studies of food products, the hedonic pricing technique performed well for high price items such as wine for which taste scoring systems are available and where otherwise taste is often described in a complex and refined way. However, fresh fruits and vegetables are perceived as low involvement items whose relative value is low and whose taste is seldom associated with sophistication. But the primary reason for the exclusion of taste from applied economic studies is the challenge posed by measuring taste.

An interesting hedonic pricing study by Jordan et al. (1985) estimated the value of selected attributes of fresh tomatoes. The authors collected a large data sample including the measurements of factors directly responsible for taste such as soluble solids, acidity and pH. However, in their wholesale price equation, they excluded these measurements because the measurements could not be judged by wholesalers or retailers prior to sale (p. 142). Although this might have been the case in the past, progress in attribute measuring techniques and cultural practices allows the supply of fresh produce with a guaranteed level of degrees Brix (°Bx). Suppliers of some fruit were forced by the intense competition to include taste measures in their own standards for grades. The progress in non-destructive testing will allow for an accurate measurement of taste indicators using, for example, a laser beam to read the sugar levels in fruit (Anonymous, 2005).

Another approach applicable in studying the economic importance of taste is the contingent valuation (CV) technique which solicits consumer opinions about their willingness to pay for specific attributes of an existing, modified or novel product. The proliferation of willingness-to-pay (WTP) studies, however, largely ignored fresh produce and the influence of taste. Although taste is difficult to measure, willingness-to-pay studies often addressed payment for attributes equally if not more difficult to quantify, for example, landscape features, food safety or environmental characteristics. The WTP studies led to the investigations of willingness-to-accept (WTA) studies. Few studies focused on food and none accounted for taste. However, the WTA studies may be applicable to examination of taste as a factor in fresh fruit and vegetable demand because for some consumers health concerns override taste preference. These consumers eat produce because of the perceived health benefits, although produce taste alone would not encourage consumption. Because the prevention of diseases and health maintenance are frequently mentioned factors motivating consumers to consider eating fresh produce, yet some are unwilling to compromise on taste (see Verbke, 1996, for the review of relevant studies); WTA studies can provide insights about the produce purchase behavior of some population segments. Sensory acceptability, including taste, drives food acceptance according to existing studies (for example, Sosa and Hough, 2006).

Experimental auctions have been applied to price food attributes and the WTP. These studies are costly and, therefore, often involve a very limited number of participants. However, the experiment offers to economists a chance to exercise
more control over the environment in which a purchase decision is made than observations from a retail outlet. By controlling the stimuli, researchers are able to elicit responses closely resembling the actual behavior in real world setting. A study by Melton et al. (1996) applied experimental auction methods to examine a fresh food item with multiple quality attributes including taste. Predictably, it was not fruit or vegetable, but meat (pork chop). However, their results rejected the equality between appearance and taste as a source of information for consumers to make the choice. Although experimental auction results are difficult to generalize, this particular study suggests the need for studying taste as an independent attribute relevant to the food choice and the WTP.

3.2.2 Taste and sales
Consumers seem to infer the quality of a food product (Grunert, 2005), including taste from quality cues, and their purchase and consumption decision are not risk-free. Taste measurement is difficult because consumers have difficulty expressing their taste perceptions in a way that can be measured in a way similar to many chemical or physical food properties. Therefore, taste is measured indirectly using ordered descriptors or scales. Scales originated in psychological studies to measure emotions. Among commonly used scales in consumer studies is the hedonic scale consisting of an odd number of options, where the middle category is interpreted as neutral, i.e., an individual choosing the category neither likes nor dislikes the taste of food (Resurreccion, 1998). The shortcoming of a scale is that, although it allows a consumer to identify the placement of preferred taste within the scale, it limits the accuracy by forcing the selection of categories separated by the same distance along the scale. The accuracy increases if the presented scale offers more options, but too many options present a difficult choice for the consumer because multiple comparisons across the presented options require advanced cognitive skills.

To account for the connection between economics and the obvious yet difficult to measure preferences, Fishbein (1963) and Fishbein and Ajzen (1975) proposed the theory of reasoned action. The theory establishes a causal relationship between attitude and observed behavior, for example between a measure of attitude toward a vegetable and the purchase decision. The purpose of measuring attitude is to examine the strength of the relationship between attitude and behavior. According to this approach, a fruit or vegetable possesses many attributes, presumably, taste is one of them that influence the attitude toward it. The consumer believes and evaluates attributes contained in the product. Once the data are available and a model specified, the effect of each attribute on the overall attitude towards a particular fruit or vegetable can be measured by the magnitude and sign of the estimated coefficient (Steenkamp, 1997).

An example of the empirical application of the theory of reasoned action is the study of attitudes toward peanuts and their consumption (Moon et al., 1999). After establishing that Bulgarian consumers liked the taste of roasted peanuts and many admitting that they would like to eat more of them, the consumption equation included the taste as an explanatory variable. Such studies typically are conducted
using survey data because time series data that include a measure of taste preference are not available, if at all collected, on a consistent basis. Data limitations regarding the influence of taste on consumption frequency, price and quantity consumed prevent any extensive and rigorous analysis. Consequently, the examination of taste influence on fresh produce consumption is reduced to case studies and constrained to broad generalizations other than confirming the relevance of taste to purchase and consumption.

3.2.3 Standards for grades

Standards for grades have been developed to facilitate trade by eliminating the need for visual inspection. For commodities where intrinsic attributes are important, they were augmented by tests verifying the content of a specific ingredient, for example the presence of bitter almonds in the lot of in-shell almonds delivered to a shelling plant. The key requirement of the test on internal attributes is its accuracy and speed because the decision to accept or reject a shipment has immediate economic consequences. A disputed test result may lead to additional measurements, for example, in the case of bitter almonds, but if reconfirmed the shipment cannot be sold for human consumption. Taste testing of other temperate zone nuts is unusual at the grower–sheller level because the only problem could be the presence of rancid nuts, which is uncommon if nuts are sold during or shortly after nut harvest. Rancidity in nuts occurs over time, especially if temperature during storage is relatively high, but large volumes of nuts are seldom stored by a grower and, therefore, the incidence of rancidity is an issue at wholesale or retail trade.

The use of standards for fresh fruit and vegetable grades, whether developed by a government agency, the industry or even an individual company, is voluntary in the United States. In the case of fresh fruits and vegetables, taste is commonly disregarded because as an internal attribute the available inspection techniques do not allow for quick and non-destructive taste measurement of every fruit or vegetable. However, in recent years, taste has been recognized as the important attribute in the strategy to differentiate one variety from competing varieties of the same or similar product. To strengthen its competitive edge, growers and marketers apply the Brix test to account for the soluble solids content which primarily measures the content of sugars in a product. Alternative measures of taste are also applied. The Brix measure is little understood by consumers and is applied at the wholesale trade. It is supposed to account for sweetness and allows such claims at the retail level because buyers understand ‘sweetness’ and sweetness is often a preferred attribute.

Brix measurement is used by apple growers to improve the success of apple marketing. Because the sales of ‘Red Delicious’ apples have been declining over time as other varieties have been introduced on the market (Carew et al., 2006), growers and traders introduced a guarantee of Brix content. By assuring wholesale buyers or supermarket chains of the minimum Brix content, the sellers differentiate their apples in expectations of improved sales or higher prices than without any assurances. The emphasis placed on Brix readings is only in part justified.
Ultimately, the perception of taste is associated with the ratio of acid to sugars. Moreover, some consumers have shown preference for tartness rather than sweetness, for example, apple consumers in Great Britain, or more bitterness than sweetness, for example the consumers of grapefruit in France versus Germany. Processed fruit marketers often guarantee the specific Brix level. For example, tart cherry juice concentrate manufacturers guarantee 68 Brix readings in their product (www.traversebayfarms.com, 2007). However, in the case of fruit juices Brix level can be manipulated by blending unless regulation disallows such practice.

Another example is the guaranteed sweetness of Vidalia onions. Vidalia is a registered trademark of several sweet onion varieties grown in the designated geographical area within the state of Georgia in the United States. Although the trade mark protection was the initial way of differentiating the onion from other onion varieties, over time as more sweet onion varieties have become available, some growers choose to distinguish their Vidalia onions from those grown by farmers in the region. Whereas the sweetness of Vidalia onions was to differentiate the product from the conventional, pungent onion varieties, the stricter definition of sweetness some growers apply is primarily a merchandising technique. The certification of sweetness must be based on well-defined sampling procedures or it will not be meaningful. The discussion among Vidalia growers continues about the procedures, the measures of sweetness, and the cost of the additional sampling and testing if the industry attempts to separate itself from other domestic and imported sweet onions (Cable, 2007a; 2007b). The sweet taste results from the combination of production practices and variety selection. In the case of the Vidalia onion, definition of what represents a true Vidalia onion was declared by the law (Official Code of Georgia, 1987).

Texas sweet onions, which appear on the market earlier in the season than Vidalia sweet onions, have been already tested for their taste for several years. The unofficial industry standard defines sweetness through pyruvic acid content (Lee, 2007). A level of the acid below 5.0 classifies Texas onions as sweet. Opinions about the importance of the certification vary and some traders feel it is crucial, while some retailers view it as less paramount (Bareuther, 2007). Interestingly, the discussion about the measures of sweetness in onions largely ignores consumers.

Conventional standards for grades omit measures of taste. Many in the fresh produce business are aware of it, but the system seems to function without measuring taste. Only occasionally taste becomes a focus of a marketing and promotion. The Golden Sweet Pineapple variety that was introduced on the American market in the mid-1990s is credited with the doubling of fresh per capita consumption of pineapples between 1995 and 2005 (USDA, 2003). The variety, developed by a major fruit trading company, is patented, was heavily promoted once introduced, and benefited from the innovative merchandising including fresh cut preparation.

Domestic standards for grades developed by the USDA are applicable to fruits and vegetables on the voluntary basis. Because the standards are used voluntarily, there exists a great variation in the amount of a specific fruit or vegetable that is inspected for quality (Nichols, 1996). A number of the standards for grades covers
individual fruits and vegetables, but none explicitly accounts for taste. Attributes that indirectly influence taste, however, are included; for example, maturity, color, condition, foreign matter, disease or pest damage. Taste varies across produce subject to grading, but information about the attribute relevant to its gradation and the measurement of taste are not readily available or accurate. Consequently, taste itself does not meet the three requirements necessary for an attribute to serve as a grade standard (The Ohio State University, 1991). Only the development of non-destructive, inexpensive and fast testing methods will create conditions for taste to be included in standards for grades and could revolutionize the grading system.

3.2.4 Taste and international trade
Taste becomes an attribute of global relevance. With the observed trade liberalization and expectations of further liberalization in trade of agricultural commodities and food products, taste or its indirect measures, become subject to international negotiations. In particular, the content of soluble solids as reflected in Brix readings is proposed for inclusion in the international standards for individual fruits. For example, New Zealand proposed threshold Brix readings for the international trade in kiwi (United Nations Economic Commission for Europe, 2003). New Zealand, which has been very innovative in its horticultural product development, postharvest and trade, emphasizes the measurement of an attribute that seems to reflect consumer preferences.

Brix level guarantees are already applied in international trade of processed fruit products. For example, Indian mango pulp exporters offer shipments of pulp with a specific Brix level. Needless to say, it is rather more possible to assure buyers of the Brix level in fruit pulp than in a shipment of fresh fruit because the pulp manufacturing process allows mixing of batches to achieve a pre-specified level of soluble solids. In fresh fruit shipments such guarantees are potentially less accurate because they are based on destructive testing of a sample of fruits. However, many fruit growers, e.g., Washington apple growers in the United States, are undertaking this effort in order to reach the high priced fruit market in Japan and other countries of South-East Asia and the Middle East.

The European Union is developing standards which may include Brix readings. Moreover, these standards will recognize variety differences of specific fruits. Growers can influence the soluble solids content, which are primarily sugars in fruit, by cultural practices controlling the fruit size. Thinning apples or peaches, for example, will affect the accumulation of sugars, and both manual and chemical thinning methods can be applied. Manual thinning assures great control but is also costly. The relationship between size and taste applies to vegetables as well. For example, the pungency of onions varies with size. For sweet onion growers, who want to adopt standards guaranteeing sweetness, the link to size poses a challenge (Cable, 2007b) because the current grades emphasize size (e.g., medium, jumbo), while the future grades would add the measure of sweetness (i.e., the absence of pungency).

The importance of taste as related to maturity has been subject to the discussion
on the development of international agricultural quality standards (UNECE, 2003). The discussion involves the maturity requirements for apples, kiwi, peaches, nectarines and table grapes, among others. International standards are of interest to several international organizations including FAO, OECD and WHO, but discussions have revealed the need for determining methods for fruit maturity and sampling. Moreover, deliberations have recognized the difference in taste preference between ready-to-eat, sweet and soft fruit and firmer, less mature fruit. The various taste preferences make the development of a single standard difficult and, consequently, create an opportunity to segment the market. In fact, if the standards are too rigid, they may prevent some marketing opportunities from being realized. For example, British consumers showed preference for apples that taste tart, while, traditionally, American consumers like apples for fresh consumption to be sweet. Similarly, French and German consumers were found to differ in their preferences in the taste of grapefruit with the French preferring bitter tasting grapefruit when the German buyers expected their grapefruits to be relatively sweet. It is likely that if international standards are developed they will refer to specific varieties such as in the case of table grapes (UNECE, 2003). Grape consumers indicated taste as the most important attribute (The Packer, 2004).

Moreover, growing conditions may affect the content of sugars and acids (sugar/acid ratio), influencing the taste of the same variety grown in different countries or regions. In UNECE recommendations for apples, the requirements regarding the content of soluble solids essential in determining taste examine the issue by variety. The conclusions point to Good Agricultural Practices (GAP) as a necessary and sufficient tool of assuring the minimum sugar content and influencing the taste (UNECE Recommendations for Apples, 2004). It seems that currently a number of these issues have been specific to a contract and the risk of failed sales at retail is either taken by one party or shared between contracting parties. Although international standards which include taste measures aim at facilitating trade, private negotiations between buyers and sellers offer flexibility. For example, given the growing conditions, the accumulation of soluble solids (mostly sugars in fruit) fluctuates from season to season and may be reflected in perceptible taste differences. Under the highly regulated industry, trade may be impaired and consumers denied an option to eat a specific kind of produce or a particular variety. In addition, the timing of production affects the taste of some fresh produce resulting in the same variety tasting differently depending on the season (Schreiner et al., 2002). If taste is indeed a key factor influencing the purchase and will be regulated, planting of, for example, radish varieties may have to be regulated as well.

3.2.5 The political economy of taste
The inclusion of measures of taste in standards for grades becomes an issue of international negotiations. The sweetness of grapes or apples, the bitterness of some vegetables or pungency of radishes and onions may have to be measured and compared with the allowable (yet to be determined) limits. Taste becomes a
tool that growers impose on trade in order to increase their negotiating power and increase sales revenues. By introducing a taste measure, growers differentiate the product and may attempt to segment the market. The homogeneous market of, for example, onions or potatoes, breaks down into several markets differentiated by taste measures. Marketers apply marketing and merchandising techniques to build the perception of taste variation justifying price premiums. Market efficiency may be lost and transaction costs may increase, yet consumers may accept higher prices for differentiated products resulting from the enforcement of taste-based standards. Because sharing taste perception is impossible (de Gariner, 1997), the inherent subjectivity of taste perception creates conditions exploiting heterogeneity of consumer preferences. In an economic sense, there appears to be a welfare loss and the transfer of gains away from consumers to suppliers and additional costs to the society as a whole. On the other hand, Grunert (2005) noted that heterogeneous markets due to quality difference create market opportunities for risk-takers targeting specific segments on the global market. Governments may reduce the risk of trading in fresh produce by sharing the cost of research into economically relevant taste studies. However, the modification of standards for grades to account for taste measures, if they are intended for the mandatory use, will induce new monitoring and enforcement costs, which will persist.

If the transfer of gains away from consumers to growers resulting from marketing taste-differentiated produce does not occur, the government may have to subsidize the growers using tax revenues. A less expensive mechanism may be to allow market segmentation, product heterogeneity and price structure tied to fresh produce taste rather than some form of subsidies to production. If the domestic market is too small to generate a sufficient sales volume, governments may encourage exports or, in the case of foreign competition, design schemes restricting the entry of imported fresh produce on the domestic market using taste measures as an argument. Because produce consumption is increasingly important for healthy living and produce contains functional ingredients, which link to taste has not been fully researched, taste and its role in trade may become increasingly important.

3.3 Taste, genetic predisposition and economics

Although unripe fruit is being blamed for sluggish sales or decline in consumption, the issue has yet to be thoroughly examined. If indeed there is an economic gain in supplying ripe fruit or matured vegetables, the gain must be sufficiently large to offset costs associated with likely losses due to shipment of produce that is more susceptible to damage than produce in the early stages of maturity. Without adequate economic gain, managers of individual supply chain links lack economic incentives to change the nature of their operation. The absence of a clear market signal rewarding suppliers of more rather than less mature fresh produce may have its roots in the genetic predisposition to taste among consumers.
3.3.1 Genetic predisposition
Numerous studies have demonstrated that the perception of taste varies due to genetic predisposition. The studies focused primarily on the ability to perceive bitterness and examined taste blindness to bitter compounds (Tepper, 1998). The taste blindness varies around the world with the population of western Africa showing more sensitivity than, for example, the population of India. Heritability was found to influence the preference for one-third of foods (Krondl et al., 1983) including grapefruit juice and green beans. It has been established that the preference for sweetness among children has its genetic basis (Mennella et al., 2005) and is associated with sensitivity to bitter taste. The sensitivity interferes with the consumption of selected vegetables and presents a potential nutritional challenge. It also offers ideas for effective marketing and merchandising of produce to households with children. Freshness or organic origin of produce is unlikely to be a sufficient argument despite the strong preference of consumers for these two attributes.

In the era of trade liberalization, differences in taste preferences are quite relevant to growers and marketers. The ability to taste bitter substances is associated with a specific gene, which appears to be distributed with similar frequency across Africa, Asia and Europe (Wooding et al., 2004), whereas the detection of bitterness in fresh produce varies.

Recently, scientists established a genetic link to the sensitivity to sour taste. The study compared sour tasting sensitivity between twins using citric acid as the source of the sour taste (Wise et al., 2007). This finding and the earlier established connection between genes and perception of other tastes creates an opportunity for marketing fruits and vegetables. The anecdotal observations, for example, indicated that there is a group of consumers who showed preference for fresh peaches in the early stage of maturity when the soluble solids content is relatively low and the taste rather than sweet could be defined as ‘tart’ or ‘sour’. The preference for sour taste seems to justify the marketing of some fruit before it develops a fully matured flavor. However, if the standards for grades used in commerce establish a minimum Brix reading as the mandatory attribute of fresh produce shipment, chances of the exploitation of the apparent market niche will be reduced or eliminated.

3.3.2 Demographics
Demographic characteristics are often used to profile consumer segments in economic studies. These characteristics are fairly stable and, therefore, the developed profile remains relevant for a period of time. The stability offers a chance of applying the profile for actual marketing purposes by the fresh produce suppliers. Among demographic household characteristics important for studying the effect of taste on purchase and consumption are gender, age and the presence of children in the household.

Taste perception varies between genders. Women tend to be more discernible tasters than men. They are also more often responsible for food selection, purchase
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and preparation, making them key decision-makers in terms of what and how much fresh or processed produce is purchased, how it is prepared, and when and in what form it is served. Many female produce buyers will make purchasing decisions that reduce the risk of buying items they and their household members dislike or experience unpleasant taste.

Taste perception declines with age. The importance of age is justified by the steady increase in the elderly population (Laureati et al., 2006). Because older buyers tend also to have more disposable income in the United States, they more readily accept the relatively higher price for produce and due to health considerations, older consumers are eating more fresh produce than many other population groups. ‘Vine-ripe’ tomatoes or ‘tree-ripe’ fruits that are priced higher than conventional produce target older, wealthier consumers. This potential market segmentation according to taste perception offers a shift in the marketing focus away from the income constraint and toward taste. The amount of purchased and consumed produce may not increase, but the price paid can increase as long as the taste meets the buyers’ preferences. For example, older consumers prefer higher sweetness intensity than younger consumers (De Jong et al., 1996) suggesting that produce that can be labeled as ‘sweet’ may attract repeated purchase. In addition, educating consumers about produce preparation by adding flavor can compensate for loss of taste perception (Schiffman and Warwick, 1993) and encourage produce consumption. Retail outlets targeting the elderly may organize sample tasting and preparation shows at times when the targeted buyer segment is more likely to shop.

Taste perception also develops as children grow. The preference for sweetness is common among children, who show sensitivity to bitter taste. Avoidance of bitter tasting produce, especially Cruciferae vegetables may lead to insufficient supply of certain nutrients and shape taste preferences that are not optimal from the standpoint of health diet. Some children show a declining sensitivity to bitter taste as they grow, but some never lose this genetic predisposition because among adults are also those with heightened sensitivity to bitterness as proved in testing mothers and children (Menella et al., 2005). From an economic and marketing standpoint the presence of children with specific taste sensitivities offers opportunities to target sales. Currently, it is accomplished by simply offering a selection of various fresh produce without specific efforts to attract buyer attention to taste.

Efforts to teach children the benefits of eating fruits and vegetables may have increased recently, but the role of taste in these programs is not clear. For example, serving a spinach dip means covering the taste of spinach by adding an ingredient containing lots of fat, damaging the overall dietary effect. The ultimate effect of many of these programs is, hopefully, a combined effect of in-school and outside the school efforts to shape long-term eating habits including teaching about various tastes. Familiarity with taste may influence produce selection and purchase later in life. Until now the socialization of children, i.e., the process of acquiring skills, knowledge and attitudes needed to function like consumers, has included food choices but little in terms of fresh produce. Isler et al. (1987) reported that 55% of requests by children 3–11 years old referred to food and only 3% of all
requests were about fruits and vegetables. In contrast, 24% of requests were about dessert foods, 17% about candy and 7% about breakfast cereals, suggesting that sweetness mattered to children, but was not associated with fruits or vegetables.

### 3.4 Innovation and taste

Taste preferences are shaped over time and are slow to change. In the United States, three fruits, i.e., bananas, apples and oranges, accounted for 50% of total daily fruit servings in 2000. The preference for a fruit that is ‘convenient’ to eat molds taste expectations. The lack of familiarity with taste is a barrier that has its economic consequences. Any new fruit introduced into the market place requires substantial up-front investment in the information campaign educating consumers about the availability and uses of new fruit. Consumers are, generally, unwilling to try ‘exotic’ fruits and have to be encouraged for an extended period of time and through a combination of efforts including informational materials, in-store sampling, promotional pricing, etc. The reaction of consumers toward the introduction of an unfamiliar fruit complicates the economic analysis because economists assume that consumers are able to compare the satisfaction (utility) derived from the consumption of novel fruit to the satisfaction level obtained from choices available prior to the appearance of a new item. This impractical assumption, however, does not prevent the analysis, which is prevented primarily by data which limit observations to past consumptive behavior.

The consumption of several fruits increased markedly in recent years, but the three dominant fruits remain the same, i.e., bananas, apples and oranges (various kinds), although their consumption remains fairly stable. Efforts are being made to increase their consumption by addressing the taste. For example, the examination of sugars-to-acid ratio in oranges may lead to new industry standards different from the current 8-to-1 ratio because consumers do not like the taste (Jorgensen, 2007). The consumption of fruit that increased in popularity in the past decade or so, includes table grapes, melons, strawberries, blueberries and other berries. All of these fruits have been well known to consumers, but eaten in smaller quantities as a fresh fruit in the past. Grapes and melons do not store well over a longer period of time and their increased consumption is a result of increased imports from South and Central America. Accessibility and competitive pricing were important, but the consistent taste, especially of berries, was the factor that encouraged a sustained consumption increase. Taste improvement resulted from the use of new varieties developed in breeding programs.

Relatively novel fruits, especially tropical fruits, must overcome consumer resistance. In 2002, 80% of consumers did not taste mangoes, but 70% did by 2007. Exporters stopped shipping unripe fruit because it contributed to unpleasant consumer experience and now ship ‘retail-ready fruit’ (Ohlemeyer, 2007b). However, some mango varieties taste tart even when ripe. Recently, consumers contacted retail outlets about the unexpected taste suspecting chemical contami-
nation (Ohlemeier, 2007a). The reason was a wide range of mango varieties and tastes and inadequate consumer education.

Fundamental research into flavor perception may lead to genetic developments in produce breeding. For example, recent studies linked the aroma to the possibility of enhancing or reducing flavor perception (Petit et al., 2007). Although commenting on the findings, Daniells (2007) stated that taste is a key driver in global food industry; what is its taste and what is its economic value are debatable. Another example provides an illustration how an innovation may lower the economic value. Thailand exports $90 million worth of durian and a new variety has been developed, which is free of its intense smell (Halliday, 2007). However, durian consumers maintain that the smellier the durian the better its taste and, if the new variety will boost its market potential remains to be seen. Consumer acceptance, essential for purchase, may increase among consumers not familiar with durian once the smell, offensive to some, is gone.

Innovation in taste also involves the return to the so-called ‘heritage’ varieties. In the United States this trend is most noticeable in the return of old tomato varieties. These varieties tend to have superior flavor resulting from taste and aroma than the newer varieties, which were bred to lengthen shelf life. To extend shelf life, the selection reduced the content of substances responsible for the aroma needed to fully enjoy the flavor. An example of the re-evaluation of old varieties in terms of their taste perception is a study of Italian apple cultivars in order to determine the sweetness and, possibly, expand production of old varieties with taste preferred by consumers (Bignami et al., 2003).

### 3.4.1 Organic production techniques

Organic food sales have been rapidly growing in a number of countries across the world (Bourn and Prescott, 2002). Fresh organic fruit and vegetable production and sales are also increasing. Although the primary motive for organic produce purchase is the preference for food that was raised without the application of synthetic chemicals, taste of organic produce also motivates buyers.

The superior taste of organically produced fruits, vegetables or nuts has not been consistently established. Boum and Prescott (2002) reported difficulty to confirm that organic products tasted better than conventional products. Weibel et al. (1999) found no differences in sweetness or tartness of McIntosh and Cortland apple varieties. Both varieties are becoming less important for the apple market and may not necessarily be best suited for organic production; for example, McIntosh is susceptible to scab, which affects the appearance, but also could be detrimental to taste perception.

Tomatoes have long been perceived as lacking taste. Vogtmann et al. (1993) reported that two tomato varieties were evaluated higher for taste than the same variety producing fruits under conventional practices. A trained panel comparing organic and conventional tomatoes did not find differences in their sweetness, acidity or bitterness (Johansson et al., 1999). The same study tested taste of carrots grown using organic and conventional methods in two subsequent seasons. The
A recent poll conducted by the Soil Association among consumers in the United Kingdom suggests that taste is almost as important as the avoidance of pesticides named by 95% of 813 respondents (Food Navigator.com, 2005). In particular, organic fruits and vegetables tasted better than non-organic according to 72% of respondents. Another survey, commissioned by a government agency, surveying United Kingdom consumers reported that taste was among the top three motivating forces responsible for consumer purchase of organic foods (Food Navigator.com, 2005).

From a marketing standpoint, the price premium received by suppliers of fresh organic produce cannot be linked to the consistent superior taste, but this may not matter. Consumers paying the premium are motivated by other factors than taste, and, possibly, some rationalize the purchase by naming the taste. From a marketing standpoint, it matters that a segment of buyers is willing to purchase organic produce because of perceived superior taste at a premium price. The superior taste perception may result from the reduced exposure of produce to environmental conditions (e.g., loss of moisture) resulting from the short transportation route from a nearby field to an outlet.

3.4.2 Postharvest handling and processing

Climacteric fruits are very sensitive to the exposure to temperature and gases after harvest, which can alter the taste. Mealiness as a result of chilling injury in peaches and nectarines has been researched and the new postharvest handling guidelines are often variety specific. Tropical fruit sensitivity to relatively low, but above freezing, temperatures is obeyed in the supply chain, but not universally applied by consumers in the home setting. Lack of familiarity with proper handling methods can prevent tropical fruits from wider acceptance and deny sales. New models of home refrigerators enhance the control consumers have over the relative humidity and temperature in fresh produce storing compartments, but it is doubtful that these improvements induce segmentation of consumers according to the fresh produce taste preferences.

Novel processing techniques enhance taste. Steam blanching of vegetables prior to freezing was reported to improve their taste (Anonymous, 2004). Because the new method lowers the cost of processing, it may gradually replace procedures based on currently available equipment. However, unless consumers are informed about the taste enhancing feature of the new technique, the benefits of the method will be limited to its cost-saving nature.

Efforts to add mouthfeel and new texture, which influence taste perception yielded a product named ‘taste strips’ (Food Manufacture, 2005). The product is a continuation of vegetable flavored foams dispensed from a spray can. The novel products include snacks with the taste of tomato, spinach or carrot based on gelatin and collagen. Taste strips look like noodle strips but are made of jellied vegetable purée. These products emphasize convenience, yet this attribute is tied to the specific taste, mostly vegetables, and intended to use as a garnish on dishes.

Fresh cut processing offers opportunity for increased sales of fruits and
vegetables, but the driving attribute of sales is convenience. Taste of fresh cut vegetables differs little from a salad prepared from individually bought ingredients. Fresh cut fruits, however, appeal not only on convenience, but also color and taste. Fresh cut tropical fruit includes mangoes, papaya and pineapple, which, once the varieties are known, taste sweet. Sweetness is the taste consumers expect when eating these fruits. However, the budget constraint may prevent many from purchasing fresh cut fruit, but such fruit has been included in restaurant menus, where meeting taste expectations is essential.

3.5 Conclusions

Sensory factors determine food choice and intake (Laureati et al., 2006). Food choice and intake have economic implications at the household level and at the sectoral level. Choices and the amount consumed generate supply response, resource allocation in production, stimulate innovation in postharvest handling, guide breeding efforts, and influence profits of growers, distributors and retailers. Taste perception is part of a complex process involving aroma, texture, olfaction and mouth feel, among others, where the timing of each sensation is relevant. The interaction of sensory modalities is non-linear in nature (Bult et al., 2007) complicating the measurement accuracy. Trained tasting panels identify the tastes nuances such as intensity of sweetness or the bitter aftertaste, which may provide guidance in the preparation of a marketing campaign involving promotion and consumer education. Stressing taste to appeal to consumers commonly involves ‘sweetness’ in the case of fruits and absence of pungency or undesired harshness in the case of vegetables. Consequently, taste panel results provide potential product descriptors and are an indication of future product acceptance, but are not an accurate purchase predictor.

In economic analysis the data volume and quality, i.e., are two areas that a researcher wants to control. Data quality in examining taste effect on price, sales volume, or consumer purchase refers to how the taste perception is measured. Minimizing a bias resulting from taste measuring is essential for the accuracy of model estimation, which is intended for making predictions. Economists cannot control the environment in which the purchase decision is made and have a difficulty separating effects of product attributes, environmental features or consumer characteristics. Moreover, there is a lack of rigorous studies linking the taste perception during eating and the subsequent purchase decision. It has been established, however, that, in general, consumers tend to remember bad experiences for a longer period of time than the positive experience. It is, therefore, likely to link the absence of purchase with taste experience, but such a study requires a large data set because the lack of purchase results from various causes, only one of which is the past experience of taste. Large data sets may be expensive to collect, but necessary to accurately quantify the value of taste. Jordan et al. (1985), working as the interdisciplinary team, collected information about price, soluble solids and acidity of tomatoes, showing that taste relevant measures could be
included in the hedonic pricing model though their model specification omitted such variables. But, from a practical standpoint, the relevance and, therefore, economic importance of flavor components suggests the need to gather information about the relative content of sugars and acid. Progress in non-destructive testing will eventually make such data available at relatively low cost enabling in-depth economic analysis. Whether such studies will be supported by the industry remains to be seen.

Efforts have been made to inform consumers about what fresh produce is in season, implying that such produce is likely to have superior taste. This is true in general, but produce purchased at various locations may taste distinctly different. The system of certifying a geographical production area practiced in many countries, most notably in the European Union, provides an implicit taste measure. However, unless augmented by specific references to taste, e.g., sweet Vidalia onions, the same type of produce originating from various production areas still may vary in taste, yet consumers will be unable to compare taste and price according to taste preference. Wine marketers constructed a scoring system serving as guidance in the purchase of wines of the same type produced from the same grape variety across the world and across various vintages. Fresh produce is not likely to offer similar scoring opportunities because of, for example, transportation costs and shelf life, limiting growers’ chances to extract the premium by supplying produce with distinct taste advantage.

Taste can be easily abused. Fresh produce taste is affected by inattention to maturity stage at harvest, poor postharvest handling, and careless treatment by consumers on the way home (e.g., temperature abuse in a car) and at home. All these reasons could negatively influence future sales volume or prices. However, more importantly, taste is an easy subject of abuse because taste perception varies among individuals. Taste differences result from genetic, environmental, demographic and economic factors. The multiplicity of causes behind taste differences invites many to exploit them for their purpose. Fresh produce marketers apply merchandising techniques using the taste as a tool influencing the purchase at the point of sale. Growers apply production techniques that have confirmed taste enhancing effect such as Good Agricultural Practices or organic farming. Exporters apply indirect measures to assure buyers of taste determining compound content, while breeders struggle to develop varieties with taste superior to that of available varieties. The number of various economic agents interested in fresh produce taste and in using taste as an attribute improving their individual economic returns encourages regulators to step in. The political economy of taste is complex and untransparent reflecting national or regional economic interests in protecting markets or producers, protecting consumer welfare, and the well-being of societies.
3.6 References


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Part II

Flavour formation during growth and postharvest flavour changes
4

Formation of fruit flavour
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4.1 Introduction

Flavour is generally accepted to be a combination of aroma and taste sensations. The main compounds detected by taste in fresh fruits are organic acids and sugars that produce sour and sweet sensations though other minor components may be associated with secondary aspects of fruit taste such as bitterness, astringency or saltiness. Regarding fruits there is no doubt about the important contribution of the aroma to the overall flavour perception. It is the aroma profile that differentiates among cultivars because of the higher sensitivity of the olfactory system and the large number of volatile compounds produced in fruits, but without forgetting the existence of a close interaction between taste and aroma, with aroma components affecting taste perceptions and vice versa.

Fruit aroma is a complex mixture of a large number of volatile compounds whose composition is specific to species and often to cultivars, and is determined not only by genetics but also by environmental factors, cultural practices and postharvest handling. The chemical nature of most fruit aromas have been characterized in the past 40 years. Current analytical methodologies employing new and sensitive isolation methods, the extended use of gas chromatography coupled to mass spectrometry, or the information provided by the latest generation of electronic noses have allowed the characterization of the most complex volatile mixtures. However, the knowledge of the biochemical pathways involved in aroma formation in fruits is still a step behind.

This chapter aims to present a general vision of the currently known or hypothesized biochemistry of fruit aroma through a description of the main
biochemical pathways involved in the biosynthesis of the most important volatile compounds produced by fruits: esters, aldehydes, alcohols, ketones, terpenes and furanones. Most of these compounds derive from essential nutrients or health promoting compounds suggesting that volatile components could provide important information about the nutritional make-up of fruits (Goff and Klee, 2006). The structure of the chapter will follow the classical scheme proposed by Tressl et al. (1975) in which aroma formation is studied within the metabolism of the three main groups of flavour precursors in fruits: carbohydrate metabolism, amino acid metabolism and fatty acid metabolism, with a final section specifically devoted to the formation of volatile esters, probably the largest and most important class of volatile compounds identified in fruits.

4.2 Carbohydrate metabolism

There are two main groups of aroma compounds that come directly from carbohydrate metabolism: terpenes and furanones. Volatile terpenes are the main components of many essential oils that, for instance, determine the aroma of most citrus cultivars. Furanones, having a pleasant sweet aroma, contribute to many fruit flavours. The biosynthesis of terpenes is associated with the isoprenoid pathway, while furanones seem to be produced from intermediates of secondary pathways of carbohydrate metabolism such as the pentose phosphate cycle.

4.2.1 Isoprenoid pathway

Volatile terpenes belong to a large family of natural compounds known as isoprenoids with many physiological functions assigned. The isoprenoid biosynthetic pathway generates both primary and secondary metabolites. Among the first are phytohormones such as gibberelic and abscisic acid, photosynthetic pigments such as chlorophylls and carotenoids, and other important compounds such as ubiquinones or sterols. Among those considered to be secondary metabolites are volatile terpenoids. It is generally assumed that the early steps in the biosynthesis of volatile terpenes in fruits are the same as those involved in the formation of other isoprenoid compounds. Terpenoids are derived from the mevalonic acid (MVA) pathway (see Fig. 4.1), active in the cytosol, or the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (see Fig. 4.2) active in the plastid, both of them forming IPP (Mahmoud and Croteau, 2002; Rodriguez-Concepción and Boronat, 2002; Rohdich et al., 2003). Data on mevalonic acid pathways are consistent with the biosynthesis of one molecule of MVA from three of acetate through an enzymatic sequence including acetyl-CoA acetyltransferase, hydroxymethyl-glutaryl-CoA synthethase, and hydroxymethyl-glutaryl-CoA reductase in a two-step reaction requiring NADPH (Kleinig, 1989). IPP is formed from MVA by a series of reactions shown in Fig. 4.1. The first step is the synthesis of mevalonic acid 5-phosphate by the action of mevalonate kinase, an enzymatic activity demonstrated to be present in many plants (Spurgeon and Porter, 1980).
The final step in IPP biosynthesis is the decarboxylation and dehydration of mevalonic acid diphosphate (MVAPP), first described in orange extracts (Potty and Bruemmer, 1970a). The biosynthesis of IPP through the MEP pathway (Fig. 4.2) begins with the formation of 1-deoxy-D-xylulose-5-phosphate (DXP) catalysed by DXP-synthase (Estevez et al., 2001). In a second step DXP reductoisomerase transforms DXP into MEP (Mahmoud and Croteau, 2001). The enzyme catalysing the last step of this pathway, hydroxymethylbutenyl diphosphate reductase, has been suggested as a rate-limiting step in the MEP pathway in tomato (Botella-Pavia et al., 2004). The MVA pathway is generally considered to supply the precursors for the production of sesquiterpenes and triterpenes while the MEP pathway seems to supply the precursors for the production of monoterpenes, diterpenes, and tetraterpenes (Aharoni et al., 2005). Little is known, however, about the regulation of the MEP pathway or the possible connection.
between these two biochemical routes (Bede et al., 2006), although limited unidirectional exchange of isoprene units, such as IPP, occurs between the plastid and cytosol (Heintze et al., 1990; McCaskill and Croteau, 1998; Bick and Lange, 2003).

In both biochemical pathways IPP is used by prenyltransferases in condensation reactions to produce larger prenyl-diphosphates such as geranyl diphosphate (GDP). This reaction needs the previous isomerization of IPP to dimethylallyl diphosphate (DMAPP) carried out by the enzyme IPP isomerase. This enzyme has been purified and characterized in tomato and red pepper (Spurgeon et al., 1984; Dogbo and Camara, 1987). Prenyltransferases produces geranyl diphosphate (GPP) when DMAPP and IPP are substrates or farnesyl diphosphate (FPP) when GPP and IPP are substrates. Both enzymes, GPP-synthase and FPP-synthase were first characterized in geranium leaves (Suga and Endo, 1991). Several terpene synthase genes have been recently identified in fruits such as citrus (Lucker et al., 2006).
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2002; Sharon-Asa et al., 2003, Iijima et al., 2004), apple (Pechous and Whitaker, 2004) and strawberry (Aharoni et al., 2004).

Monoterpenes are the main fruit aroma component from the isoprenoid family. Their biosynthesis is comparatively well understood. GPP is the direct precursor of their formation through a sequence of reactions including hydrolysis, cyclizations and oxidoreductions (Schreier, 1986; Croteau, 1988; Lange and Croteau, 1999). Cyclases, the key enzymes in this sequence, are soluble proteins with molecular weights in the range of 50–100 KDa, and requiring Mn²⁺ or Mg²⁺ as cofactors (Croteau, 1992). The crucial role of cyclases in the origin of the different monoterpane structural groups has stimulated several studies on the stereochemical mechanism of cyclization (Schwab et al., 2001). Non-oxygenated monoterpenes are generally oxygenated in a series of steps involving cytochrome P450 systems and molecular oxygen (Sanz et al., 1997).

Significant progress has been made over the past few years on monoterpenoid metabolic engineering (Aharoni et al., 2005). First studies were carried out on mint and petunia species with the aim of overexpressing the gene encoding limonene synthase (Krasnyanski et al., 1999; Diemer et al., 2001). In a similar study, a gene encoding S-linalool synthase has been expressed in tomato under the control of a late fruit-ripening promoter resulting in the accumulation of S-linalool and 8-hydroxy linalool in ripe tomato (Lewinsohn, 2001).

Although monoterpenes are probably the most important class of fruit aroma components derived from the isoprenoid pathway, some other important aroma compounds, such as norisoprenoids, are derived from breakdown of carotenoids generated as primary metabolites of this pathway (Winterhalter and Rouseff, 2002). In some fruits, such as tomato, near isogenic lines differing only in carotenoid composition correlated with differences not only in the composition of norisoprenoid but also in monoterpane volatiles (Lewinsohn et al., 2005a,b). Thus, although it is widely accepted that citral, a mixture of neral and geranial (Baldwin et al., 2000) is directly formed from GPP, a carotenoid derived biosynthesis cannot be discounted in some fruits. This might be the case with tomato and watermelon, where citral is not present in fruits from those cultivars devoid of lycopene (Lewinsohn et al., 2005a,b).

Some in vitro studies point to an effective participation of oxidative enzymes such as peroxidase, lipoxygenase or other dioxygenases in the formation of some classes of volatile terpenes (Bouvier et al., 2003; Giuliano et al., 2003; Wu et al., 1999). In relation to this, regiospecific carotenoid cleavage enzymes involved in the formation of fruit aroma compounds have been isolated in quince, star fruit and nectarine (Fleischman et al., 2002, 2003; Balderman, 2005). Recently, a tomato dioxygenase able to cleave carotenoids at position 9 and thought to be involved in the formation of geranyl acetone, pseudoionone, and β-ionone has been described (Simkim et al., 2004). However, the enzymatic or non-enzymatic nature (Wache et al., 2003) of some subsequent carotenoids oxidative reactions has yet to be elucidated.
4.2.2 Furanone biosynthesis

The interest in furanones, and particularly in furaneol, the key component of this family of volatiles, during the last three decades has not only been due to their demonstrated contribution to many fruit aromas, but because furaneol has also been detected in many heat-processed foods, e.g. beef broth, roasted almonds, roasted coffee, wheat bread crust or popcorn (Schieberle, 1992; Kroh, 1994). Actually, furaneol was first reported as a product of the Maillard reaction (Hodge et al., 1963).

Furaneol (2,5-dimethyl-4-hydroxy-3[2H]-furanone) and its methylether derivative mesifurane (2,5-dimethyl-4-methoxy-3[2H]-furanone) are important aroma compounds that have been identified in many fruits such as pineapple (Rodin et al., 1965), raspberry (Honkanen et al., 1980), mango (Pickenhagen et al., 1981), arctic bramble (Kallio et al., 1984), grapefruit (Lee and Nagy, 1987), tomato (Buttery et al., 1994) and strawberry (Sanz et al., 1995). Both compounds occurring as racemates in different fruits (Bruche et al., 1991) have strong, pleasant and sweet odours. Furaneol imparts caramel burnt sugar notes at high concentrations and becomes fruity at lower concentrations (Re et al., 1973) while mesifurane is described as having a more sherry-like aroma (Hunter et al., 1974). Furaneol is the most important compound among furanones due to its low odour threshold (10 ppb) (Schwab and Roscher, 1997). In fruits such as strawberry, at least four furaneol derived compounds have been detected: free furaneol, mesifurane, furaneol-β-d-glucopyranoside and the malonyl derivative of this furaneol glucoside (Sanz et al., 1995; Zabetakis et al., 1999; Roscher et al., 1996). Furaneol and mesifurane are considered character impact compounds in the aroma of many fruits, while furaneol-glucoside and furaneol-malonyl-glucoside are non-volatile compounds that might influence the overall flavour of fruits (Zabetakis and Holden, 1996, Bood and Zabetakis, 2002).

Despite the number of studies devoted to their identification and the establishment of the importance of furanones to fruit aromas, their biosynthesis is still not well understood. Different studies indicated that furaneol is derived from carbohydrate metabolism. The first studies were carried out by Kallio (1975) on arctic bramble homogenates using labelled fructose. A hypothesis by which furaneol was formed from fructose through a series of unknown reactions involving hydrogen transfer and dehydration with a subsequent formation of mesifurane by enzymatic methylation was proposed. In the last decade most studies on furaneol biosynthesis have been carried out in strawberry where this compound can reach a high concentration in fully ripe fruits (37 µg/g) (Pérez et al., 1996a). Sanz et al. (1995) demonstrated that furaneol formation in strawberry was associated with ripening. The same authors found, by means of an in vitro growth system (Perkins-Veazie and Huber, 1992), an increase in furaneol content with time at a higher rate than field-grown fruits, with highest furanone accumulation (125%) in those fruits grown in a medium supplemented with D-fructose 6-phosphate (Pérez et al., 1999a).

Experiments using radioactively labelled substrates proved the transformation of the complete carbon chain of D-fructose into furaneol (Schwab, 1998). Further
incorporation studies with D-[2-2H]-glucose demonstrated the involvement of phosphohexose isomerase in the conversion of D-glucose to furanones, confirming the hypothesis that D-fructose-6-phosphate is a natural precursor of furaneol biosynthesis (Wein et al., 2001). Recently, Raab et al. (2006) have isolated and characterized an enzyme involved in the last step of the furaneol biosynthetic pathway in strawberry. The protein purified from ripe strawberry fruits seems to be an enone oxidoreductase (FaQR) with a 37 KDa molecular mass, an optimum temperature of 37 ºC and a broad pH optimum peaking at 7.0. The enzyme catalyses a two-substrate reaction for which an apparent $K_m$ of 3.5 mM for D-fructose-1,6-diphosphate and 30 µM for NADH have been calculated. The ripening-induced increase of this enzymatic activity correlates with the observed furaneol accumulation during ripening (Pérez et al., 1996b). Based on the findings of this study, a new natural precursor (4-hydroxy-5-methyl-2-methylene-3[2H]-furanone) and a new scheme of furaneol biosynthesis have been suggested (Fig. 4.3).

Furaneol is the key metabolite of the pathway. Radiotracer studies have demonstrated that fruits are able to convert furaneol into mesifurane and furaneol glucoside (Roscher et al., 1997). Lavid et al. (2002) identified an O-methyltransferase in strawberry capable of transferring a methyl group from S-adenosyl-L-methionine (SAM) to furaneol. The activity of this enzyme, with a native molecular mass of 80 kDa, optimum activity at pH 8.5 and 37 ºC, and an apparent $K_m$ of 5 mM for furaneol, also increases with fruit ripening (Lunkenbein et al., 2006). Though in strawberry fruits furaneol glucoside is formed at the latest stage
of fruit development (Pérez et al., 1996b) and most studies proved that furaneol is the precursor of furaneol-glucoside (Roscher et al., 1997; 1998), the mechanism regulating interconversion of furaneol into furaneol-glucoside is not fully understood. Studies with callus cultures have shown that after sugar feeding experiments the preferred storage metabolite is furaneol-glucoside (Zabetakis and Holden, 1996). Roscher et al. (1996) after fractionation of a glycosidic extract of strawberry found not only the mentioned β-D-gluco-pyranoside derivative but also a furaneol-malonyl glucoside formed through esterification.

Recent studies have also focused on the biosynthesis of other furaneol derivative, the norfuraneol (4-hydroxy-5-methyl-3[2H]-furanone) identified in raspberry, guava and tomato (Hauck et al., 2003). D-ribulose-5-phosphate seems to be the norfuraneol precursor in tomato through a biosynthesis pathway in which 4,5-dihydroxy-2,3-pentanedione is considered to be a key intermediate.

4.3 Amino acid metabolism

Amino acids represent an important source of volatile compounds contributing to the aroma of fruits and vegetables. They should be considered from two different points of view: as direct precursors of aroma compounds or as indirect precursors, forming non-volatile compounds that need a second enzymatic transformation upon cell disruption to form volatiles. The latter mechanism is mainly present in vegetables and will not be discussed in this chapter.

The metabolism of amino acids generates aliphatic, branched or aromatic alcohols, carbonyls, acids and esters. These compounds contribute to, and in some cases determine, the primary aroma of many fruits. Thus the free amino acid pool of a fruit could determine its aroma profile (Tressl and Drawert, 1973). Radioactive labelling studies have proved the transformation of amino acids such as alanine, leucine, phenylalanine or aspartic acid into volatile compounds (Myers et al., 1970; Tressl and Drawert, 1973; Yu et al., 1968) following the scheme shown in Fig. 4.4. This aroma compound biosynthesis is quite similar to that first postulated by Ehrlich in 1907 in yeast (Ehrlich pathway), and whose enzymatic basis was established by Senthesshanmuganathan (1960). It comprises three enzymatic activities: aminotransferase, decarboxylase and alcohol dehydrogenase. Tressl and Drawert’s studies on banana showed that 14C-leucine is converted into labelled 3-methylbutanol, 3-methylbutanoic acid and 3-methylbutyl esters (Fig. 4.4). A first step of transamination is inferred from the experimental data as glutamic acid is produced from 2-oxoglutarate. This hypothesis was validated after identification of the corresponding intermediary 2-oxoacid from the amino acid and the isolation of alanine 2-oxoglutarate aminotransferase in tomato (Yu and Spencer, 1969; Rech and Crouzet, 1974; Gazeu-Reyjol and Crouzet, 1976). This enzyme seems to be partially bound to mitochondrial membrane exhibits a molecular weight of 100 KDa and requires pyridoxal-5-phosphate (PALP) as cofactor, although a tight PALP-apoenzyme linkage is demonstrated as no added PALP is needed for in vitro maximum activity. The mechanism of action seems to be in
good agreement with those elucidated for other plant and animal aminotransferases (Givan, 1980). The next step is the decarboxylation of the 2-oxoacid formed after amino acid transamination. Drawert (1975) postulated that the 2-oxoacid oxidative decarboxylation occurs via an enzymatic complex similar to pyruvate dehydrogenase or 2-oxoglutarate dehydrogenase from the TCA cycle, involving as cofactors thiamine pyrophosphate (ThPP), lipoic acid, FAD, NAD and coenzyme A. This complex would produce 3-methylbutanoyl-CoA from leucine, substrate for the biosynthesis of 3-methylbutanoate esters. The involvement of ThPP as the solo cofactor would give rise to 3-methylbutanal, which is reduced to alcohol by ADH using NADH as cofactor. This alcohol was the labelled moiety found in banana esters. However, no labelled aldehyde was found in banana (Tressl et al., 1970), suggesting that 3-methylbutanal would be quickly reduced to alcohol or oxidized to 3-methylbutanoic acid by an oxidoreductase requiring NAD as cofactor. Yoshioka et al. (1981) proposed a 2-oxoisocaproate decarboxylase as being responsible for 3-methylbutanal synthesis in banana. Similar enzymatic transformations have been described for amino acids other than leucine. Isoleucine is a reported precursor of 2-methylbutanol and 2-methylbutyric acid in melon (Yabumoto et al., 1978; Wang et al., 1996a,b), apple (Hansen and Poll, 1993: Rowan et al., 1996) and strawberry (Pérez et al., 2002). Valine is a reported precursor of 2-methyl propyl esters in banana (Tressl and Drawert, 1973) and tomato (Buttery and Ling, 1993) and alanine is presumably the source of ethyl esters in strawberry (Pérez et al., 1992). Phenylalanine can also be metabolized through this pathway to 2-phenylacetyl-CoA, and this compound converted into esters of a variety of
Aromatic amino acids may also serve as important precursors leading to a family of compounds with phenolic and spicy odour notes, through a different metabolic pathway (Fig. 4.5). Cinnamic acid and its \( p \)-hydroxy derivative (\( p \)-coumaric) are the key intermediates of this pathway that was firstly postulated to operate in banana (Tressl and Albrecht, 1986) and more recently involved in the biosynthesis of \( p \)-hydroxyphenylbutan-2-one (\( p \)-HPB), also known as raspberry ketone (Borejsza-Wysocki and Hrazdina, 1994; Kumar and Ellis, 2001). \( p \)-HPB biosynthesis consists of two enzymatic steps identified in raspberry crude extracts. The first one is a condensation reaction of \( p \)-coumaryl-CoA with malonyl-CoA catalysed by \( p \)-hydroxyphenylbut-3-en-2-one synthase, releasing coenzyme A and carbon dioxide. Then, a NADPH-reductase gives rise to pHPB.

It is important to point out that the final product of the decarboxylation, the acyl-CoA or the corresponding aldehyde, could depend on the species (Sanz et al., 1997). Thus, 2-oxopentanoic acid was described as a powerful alkylating agent in strawberry segments after decarboxylation (Yamashita et al., 1978b, Drawert and

Fig. 4.5  Conversion of aromatic amino acids into volatile aroma compounds.
Berger, 1982) while incubation of apple discs with this compound produced more butanoate esters (67%) than butyl esters (21%) (Berger and Drawert, 1984). Nevertheless, contradictory data have been reported on the relative contribution of each catabolic branch to fruit aroma. Feeding experiments with in vitro grown strawberries (cv. Camarosa) showed that metabolism of L-leucine in this fruit is mainly carried out through the decarboxylating branch producing acyl-CoAs (Pérez et al., 2002) contrary to what Drawert and Berger (1982) found in strawberry slices. However, it is also possible that 2-oxopentanoic acid was reduced by other oxidoreductases before entering the ester synthesis pathway. Similar discrepancies can be found in ester metabolic studies on apple. Rowan et al. (1996) observed that incubation of Granny Smith apple tissues with isoleucine produced almost exclusively ethyl-2-methylbutanoates while Red Delicious apple tissues produced 2-methylbutyl esters in the same conditions. These authors attributed this predominance of 2-methylbutyl esters, contradicting previous data (Berger and Drawert, 1984), to a consequence of the irreversible reduction of 2-methyl butanoic acid to 2-methylbutanol occurring in Red Delicious apples. Both decarboxylating branches seem to have different regulatory mechanisms. Thus, Bauchot et al. (1998) working on ACC oxidase antisense melons found that those pathways leading to the formation of ethyl esters with branched chain acyl groups from amino acids were more strongly regulated by ethylene than those forming acetates with branched chain alcohol moieties.

Although this relationship between the formation of aroma volatiles and the free amino acids pool present in ripening fruits is well established (Schreier, 1984), volatile formation is not only determined by substrate availability (Wyllie et al., 1996) but also depends on the relative activities of the 2-oxoacid decarboxylase/dehydrogenase-type enzymes in the fruit. These enzymatic activities could be affected by cultivar, maturity stage, and even environmental conditions, either on or off the plant. In this sense, strawberry cultivar variations in two key aroma enzymes, alcohol dehydrogenase (ADH) and pyruvate dehydrogenase, account for the different susceptibility to off flavour development, that is ethanol, acetaldehyde and ethyl acetate production (Ke et al., 1994a,b; Fernandez-Trujillo et al., 1999; Watkins et al., 1999). Despite the importance of ADH for fruit aroma biosynthesis, in most fruits the specificity of ADH is not a limiting factor (Mitchell and Jelenkovic, 1995; Wyllie et al., 1996; Wyllie and Fellman, 2000; Defilippi et al., 2005a), while the decarboxylating step seems to be critical for the release of esters precursors. The biochemical and molecular characterization of fruit decarboxylases should provide valuable information to fully elucidate volatiles formation from amino acids metabolism (Moyano et al., 2004; Tieman et al., 2006).

4.4 Fatty acid metabolism

Fatty acids (FA) are the major precursors of volatile compounds responsible for the aroma of most plant products. They are catabolized through two main oxidative
pathways: β-oxidation and the lipoxygenase (LOX) pathway. It has been suggested that β-oxidation is the main metabolic pathway producing primary aroma in fruits, whereas the LOX pathway may account for the widest variety of aroma compounds from fatty acids in disrupted fruit tissues (Schreier, 1984). Nevertheless, LOX pathway actuation should not be absolutely restricted to disrupted tissues. Some studies suggest that increasing availability of FA, along with higher membrane permeability, during fruit ripening might allow the LOX pathway to become active and to function as an alternative to β-oxidation (Brackman et al., 1993; Rowan et al., 1999; Echevarria et al., 2004; Leone et al., 2006).

4.4.1 β-oxidation
Different studies involving incubation experiments with cold or labelled FA of different fruit tissues such as pear (Jennings, 1964; Jennings and Tressl, 1974), banana (Tressl and Drawert, 1973) and apple (Paillard, 1979) described the generation of aroma compounds via β-oxidation. A recent review by Baker et al. (2006) describes varied roles for this pathway in relation not only to fatty acid catabolism but also in amino acid metabolism and biosynthesis of hormonal compounds through the LOX pathway. The postulated scheme for this pathway in fruits in relation to aroma biosynthesis is shown in Fig. 4.6. Unfortunately, the information related to β-oxidation in fruits is not based on a detailed study of the enzymes involved but on simple incubation experiments (Rowan et al., 1999). In this metabolic pathway, acyl-CoAs derivatives of FA are metabolized to shorter chain acyl-CoAs by losing two carbons at every round of the cycle involving, in the case of saturated FA, the following enzymes: acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA-dehydrogenase and acetyl-CoA acetyltransferase. The two hydrogenation steps require FAD and NAD, respectively, while free coenzyme A is needed for the scission step. The resulting acyl-CoAs produced by β-oxidation will be used later by alcohol acyltransferase for volatile esters generation. Pear and apple aroma have been two classical examples of volatile formation through the β-oxidation pathway (Paillard, 1990, Suwanagul and Richardson, 1998). Varietal differences of ester composition (acetates and butanoates) in apples have been related to the last step of β-oxidation of FA, that is the transformation of butanoate into acetate (Paillard et al., 1979; Paillard, 1990). The oxidative catabolism of unsaturated fatty acids involved the actuation of auxiliary enzymes, such as enoyl-CoA isomerase (Goepert et al., 2005) and 3-hydroxyacyl-CoA-epimerase as included in the scheme of β-oxidation for linoleic acid (Fig. 4.6). These auxiliary enzymes could be implicated in the formation of the different enantiomeric compositions found among 3-hydroxyacid esters in tropical fruits (Tressl et al., 1985).

The biosynthesis of lactones, key aroma components in fruits such as peach and nectarine (γ-decalactone and γ-dodecalactone), pineapple (δ-octalactone) or coconut (γ-octalactone) is also associated with the β-oxidation pathway. In fact, most hypotheses on lactone biosynthesis in fruits put in contact the two major pathways producing aroma compounds from FA, β-oxidation and LOX (Sanz et al., 1997).
Fig. 4.6  Scheme of β-oxidation for linoleic acid.
Oxo and hydroxyacids seem to be precursors of lactone biosynthesis in fruits (Tressl et al., 1988) but despite the importance of these compounds for fruit aroma there is a lack of enzymatic studies in fruits, and micro-organisms serve as a model for studying lactone biosynthesis (Haffner and Tressl, 1996; Wache et al. 2001). Thus, Albrecht et al., (1992) using Sporobolomyces odorous as a model demonstrated that biosynthesis of γ- and δ-lactones combines hydroperoxydation of linoleic acid and β-oxidation, as well as some reduction steps of the hydroperoxide group. More recently, it was shown for the first time that enzymes present in apple fruit could be used as an effective agent for the lactonization reaction (Olejniczak et al., 2003).

4.4.2 LOX (lipoxygenase) pathway

The metabolism of polyunsaturated fatty acids (PUFAs), via the first LOX-catalysed step and the subsequent reactions, is commonly known as the LOX pathway. In plants the hydroperoxy polyunsaturated fatty acids initially synthesized by LOX are substrates of different enzyme families that generate an important number of compounds collectively known as oxylipins. In the past few years, the knowledge of the function of LOX and oxylipins in plants, especially in relation to plant defence mechanisms, has increased considerably (Porta and Rocha-Sosa, 2002; Blee, 2002; Feussner and Wasternak, 2002). In this chapter, only those metabolic branches related to fruit aroma biosynthesis will be discussed.

Many of the alcohols, aldehydes, acids and esters found in fruit and vegetable aroma are generated from the oxidative degradation of linoleic and linolenic acids, the main substrates of LOX in the plant kingdom. An overall view of aroma formation through LOX pathway is shown in Fig. 4.7. The enzymatic oxidative degradation is preceded by the action of acylhydrolases which liberate PUFAs from triacylglycerols, phospholipids or glycolipids. Though most plant LOXs prefer free FA as substrates, the activity of some LOXs with esterified PUFAs in phospholipids or triglycerides have been demonstrated (Matsui et al., 1998; Feussner et al., 1998). LOX is the key enzyme in this pathway and catalyses the regio- and stereo-specific hydroperoxidation of PUFAs with a (1Z,4Z) pentadiene structure. The Z double bond attacked by oxygen moves into conjugation with the neighbouring Z double bond and assumes an E configuration. Depending on the plant source and the isoenzymes present, oxygen incorporation can occur preferentially at C9, C13 or at either C9 or C13 in a non-specific manner. In either case, the hydroperoxide group has an S enantiomeration (Vick and Zimmerman, 1987). Typical LOX contains a non-haem iron atom in the active site that alternates between Fe²⁺ and Fe³⁺, having a molecular weight around 75–95 KDa (Sanz et al., 1997). A number of plant LOX sequences are now available making possible the elucidation of the relationship between both LOX sequences and structures and their regiospecificity and activity (Casey and Hughes, 2004). According to their overall sequence similarity, plant LOXs can be classified into two families. Those having no transit peptide and having high sequence similarity are type 1-LOX and those having a putative chloroplast transit peptide sequence are type 2-LOX. All
plants contain multiple isoenzymic forms of LOX. Thus, five tomato LOX genes have been shown to be expressed during fruit ripening, four of them mainly producing 9-hydroperoxides while TomloxC, a chloroplastic isoform, can use both linoleic and linolenic acid and produces 13-hydroperoxide products critical for biosynthesis of tomato aroma (Chen et al., 2004).

The LOX pathway has long been recognized as responsible for the generation of ‘green’ odour notes in plant products. The review by Hatanaka (1993), although mainly focused on green leaves, serves as an excellent guide to the understanding of the catalytic and mechanistic aspects related to the LOX pathway in any plant material. Numerous papers have described the importance of LOX to aroma biogeneration in fruits such as apple (Rowan et al., 1999, Defilippi et al., 2005a), cucumbers (Galliard and Phillips, 1976; Feussner and Kindl, 1992), tomatoes (Galliard and Mathew, 1977, Smith et al., 1997; Yilmaz et al., 2002; Chen et al., 2004), banana (Jayanty et al., 2002), strawberry (Pérez et al., 1999; Leone et al., 2006) or olive (Olías et al., 1993; Salas et al., 1999; Pérez et al., 2003). Though
some readily volatile compounds (C5 carbonyls) might be generated through an additional branch of the LOX pathway from LNA (Gardner et al., 1996), the first readily volatile compounds formed in this pathway came from the cleavage of FA-hydroperoxides catalysed by the enzyme hydroperoxide lyase (HPL). HPL is a membrane bound enzyme that was first suspected in banana by Tressl and Drawert, (1973) and its presence was later demonstrated in many plant leaves and fruits (Hatanaka, 1993). The cleavage catalysed by HPL takes place between the carbon that contains the hydroperoxide group and the proximate ethylenic carbon, giving rise to C6 or C9 carbonyl compounds and the corresponding oxoacids. Products from 13-hydroperoxylinoleic acid are hexanal and 12-oxo-(9Z) dodecenoic acid. When 13-hydroperoxylinolenic acid is the substrate, the same oxoacid is obtained and the aldehyde is (3Z)-hexenal. The products of HPL with 9-hydroperoxides of linoleic and linolenic acids are the 9-oxononaic acid and (3Z)-nonenal or (3Z, 6Z)-nonadienal, respectively. HPL enzymes belong to the cytochrome P450 protein family and have been characterized in different fruits such as pear (Kim and Grosch, 1981), cucumber (Galliard and Phillips, 1976), tomato (Riley et al., 1996; Matsui et al., 2001), melon (Tijet et al., 2001), citrus fruits (Gomi et al., 2003), watermelon (Fukushige and Hildebrand, 2005), guava (Tijet et al., 2000), strawberry (Pérez et al., 1999) and olive (Olías et al., 1993; Salas and Sanchez, 1999; Luaces et al., 2003).

Though we are far from a detailed understanding of the regulation within the LOX pathway in plants (Feussner and Wasternak, 2002), several studies have provided new insights of the relative contribution of LOX and HPL in terms of aldehyde production. Thus, it is likely that hexanal and 3-hexenal production is determined by substrate availability to HPL rather than by the abundance of HPL activity, which seems to be constitutively present (Vancanneyt et al., 2001). LOX depleted gives rise to a marked reduction of C6 and C5 aldehydes and alcohols (Leon et al., 2002). Several attempts to modify tomato aroma, by acting on the LOX pathway, have been carried out with different results. Genetic manipulation of desaturases in order to modify FA composition (Wang et al., 1996a,b), or ADH (Speirs et al., 1998) effectively changed the aroma profile of transformed tomato fruits, while antisense suppression of LOX (Griffiths et al., 1999) or overexpression of a 9-HPL did not cause a significant aroma alteration (Matsui et al., 2001). More recently, Myung et al. (2006), studying aldehyde biosynthesis in strawberry, hypothesized that fruits may exhibit different metabolic flows through the pathway of LOX and HPL for the production of Z,3-hexenal and E,2-hexenal. In relation to product specificity it is well documented that, in most plant products, HPL specificity determines aroma composition despite the specific action of LOX. Thus, though pear LOX forms mainly 13-hydroperoxides (Kim and Grosch, 1978) pear HPL is specific for 9-hydroperoxides (Kim and Grosch, 1981). In a similar way cucumber LOX produces 13/9-hydroperoxides in a ratio 85:15 while cucumber HPL exhibits the higher specificity for the latter substrate, explaining the important amount of C9 compounds in the aroma of cucumber fruit (Wardale and Lambert, 1980, Matsui et al., 2000). The biosynthesis of virgin olive oil aroma through the actuation of olive LOX/HPL is another example of the different
importance of LOX and HPL in terms of volatile composition (Olías \textit{et al.}, 1993; Salas \textit{et al.}, 2000, Luaces \textit{et al.}, 2003).

In most plants, compounds with a (Z,3)-enal structure are rapidly isomerized to the (E,2)-enal form. There is not a clear consensus on the chemical or enzymatic nature of this isomerization step (Hatanaka \textit{et al.}, 1986; Myung \textit{et al.}, 2006). Before or after the isomerization of unsaturated carbonyls formed by HPL, ADH catalyses the reversible reduction of C6-aldehydes to C6-alcohols in a reaction dependent on pyridine nucleotides. In olive pulp, Salas and Sanchez (1998) characterized an NADP-dependent ADH present in the pulp tissue of developing fruits displaying a clear preference for C6 and C9 aldehydes. ADH is a metalloprotein possessing sulfydryl groups in the catalytic site and with two probably identical subunits of molecular weight 45 kDa (Sanz \textit{et al.}, 1997) that have been characterized in different fruits (Yamashita \textit{et al.}, 1978a; Bicsack \textit{et al.}, 1982; Longhurst \textit{et al.}, 1990, Ke \textit{et al.}, 1994a,b; Salas and Sanchez, 1998; Chervin \textit{et al.}, 1999). Most ADH genes expressed in fruit isolated so far belong to the medium-chain zinc-binding subfamily of ADHs (Chase, 1999). As has been stated previously in relation to amino acid metabolism, in most fruits the specificity of this enzyme is not a limiting factor for the biosynthesis of volatile compounds from FA metabolism (Defilippi \textit{et al.}, 2005a,b), though a change in ADH activity effectively alters the balance between C6 aldehydes and alcohols and might affect HPL regulation (Bate \textit{et al.}, 1998; Speirs \textit{et al.}, 1998). Very recently, two new ADHs have been isolated and characterized in melon (Manriquez \textit{et al.}, 2006). Both are positively regulated by ethylene and operate preferentially as reductases of aldehydes into alcohols that are indeed substrates for the biosynthesis of volatile esters in melon (El-Sharkawy \textit{et al.}, 2005). Alcohols resulting from ADH activity are natural substrates for the ester-forming enzyme, alcohol acyltransferase (AAT). Thus, saturated and unsaturated C6-alcohols formed through the LOX pathway can be esterified with acyl-CoA moieties to produce hexyl, (2,E)-hexenyl and (3,Z)-hexenyl esters (Olías \textit{et al.}, 1993; Salas, 2004). The occurrence and characteristics of the enzyme AAT will be outlined below.

4.5 Ester formation

Volatile esters formed by esterification of alcohol and carboxylic acids constitute one of the largest and main group of volatile compounds identified in fruit aroma (Sanz \textit{et al.}, 1997). Biogeneration of volatile alcohols and acids is generally well explained through the enzymatic routes previously described, but specific information on the final esterification step has only be obtained in the last few years. Volatile esters are important contributors not only to the aroma of fruits but also to the flavour of fermented products. In fact, the mechanism of ester formation is better known in micro-organisms in which two different enzymes seems to be implicated: AAT and esterase. AAT catalyses the transfer of an acyl moiety from an acyl-CoA intermediate on to the corresponding alcohol, while esterase functions mainly by hydrolysing esters, although an ester-forming activity by esterase
has also been reported (Yoshioka and Hashimoto, 1981; Yamauchi et al., 1989; Malcorps and Dufour, 1992). Both enzymatic activities have been described in fruits (Ueda and Ogata 1976), where esterase has only hydrolytic activity and ester formation is a CoA-dependent reaction (Ueda and Ogata, 1977). The ester-forming capacity of different fruits was initially studied using whole fruits or tissue discs and did not involve deeper biochemical aspects. Esterification of added alcohols to banana pulp discs was investigated as early as 1970 by Myers et al. and further studies on ester formation were carried out in various fruit tissues (Yamashita et al., 1975; Ueda and Ogata, 1977). These investigations showed that ester-forming activity was related to fruit ripening (Yamashita et al., 1977) and gave initial information on the relationship between substrate specificity and ester composition (Ueda and Ogata, 1978; Yamashita et al., 1979).

The first AAT enzyme characterized in fruits, described as an alcohol acetyltransferase, was localized by Harada et al. (1985) in the soluble fraction of banana pulp cells. A similar enzyme, only active on acetyl-CoA, was identified in apple (Fellman et al., 1991) while the third fruit AAT, active with different acyl-CoAs, was purified and characterized in strawberry (Pérez et al., 1993), where a clear correlation was observed between AAT activity and flavour quality of different strawberry cultivars along ripening (Pérez et al., 1996, Olías et al., 2002). Olías et al. (1995), using crude enzymatic extracts from banana and strawberry fruits, observed important differences in both fruits AAT activities towards short aliphatic alcohols and acyl-CoAs. Strawberry AAT displayed the highest activity with hexanol and acetyl-CoA, while very low activity was found with these two substrates in banana. In a similar study, Ueda et al. (1992) also found a clear relationship between specificity of the ester-forming enzyme system of banana, melon and strawberry and their characteristic aroma pattern. More recently, Holland et al. (2005), also working with fruit extracts, have found different enzyme levels and substrate specificities in AAT enzymes from two apple cultivars. AAT activity from Granny Smith fruits uses almost exclusively hexanol and Z,3-hexenol, while the enzyme from Fuji fruits, being considered one of the most aromatic apple cultivars, accepted a broader range of alcohols.

The level and/or characteristics of an enzyme responsible for the final step of the biosynthesis of a particular volatile is not the only limiting factor. This is particularly true in the case of enzymes such as the AAT that are able to use different substrates so that the final volatile profile of a given fruit might depend on the availability of precursors for those substrates (Dudareva et al., 2004). This is the case of banana, where the substrate specificity of AAT does not explain the composition of the branched-chain esters found in this fruit aroma (Wyllie et al., 1996), and availability of substrates from amino acids metabolism seems to be the key process in ester biosynthesis (Wyllie and Fellman, 2000). On the other hand, Souleyre et al. (2005) reported recently that apple AAT preference for acetate ester formation depends upon substrate concentration. Thus, at low concentrations of alcohol the enzyme prefers 2-methylbutanol over hexanol and butanol while at high concentrations of substrate hexanol is used at the greatest rate. In the past few years a combination of appropriate biochemical knowledge with gene expression
data has provided very valuable information to enable the elucidation of the role of AAT in aroma formation during fruit ripening. AATs belong to a recently discovered family of plant acyltransferases called BADH (St Pierre and De Luca, 2000). BADH acyltransferases directly involved in volatiles generation have been investigated in flowers (Dudareva et al., 1998; 2004; Shalit et al., 2003; Boatright et al., 2004) and in fruits such as strawberry (Aharoni et al., 2000; Nam et al., 1999; Aharoni et al., 2004), apple (Defilippi et al., 2005; Souleyre et al., 2005; Holland et al., 2005; Li et al., 2006), banana (Beekwilder et al., 2004), melon (Shalit et al., 2001; Yahyaoui et al., 2002; El-Sharkawy et al., 2005) and grape (Wang and De Luca, 2005). Though all of them belong to the BADH family, AAT proteins from different fruit species are highly divergent. Curiously, some proteins with very low amino acid identity, for instance strawberry and melon (SAAT and CmAAT1) having only 22% identity, have quite similar substrate preference (Aharoni et al., 2000; Yahyaoui et al., 2002). On the contrary, AAT genes from wild and cultivated strawberries (VAAT and SAAT) are closely related but the activity of both recombinant enzymes is quite different (Beekwilder et al., 2004). The AAT from wild strawberry is much more active on short alcohols in agreement with the substrate preferences reported in fruit enzymatic extracts from both wild and cultivated berries (Olías et al., 2002). In some cases the substrate preference of recombinant AAT enzymes does not reflect the ester profile of the corresponding fruit. Thus, the banana recombinant enzyme exhibits very low efficiency for synthesizing isoamyl acetate, the key component of banana aroma (Beekwilder et al., 2004). In other fruits such as melon the different specificity of the multiple AAT proteins codified by the genes identified (CmAAT1, CmAAT3 and CmAAT4) effectively accounts for the great diversity of esters formed in the fruit (El-Sharkawy et al., 2005).

Despite the low sequence homology found among the AAT genes identified so far, AAT proteins exhibited some common characteristics. All fruit AAT genes identified so far encode proteins ranging from 419 to 479 amino acid residues which correspond to an average molecular weight of 51–55 KDa. These data are in good agreement with the molecular weight of the native AAT proteins purified in banana (40 KDa), strawberry (48 KDa) or grape (50 KDa) (Harada et al., 1985; Olías et al., 2002; Wang and DeLuca, 2005). The inhibitory effect of zinc and sulfhydryl reactive compounds on the activity of native and recombinant AATs (Pérez et al., 1993; Souleyre et al., 2005) suggest the implication of cysteine residues in the substrate pocket and/or catalytic region of the enzyme. A threonine residue seems also to be critical for AAT activity as pointed out by El-Sharkawy et al. (2005), after site-directed mutagenesis on a cloned melon AAT gene (CmAAT2) that lacked AAT enzymatic activity. Most of the native and recombinant proteins exhibit a broad pH range of activity between 7 and 9, and an optimum temperature around 30 °C (Sanz et al., 1997; Souleyre et al., 2005; Wang and DeLuca, 2005). Most AAT proteins also have a similar expression pattern during fruit development and ripening. The expression of genes from wild and cultivated strawberries (Aharoni et al., 2000; Beekwilder et al., 2004), banana (Beekwilder et al., 2004), melon (El-Sharkawy et al., 2005) and grape (Wang and DeLuca, 2005) is strongly
induced during fruit ripening. Ethylene has proved to be a major regulator of the activity in melon and banana (Flores et al., 2002; Medina-Suarez et al., 1997) and experiments carried out with transgenic apple fruits, suppressed in ethylene biosynthesis, and with the ethylene antagonist 1-MCP also suggest a regulatory effect of ethylene on AAT (Defilippi et al., 2005a; Li et al., 2006).

4.6 Conclusions

As we have seen throughout this chapter, fruit aroma formation is a complex process in which quite different pathways are involved. These biochemical routes are interconnected and most of them are not exclusively devoted to aroma formation but also give rise to some other important plant metabolites which have many different biological functions. In fact, the physiological significance of plant volatiles constitutes a research line of increasing importance in plant science. Major progress has come in the past few years from the use of molecular and biochemical techniques in terms of the characterization of key enzymes and identification of new intermediate and final aroma compounds, but more research still needs to be done. Up to this point it is clear that two main factors control fruit aroma composition; the selectivity of a group of key enzymes and the availability of appropriate substrates but there is limited information about the regulation of these catalytic processes. On the other hand, it is also clear that a single enzymatic step will rarely control aroma formation and in most cases an entire metabolic pathway should be studied in order to understand the aroma of a given fruit. Though genetics determine the enzyme system and precursors involved in aroma formation, many other factors such as soil nutrition, growing environment, stage of maturity and postharvest conditions might affect the aroma of fresh fruits and must be taken into account in any attempt of improving fruit flavour. In the next few years fruit flavour formation should become a multidisciplinary research area that brings together different scientific disciplines such as flavour chemistry, biochemistry, physiology, molecular biology and plant genomics.

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5

Formation of vegetable flavour

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5.1 Introduction

Vegetables comprise over-wintering storage organs, leaves, stems and some modified flowers. The factor that unites them is that they give variety in flavour and colour to food, rather than the bulk of the calories. In addition, health benefits are conferred through the supply of vitamins, roughage and nutraceuticals. The objective of this chapter is to focus on what is known about the biosynthesis and physiology of the flavour compounds of the major vegetable crops consumed within the EU and USA. These are carrots, the Brassicas (cabbage, broccoli, cauliflower, turnip, swede, Brussels sprouts) and Alliums (onion, garlic, spring onion, leeks). Table 5.1 indicates the flavour compounds found in these vegetables and the sections in this chapter where each is discussed.

Vegetable flavour comes from an interplay of sensory factors. One is from secondary metabolites, namely low molecular weight organic compounds often restricted to a limited number of plant families or species. Although over 100 000 have been described, a species or cultivar will have a characteristic complement, which may consist of a few up to hundreds. These make the major contribution to the flavour of the vegetable. However, carbohydrate storage compounds, especially mono- and di-saccharides and the texture of the plant material derived from structural polymers, also make important contributions. The flavour may be affected by growth conditions, storage, preparation and cooking that the vegetable has undergone. Finally, human perception of flavour is influenced by numerous psychological and cultural factors surrounding food and meals.
Table 5.1  Major vegetable flavour compounds

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<td>Alk(en)yl cysteine sulphoxides</td>
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5.2 The major flavour compounds in vegetables: secondary metabolites

5.2.1 An overview of the role of secondary metabolites in flavour

Secondary metabolites have a major role in vegetable flavour. Their roles within plants are now considered to be part of a complex defence strategy developed from interaction with predators, pathogens and the environment, as well as contributing to colours and scents (Hadacek, 2002; Pichersky et al., 2006). The chemicals may have additional roles as storage of carbon, nitrogen and sulphur resources. Each plant species contains characteristic secondary metabolites, but information on the number, level and location has been constrained by extraction and analytical methods. Developments in analytical technology, especially on-line mass spectrometry, have seen a marked increase in the spectrum of secondary compounds identified within many plants.

From the point of view of vegetable flavour, human tastes, both gustatory and visual, also influence the identity and level of secondary metabolites. The wild relatives of vegetables indicate the flavour traits that existed prior to domestication. Vegetable crops have been selected for qualities including flavour, colour, shape, keeping, palatability and yield that have emphasised different factors from those found in the wild relatives. For example, the human preference for sweet flavours has increased the sugar content of many vegetables, and decreased levels of compounds perceived as bitter (Drewnowski and Gomez-Carneros, 2000).

The major classes of secondary metabolites that act as flavour compounds are the terpenoids (also known as isoprenoids or terpenes), glucosinolates, alk(en)yl cysteine sulphoxides and phenolic compounds. Individual terpenoids are perceived to have a wide range of flavours and aromas which blend into the characteristics of carrot. After enzymic cleavage the glucosinolates produce the typical flavours and aromas of the Brassicas. Methyl cysteine sulphoxide also contributes to Brassica flavour, while cleavage products of this and other alk(en)yl cysteine sulphoxides produce the pungent and sulphurous flavours characteristic...
of Alliums. Phenolic compounds generally produce bitter and astringent flavours and have been detected in all groups of vegetables.

5.2.2 Terpenoids

The terpenoids are one class of secondary metabolite synthesised from isoprene units and have roles in signalling and response to stresses (Tholl, 2006) as well as contributing to flavour. Diversity and biosynthetic flexibility of response are therefore key attributes. Individual species probably have a unique complement of as many as 100 terpenes, with additional differences among individuals (see Chapter 4 of this book).

Plants have two biosynthetic pathways leading to the central intermediates for all isoprenoids; the mevalonate pathway (MVA) within the cytosol and the mevalonate-independent methylerthritol phosphate pathway (MEP), that operates within plastids, as summarised in Fig. 5.1 (Rohmer et al., 1993; Bouvier et al., 2005). Pyruvate and glyceraldehyde-3-phosphate are the initial substrates of the MEP pathway, which yields, via deoxy-xylulose-5-diphosphate (DOX), methylerthritol phosphate as the first isoprenoid-committed intermediate.

Both routes lead to the key five-carbon intermediates isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). These are used by prenyltransferases to produce the prenyl diphosphate precursors of the terpenoids, namely geranyl diphosphate (GPP; 10 carbon), farnesyl diphosphate (FPP; 15 carbon) and geranylgeranyl diphosphate (GGPP; 20 carbon). Members of the terpene synthase superfamily are responsible for the formation of terpene skeletons from these three intermediates, often producing several products from a single substrate (Chappell, 2002). Further modifications such as hydroxylation, glycosylation or reduction may also occur, resulting in the tremendous structural variety of the terpenoids where over 20 000 different natural oils, resins and volatiles have been identified (Tholl, 2006).

Both GPP and GGPP are synthesised primarily within plastids as intermediates in the synthesis of isoprene, monoterpenoids, diterpenoids and tetraterpenoids as well as other isoprenoid secondary metabolites (Chappell, 2002). Sesquiterpenoids and triterpenoids are largely synthesised by the cytosolic mevalonate pathway from IPP and DMAPP following the key intermediate FPP. However, the parallel biosynthetic pathways in different compartments offer additional possibilities for metabolite diversity and environmental responses. There is clear evidence that the two pathways are not completely isolated in that both are required for synthesis of some sesquiterpenoids, necessitating exchange of the biosynthetic prenyl diphosphate intermediates between cytosol and plastid under certain conditions, such as stress (Piel et al., 1998). Although there is limited information on this subject, it appears to differ between plant species and to be affected by cross-talk between environmental factors (Bick and Lange, 2003; Hampel et al., 2005). An exporter of prenyl diphosphates across the plastid envelope membrane has been characterised in chloroplasts from spinach leaves (Spinacia oleracea), kale (Brassica oleracea) and Indian mustard (Brassica juncea) (Bick and Lange, 2003).
mechanism involves proton symport and may be gated by calcium. Transport processes occur for IPP, FPP and GPP but not for GGPP.

The contributions of the MVA and MEP pathways to the biosynthesis of mono- and sesquiterpenoids in carrots was evaluated by Hampel and colleagues in excised root phloem and in xylem tissue using deuterated precursors of the two pathways. This showed that both mono- and sesquiterpenoids are synthesised using the MEP route, while the MVA pathway only synthesises sesquiterpenoids (Hampel et al., 2005). The labelling patterns indicated that IPP and/or DMAPP, or GPP formed from MEP, could be transported from plastids to the cytosol for sesquiterpenoid biosynthesis but not in the other direction.

The roles of most individual terpenoids are unknown but presumed to be within the area of interaction with the environment, especially in responses to stresses
including microbes, insect predation, photooxidation and heat and also as attractants for pollinators and predators of herbivorous insects (Tholl, 2006). The odour thresholds for human perception of individual terpenoids differs substantially (α-pinene 6 µg ml⁻¹; terpinolene 200 µg ml⁻¹, Whitfield and Last, 1991). A further dimension is added to this already complex mix through human selection for appearance, palatability and storage properties of vegetables. The differences in terpenoid spectrum between cultivated and ancestral vegetable varieties will include both coincidental and deliberate changes but there are only a few examples where these are attributed to functionally defined genes.

5.2.3 Glucosinolates

The glucosinolates (GS) are the stable precursors to volatile and reactive flavour compounds found within the order Capparales of the Brassicaceae. They probably evolved from the widespread system of cyanogenic glucosides and corresponding O-β-glucosidases (Halkier and Gershenzon, 2006). Their natural function is as defence compounds with well-documented toxicity and feeding-aversion effects, in addition to anticarcinogenic properties (e.g. Stoewsand, 1995; Talalay and Fahey, 2001).

More than 120 GS are known (Halkier and Gershenzon, 2006; Fahey et al., 2001). They all share a core comprising thioglucose and thiohydroximate moieties with an aliphatic, aromatic or indolic side chain. Since completion of the Arabidopsis thaliana genome sequence there has been major progress in understanding the biosynthetic steps towards this structure (Grubb and Abel, 2006). The pathway is summarised in Fig. 5.2. The first committed and key regulatory step is the oxidation of aromatic and aliphatic amino acids (alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophan, methionine) to their aldoximes by substrate specific cytochromes P450 of the CYP79 family (Mikkelsen et al., 2002). Subsequent enzymes, although specific for GS biosynthesis, have low substrate specificity and generate a series of end-products around a core structure. The next step is further oxidation of the aldoxime by cytochromes P450 from the CYP83 family and conjugation to (usually) cysteine as S-donor with cleavage, probably by a C–S lyase, to give a thiohydroximic acid. This is followed by transfer of glucose from UDP-glucose (generating thioglucose) and finally by transfer of sulphate from phosphoadenosine-phosphosulphate to create a sulphonated oxime and so finish the GS core (Halkier and Gershenzon, 2006).

More than one enzyme is probably required for each of these steps in many species to produce the range of GS that have been identified consistent with the limited substrate specificity of the few enzymes that have been studied in detail so far. Diversity is introduced initially by the side chains derived from the initial aminoacids and from chain elongation through addition of methylene groups, most frequently in GS derived from methionine or phenylalanine (Fahey et al., 2001). Further diversity is created through a series of secondary modifications to both side chain and core including hydroxylation, methoxylation, sulphation, glucosylation
Fig. 5.2 Outline of the biosynthesis of glucosinolates and consequences of cleavage by myrosinase. The major stages are shown, modified from Halkier and Gershenzon (2006) and Grubb and Abel (2006) and as described in Section 5.2.3. Stages after tissue damage are indicated below the horizontal dotted line.

or oxidation of the side chain sulphur leading to its loss and formation of a terminal double bond (Halkier and Gershenzon, 2006).

On tissue damage, GS are hydrolysed by the enzyme myrosinase (thioglucosidase EC 3.2.3.1). GS and myrosinase are separated in intact tissue through myrosinase being located within specialised myrosin cells scattered among all organs of the Brassicas, and also possibly by subcellular compartmentation of GS to the vacuole (Halkier and Gershenzon, 2006). The hydrolysis products are glucose, sulphate and unstable aglucone intermediates that undergo further abiological changes to yield isothiocyanates, nitriles and other compounds depending on pH, metal ions and further protein factors (Bones and Rossiter, 2006). These are the direct source of the flavours of vegetables that contain GS. The isothiocyanates have a major defence function but their level is reduced in the presence of epithiospecifier protein (ESP), which promotes the production of sulphur-containing nitriles (epithionitriles), although the enzyme mechanism is unclear (Burow et al., 2006).
5.2.4 Alk(en)yl cysteine sulfoxides

These sulfoxide-containing cysteine derivatives are the precursors of the characteristic volatile flavour compounds of Alliums, although one (methyl cysteine sulfoxide) is distributed more widely. The Alliums are unusual in that their flavour and odour depends on differing levels of only four flavour precursors, with sugars providing sweetness to the flavour. The spectrum of alk(en)yl cysteine sulfoxides (CSOs) differs between Allium vegetables and gives rise to their characteristic odours (Lancaster and Boland, 1990).

The stable CSO flavour precursors are cleaved by the pyridoxal-phosphate dependent glycoprotein alliinase (alliin lyase, EC 4.4.1.4) to yield pyruvate, ammonia and reactive, volatile, sulphur compounds. The latter undergo further non-enzymic reactions resulting in the changing taste and smell of Allium tissue over time during cooking or food processing (Fenwick and Hanley, 1985; see also Chapter 11 this volume). Methyl cysteine sulfoxide (MCSO) is present in most Alliums and also in some other species and its cleavage products are the source of ‘cabbage’ or ‘fresh onion’ odours. Allyl cysteine sulfoxide (ACSO) is the major flavour precursor in garlic (A. sativum) and the source of diallyl thiosulphinate (allicin), its characteristic odour and flavour. It is also present in leeks (Allium porrum). Trans-prop-1-enyl cysteine sulfoxide (PeCSO) is the source of the typical onion (A. cepa) odour, along with products of propyl cysteine sulphoxide (PCS0) and MCSO (Lancaster and Boland, 1990). The lachrymatory effect of onion odour is caused by thiopropanal-S-oxide, formed through the action of lachrymatory factor synthase on the immediate product from allinase cleavage of PeCSO (Imai et al., 2002). The CSOs are located within the cytoplasm and only come into contact with allinase after tissue damage (Lancaster et al., 1989). Allinase appears to be present in the vacuole of all tissues in onion (Lancaster et al., 1989) while it is confined to the bundle sheath cells of garlic, again probably within the vacuole (Ellmore and Feldberg, 1994).

Alliums also contain substantial amounts of γ-glutamyl cysteine sulphotides and glutathione derivatives, and their relationship to CSO biosynthesis remain to be completely resolved. Two routes have been proposed for the biosynthesis of CSOs (reviewed in Jones et al., 2004), outlined in Fig. 5.3. One (Fig. 5.3B) is from glutathione with γ-glutamyl peptides (γGPs) as intermediates and has parallels with glutathione degradation (Leustek et al., 2000). It involves S-alk(en)ylation of the cysteine in glutathione, trans-peptidation to remove the glycyl group, oxidation of the cysteine sulphhydryl to yield a sulphone and finally removal of the glutamyl group to give the final CSO (Lancaster and Shaw, 1989; Randle et al., 1995). An alternative route (Fig. 5.3A) with analogies to cysteine synthesis, has been proposed through direct alk(en)ylation of cysteine or thioalk(en)ylation of O-acetyl cysteine (Granroth, 1970) in a similar way to the biosynthesis of several other secondary metabolites mediated by O-serine acetyl transferase and cysteine synthase (Ikegami and Murakoshi, 1994).

There is experimental evidence to support both routes and the proposed biosynthetic enzymes are now starting to be obtained from Alliums and studied in detail. The differences between studies on excised leaves, which indicated a major
role for γGPs as intermediates in biosynthesis (Lancaster and Shaw, 1989), and studies using intact, sprouting onion bulbs or garlic cloves which suggested they had a minor role (Edwards et al., 1994) may reflect different biosynthetic routes within tissues in different physiological states. This is supported by recent information about a membrane-associated γ-glutamyl trans-peptidase cloned from sprouting onion bulbs. It had a high substrate affinity for glutathione and glutathione conjugates but kinetic analysis indicated that the major onion γ-glutamyl peptide, γ-glutamyl-trans-prop-1-enyl cysteine sulfoxide was unlikely to be the substrate in vivo (Shaw et al., 2005). A serine acetyl transferase has recently been characterised from onion (McManus et al., 2005) which is expressed in leaves and up-regulated in response to low sulphur availability. The serine acetyl transferase and cysteine synthase from garlic chives (A. tuberosum) have been studied in detail. One particularly interesting property of this cysteine synthase was that high levels of substrate did not inhibit activity (Ikegami et al., 1993; Urano et al., 2000). This property would be likely if cysteine synthase was involved in CSO synthesis as proposed by Granroth (1970), to allow sufficient flux through the pathway. Cysteine synthase can use a variety of compounds in vitro as acceptor for the alanyl group from O-acetyl serine resulting in a range of β-substituted alanine secondary metabolites including S-methyl cysteine and S-allyl cysteine when methyl or allyl sulphide are provided as acceptors (Ikegami and Murakoshi, 1994). Garlic also contains cysteine synthases that differ in their substrate specificity (Jones et al., 2004).

Fig. 5.3 Outline of the biosynthesis of alk(en)yl cysteine sulfoxides. The presumed biosynthetic steps are shown, modified from Jones et al. (2004) and as described in Section 5.2.4. A indicates the proposed route via cysteine or O-acetyl cysteine (Granroth, 1970). B indicates the proposed route via glutathione (Lancaster and Shaw, 1989).
MCSO also occurs in other genera (Brassica, Raphanus, some Phaseolus and Vigna) and in some members within the families Compositae and Umbelliferaceae (Benevegna et al., 1989). It is cleaved by the enzyme C–S lyase (EC 4.4.1.8) to yield pyruvate, ammonia and methane sulphenic acid (Chin and Lindsay, 1994). This enzyme is distinguished from alliinase through its ability to cleave L-cystine as well as CSOs and activity is present in the cytosol and vacuole of all tissues of broccoli, although it is highest in the inflorescence (Ukai and Sekyia, 1999). Three distinct isoforms (a, b, c) have been reported. The b-isoform was the most abundant and consisted of a tetramer (160 kDa) composed of four identical subunits (40 kDa each) that did not appear to be glycosylated (Ukai and Sekyia, 1999), although an earlier report concluded that one trimeric isoform was glycosylated (Hamamoto and Mazelis, 1986). MCSO was cleaved by the b-isoform (K_m 9.8 mM) although L-cystine was a better substrate (K_m 0.9 mM) (Ukai and Sekyia, 1999). A C–S lyase has been found in Alliums in addition to alliinase (Ukai and Sekyia, 1999). As understanding of enzymes capable of cleaving cysteine and its derivatives has increased, it has become apparent that they have roles in primary and secondary metabolism including within the biosynthesis of methyl methanethiosulphinate and in catalysing donation of reduced S in glucosinolate synthesis (Kiddle et al., 1999).

5.2.5 Phenolic compounds

Plants contain around 8000 compounds within the classes of phenolic compounds united by possession of at least one aromatic ring with a hydroxyl substituent (a phenol group) (Robbins, 2003). They include anthocyanins, coumarins, flavones, stilbenes, cinnamates, hydroxyl benzoic acid derivatives, lignans and chalcones. Biosynthesis commences from the shikimate pathway using intermediates from carbohydrate metabolism, and proceeds through phenylpropanoid pathways as summarised in Fig. 5.4, but requires many further specific enzymes to produce the diversity of aromatic compounds (Herrmann, 1995; Herrmann and Weaver, 1999). 3-Deoxyarabinoheptulosonate-7-phosphate synthase (EC 4.1.2.15) catalyses the initial step that controls carbon flows to yield shikimate and then chorismate prior to synthesis of the aromatic amino acids tryptophan, tyrosine and phenylalanine. After phenylalanine synthesis, the enzyme phenylalanine ammonia lyase (PAL, EC 4.3.1.5) transforms it into cinnamic acid through non-oxidative deamination in the three steps of general phenylpropanoid metabolism (Robbins, 2003).

Bitter, sweet, pungent and astringent tastes have been attributed to individual phenolics and they can also contribute to aroma. They also show pharmacological activities, acting as antioxidant, anti-inflammatory, antitumoural and oestrogenic agents. Phenolics have been suggested as the explanation for epidemiological evidence of health benefits from a diet high in fruit and vegetables in prevention of cardiovascular disease and some types of cancer (Block et al., 1992; WHO/FAO, 2002). The food industry also has a major concern to prevent enzymatic browning during vegetable processing because of the unattractive visual and flavour consequences, and phenolic compounds are involved in this process.
Fig. 5.4 Outline of the biosynthetic pathways for phenolic compounds. The initial stages to phenylalanine are summarised. Several of the major classes formed via the phenylpropanoid pathway are indicated, modified from Herrmann (1995), Herrmann and Walker (1999) and Robbins (2003) and as further described in Section 5.2.5.

Cinnamic and benzoic acids are present in most plant tissues, linked to structural components such as the cell wall (and they thus have an important role in the textural aspects of flavour) and a range of other molecules such as sugars, polyphenols, organic acids and terpenes (Robbins, 2003). Chlorogenic acids are esters between trans-cinnamic acid derivatives and quinic acid, also known as hydroxycinnamates. The most common is 5-O-caffeoyl-quinic acid (5-CQA) but related compounds with many different acyl groups have been identified (Clifford, 1999). The clustering of phenolic hydroxyls are the basis of their astringent taste, although even compounds with only one dihydroxyphenyl group such as catechin, epicatechin and 5-CQA can taste astringent (Clifford, 1997).

Some lettuce varieties (iceberg, butterleaf) are very low in flavonoids and caffeic acid derivatives, while other red-hued varieties (lollo rosso, oak leaf) contain large amounts of flavonols, caffeic acid derivatives and anthocyanins...
Levels vary considerably between cultivars, and are lower in inner leaves due both to genetic factors and lack of light (Hohl et al., 2001). Texture is an important component of lettuce flavour (Delaquis et al., 2000). Consideration of breeding lettuce varieties with higher flavonoids in internal leaves depends on whether or not they contribute to flavour.

### 5.3 Carrot flavour

#### 5.3.1 Overview

Carrots are the major commercial root vegetable in many countries and considerable effort has therefore been devoted to understanding the complex nature of their flavour. It has been mainly attributed to terpenoids and sugars, with perception of sweetness being crucial to a pleasant flavour. Commercial carrot varieties differ in their sugar contents (Seljåsen et al., 2001a). Terpenoids provide the characteristic carrot flavour and aroma but can give an undesirable quality of harshness to the flavour at higher levels. Cooking by boiling removes the greater proportion of the terpenoids and is therefore beneficial from a flavour point of view since there is then less possibility that the sweet taste will be masked (Alasalvar et al., 1999). The cultivar and cultivation conditions play a part, as do postharvest storage and processing. For the fresh carrot market, and especially for the developing sector of fresh carrot products (carrot snacks, ready-prepared carrots, ready-prepared salads) it is important to understand the effects of processing on flavour to provide a product that tastes good and is hygienic. The consequences of damage through peeling and slicing, as well as storage are therefore important.

There is good evidence that carrot pests use cues including the volatile foliar terpenoids to identify their host plant. Understanding details of these interactions could be valuable in designing integrated pest management systems and selecting varieties for organic production systems. This opens the possibility of selecting varieties that attract predators of pests, but not the pests themselves (Kainulainen et al., 1998). One strong message from reports of carrot flavour analyses is that there is substantial variation between and within even commercial cultivars, and the basis of desirable and undesirable flavours is not well defined. With greater understanding of the chemical basis of carrot flavour, this variation would be the basis of selecting new varieties.

The role of other compounds in carrot flavour is less definite. The distinctive carrot colour comes from flavourless carotenoids and anthocyanins (orange, primarily β-carotene; purple, β-carotene and anthocyanins; red, β-carotene and lycopene; white, lutein), which are the major vegetable source of pro-vitamin A and provide well-documented health benefits (Surles et al., 2004). The role of colour in consumer appeal has been tested in taste trials, which indicated that flavour rather than colour was the decisive factor (Alasalvar et al., 2001; Surles et al., 2004). However, some monoterpenes (e.g. β-ionone, 4-methyl-5-hepten-2-one) can be formed from carotenoids and may be a route to off-odour in freeze
dried carrots (Kjeldsen et al., 2003). A large number of phenolic compounds have been detected in carrots which have a role in browning. The most abundant is 5-CQA (Alasalvar et al., 2001), which may contribute to bitter and off flavours. The bitter flavoured isocoumarin 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (6MM) has frequently been detected, especially after tissue damage or ethylene exposure, but normally at levels below human perception (e.g. Seljåsen et al., 2001b). Volatile phenylpropanoids are commonly found in carrot steam oil but not in raw carrot extracts. Eugenol methyl ether, eugenol, elemicin and myristicin were detected in the aroma of shredded refrigerated and frozen carrot (Kjeldsen et al., 2003).

5.3.2 Terpenoids in carrot flavour
Carrot flavour is based on a mixture of volatile and non-volatile components, affected by both genetic and environmental factors. In addition to sweetness from stored sugars (sucrose, glucose, fructose) which is generally perceived as the most attractive characteristic by consumers (e.g. Martens et al., 1985; Varming et al., 2004), more than 90 volatile compounds have now been identified with mono and sesquiterpenoids by far the most abundant.

Many recent studies have combined sensory evaluation, often using trained panels and a rigorous experimental design, with chemical analysis to determine the most important contributors to carrot flavour and aroma. The full details of how interactions between compounds give rise to the sweet, bitter and harsh flavours reported in sensory evaluations has not been fully resolved, although the importance of several compounds has become apparent. The conflicting evidence that carrot roots grown at lower temperatures contain more sugars, but taste less sweet and more bitter (e.g. Rosenfeld et al., 1997) has a persuasive explanation in a simultaneous increase of terpenoids and sugars masking the sweet flavour.

Different amounts and identities of flavour compounds have been found in different studies. This may be attributable in part to analytical methods and changes caused by sample processing as well as real differences due to cultivar, growth or storage conditions. For example, terpin-4-ol and α-turpineol have been detected in some studies of carrot aroma but not in others (e.g. Kjeldsen et al., 2003). These can both be formed by oxidation of the abundant monoterpenoid terpinolene and therefore may result from vigorous sample preparation methods.

The most abundant are usually β-caryophyllene, terpinolene and α-humulene. ‘Spicy’ and ‘woody’ components are attributed to the sesquiterpenoids β-caryophyllene, and α-humulene, while the monoterpenoids (+)-sabinene, β-myrcene, p-cymene (+)- and (–)-α-pinene, (+)- and (–)-β-pinene and (–)-α-phellandrene seem to be important for ‘carrot top’ aroma and they also attracted the descriptors ‘green’, ‘pine’ and ‘terpene-like’ (Kjeldsen et al., 2003). Several of these are perceived at very low levels (e.g. β-myrcene, odour threshold 13 ppb; p-cymene, 13 ppb; sabinene 75 ppb; Whitfield and Last, 1991) and therefore can have a significant effect on the perceived flavour. Others have much higher thresholds for perception (e.g. α-pinene 1000 ppb) so may make a lesser contribution. The
monoterpenoids (+)- and (−)-limonene, γ-terpinene and terpinolene all have odours described as ‘citrus-like’, ‘fruity’ and ‘sweet’ and also affect the flavour (Kjeldsen et al., 2003). Measurements of composition during storage has indicated that, although the total amount does not change, the levels of individual terpenoids may alter, resulting in subtle effects on taste (Seljäsen et al., 2001b).

Alasalvar et al. (1999) have developed a headspace GC–MS method using grated carrot within sealed vials to screen large numbers of carrot samples, requiring minimal sample preparation and reducing the possibility of artefacts. They recorded 34 terpenoids, with a higher percentage of monoterpenoids in some varieties and higher percentage of sesquiterpenoids in others. Large varietal differences occurred in both total content and levels of each terpenoid in a series of F1 hybrids with some terpenoids absent from some varieties. The use of GC–olfactometry has added valuable detail to human perception of the volatiles in carrot flavour. In one study, flavour panellists were asked to breathe the eluate from gas chromatography columns (Kjeldsen et al., 2003) and could attribute an odour to 26 of 56 individual terpenes. Panellists did not always agree on descriptions, so that statistical analysis of the majority opinion was used to detect significant differences. Each volatile has a threshold concentration for detection, which may differ in combination with other volatiles. Chemical analysis can detects terpenoids at levels below their contribution to flavour or aroma.

5.3.3 Biosynthesis and subcellular location

There is little information about the biochemistry of terpenoid formation specifically within carrots. The different amounts and proportions of terpenoid in carrot roots and leaves indicates that some aspects of biosynthesis are organ-specific (Habegger and Schnitzler, 2000). A detailed study of carrot leaves by Kainulainen et al. (1998) identified 50 monoterpenoids, sesquiterpenoids and propylbenzenes, which changed as the leaves aged. There were significant different in composition between leaflets and petioles, and between individual carrots within the two varieties examined. However, the distinctly different environments of the open field and glass house did not affect the composition. The components in leaflets were myrcene, sabinene, (E)-β-ocimene, limonene, germacrene D and (E)-β-caryophyllene. Some compounds (α-asarone, methylisoeugenol, propylbenzenes) were present in young leaves but almost absent in older ones. Over half the terpenoid found in young (five weeks old) petioles was methylisoeugenol. In contrast, the major terpenoids in wild carrot leaves were reported as α-pinene, γ-terpinene and camphene with only a low proportion of myrcene (Kilibarda et al., 1996). Hampel and colleagues have shown that biosynthesis of myrcene in excised leaves proceeded exclusively from DOX, while DOX and MVA were incorporated into the sesquiterpenoid β-caryophyllene to equal levels. Transport of precursors from plastid to cytoplasm therefore occurs in leaves as well as in roots in carrot indicating that both organs can synthesise terpenoids (Hampel et al., 2005).

It has been suggested that terpenoid synthesis is confined to oil ducts in the phloem, which contain higher concentrations than xylem. A recent isotopic
labelling study using excised root phloem and xylem (Hampel et al., 2005) detected four-times higher rates of incorporation in phloem than in xylem suggesting that oil ducts were important but not completely essential. Biosynthesis in the xylem ceased after the carrots had been stored for seven days at 4 °C, hinting at post-harvest changes (Hampel et al., 2005).

5.3.4 Effects of environmental and cultivar factors
Geographical location, and thus growth temperature and rainfall have an effect on carrot flavour. When information on sugars, carotenes, root shape and sensory profiles of four varieties grown at six locations in Norway was analysed by principal components analysis, the geographical location where the carrots were grown accounted for 75% of the variation between samples (Rosenfeld et al., 1997). Those grown at lower temperatures were perceived as having a more pleasant sweet flavour, while high temperatures produced carrots with more bitter flavours. However, large root size was also a predictor of bitter flavour. A further 17% of variation related to crispness, sweetness and flavour was attributable to variety. Genetic differences between cultivars therefore accounted for much less of the difference between samples than environmental variables.

In a later study using controlled climate chambers to investigate the role of temperature more closely, the highest panel score for sweet taste and a crisp, juicy texture was for carrots grown at the lowest temperature (9 or 12 versus 15, 18 or 21 °C), while bitter taste, terpenoids and sugars all increased with increasing temperature (Rosenfeld et al., 2002). This provides strong support for the idea that terpenoids provide bitter flavour to carrots by suppressing their sweet flavour since principal components analysis showed that terpenoids were an excellent predictor of sensory variables. Levels of almost all terpenoids except the most abundant one (α-terpinolene) increased as the temperature increased. Statistical analysis indicated that α-terpinene, β-pinene, β-myrcene, caryophyllene, β-farnesene and α-humulene contributed most to the sensory variables describing carrot taste. ‘Green’ flavour correlated best with caryophyllene while bitterness, aftertaste, ‘earthy’ taste and ‘terpene’ flavour correlated with β-myrcene (Rosenfeld et al., 2002).

5.3.5 Effects of storage, washing and packing on flavour
Metabolism continues in carrot roots after harvest, converting sucrose to glucose and fructose (Seljäsen et al., 2001b). Ethylene encourages the formation of phenolics including 6MM and 5-CQA but these may not affect flavour. 6MM has a well-documented bitter flavour, distinctly detectable at above 94–100 mg kg⁻¹, and noticeable above 48 mg kg⁻¹ (Talcott and Howard, 1999). Thus when Seljäsen et al. (2001b) measured the response of carrots (cv. Yukon) to the low level of ethylene typical within carrot stores (1 µl l⁻¹), although levels of 6MM increased from less than 0.4 mg kg⁻¹ to 13 mg kg⁻¹ over three weeks, this remained below perceptible levels and was not the cause of the increased bitterness detected by a
trained sensory panel. Indeed, correlation analyses showed a significant correlation between the bitter flavour and content of the terpenoids γ-terpinene, α-pinene, limonene and p-cymene. Other compounds that may affect taste and have been detected after storage include propanol, which may have been a consequence of microbial spoilage (Alasalvar et al., 1999), and aldehydes such as octanal resulting from lipid oxidation, although measurements indicated that this also was below the threshold of human perception (Kjeldsen et al., 2003).

Varying effects of storage on the terpenoid composition of carrot have been recorded. Alasalvar et al. (1999) noted that the levels of the major terpenoids (α-pinene, sabinene, myrcene, limonene, terpinolene, γ-terpinene, β-caryophyllene, γ-bisabolene) in seven cultivars differed significantly but did not change appreciably over 28 days’ storage. In a longer study, comparison of the terpenoid volatiles in stored roots of the two cultivars Bolero and Carlo indicated that the total levels of flavour compounds increased three- to four-fold in both cultivars after 4 months of refrigerated storage, with the sesquiterpenoids increasing more than the monoterpenoids (Kjeldsen et al., 2003). This indicated that secondary metabolism, in particular biosynthesis of sesquiterpenoids, was active during the low temperature storage.

Further flavour changes occur when carrots are taken from store and prepared for marketing by washing and packing. Although a trained flavour panel recorded increases in undesirable off-flavours such as bitter taste, earthy odour and after-taste in carrots after both mechanical and hand preparation and packaging, analysis for 15 terpenoids and 6MM could not detect significant differences (Seljåsen et al., 2001a, 2004). The carrots were stored from November to March, and although the measured level of sugars decreased, the sensory panel found the carrots equally sweet on both occasions. Hand versus mechanical harvesting had no effect on flavour (as measured by taste trials and analysis of sugars, terpenoids and 6MM), but shaking at levels that caused physical damage to simulate harsh handling increased the production of 6MM and ethylene. However, even though the carrots tasted more bitter, 6MM levels were below the taste threshold (Seljåsen et al., 2001a). The proportion of diseased carrots was increased by mechanical washing and chemical products from the disease process may have started to affect flavour since the panel detected off flavours of ethanol and a ‘sickeningly sweet’ odour as storage proceeded (Seljåsen et al., 2004). Low temperature storage and packaging materials permitting good air exchange were the most significant factors in promoting good flavour in the stored carrots.

5.4 Brassica flavour

5.4.1 Overview
Volatile sulphur-containing compounds, aliphatic aldehydes and alcohols are all components of broccoli flavour, generated by enzymic or thermal breakdown of GS and MCSO. Hex–3(Z)-enol was the major constituent of an oil of volatile and semi-volatile constituents extracted by steam distillation from Romanesco-type
broccoli leaves, and this alcohol, along with dimethyl disulphide and dimethyl trisulphide were the major components from freshly disrupted inflorescences (Valette et al., 2003). Leafy Brassicas also contain the phenolic 5-CQA, the monoesters of caffeic acid, 3-feruloyl quinic acid and 3-\(p\)-coumaroyl quinic acid and sugar derivatives including sinapoyl and feruloyl glucose (reviewed in Clifford, 1999; Nilsson et al., 2006).

Selective plant breeding has reduced the levels of GS in modern Brassica cultivars compared with wild relatives because they contribute towards a bitter flavour. There are now suggestions that these efforts should be reversed, with the objective of producing customised GS profiles and especially increasing ones beneficial in cancer prevention either through introgression from wild forms (Mithen et al., 2003) or introduction of single genes (Kristensen et al., 2005). Clearly, any commercial varieties intended for consumers must achieve this goal without adversely influencing flavour. Knowledge of both the details of the control and biosynthesis of GS and how they contribute to taste will be essential.

5.4.2 Glucosinolates in Brassica flavour

The products of GS cleavage by myrosinase contribute pungent and burning qualities to Brassica flavour and include isothiocyanates, nitriles and thiocyanates. Myrosinase should be inactivated by cooking, therefore reducing the intensity of these flavours. ESP is more heat labile than myrosinase, and it is inactivated by brief boiling. An analogous thiocyanate-forming factor has been proposed, but not yet identified (Bones and Rossiter, 2006), which increased production of isothiocyanates at the expense of nitriles. The isothiocyanates are presumed to cause the pungent burning aftertaste sometimes experienced in broccoli although the GS sinigrin, the precursor of the highly pungent allyl isothiocyanate, is not found in broccoli (Baik et al., 2003).

The contribution that individual GS make to flavour is still unclear. Few studies have tried to relate the chemical composition of Brassicas to taste using consumers or trained panels. Knowledge about the flavour contribution of the myrosinase-mediated breakdown products of each GS has been hindered by lack of availability of individual, pure compounds. The recent development of methods for large-scale isolation of several GS (Song et al., 2006) may allow flavour profiles of individual ones to be gathered. However, most work to date has had to rely on comparisons of Brassica cultivars that contain mixtures of GS.

The GS sinigrin and progoitrin have been implicated as the major source of bitterness in Brussels sprouts, since higher levels reduced the number of consumers who consider that the sprouts had ‘good taste’ (Van Doorn et al., 1998). The comprehensive recent study by Klein and her colleagues supports the view that broccoli does not show a strong relationship between chemical composition and aroma or flavour. The study evaluated 19 cooked cultivars of broccoli (Brassica oleracea var. italica) that differed in GS composition and concentration using a trained sensory panel (Baik et al., 2003). HPLC–MS analysis indicated that glucoraphanin was the dominant aliphatic GS, frequently contributing up to 80%
of the total amount, followed by progoitrin and the indole GS glucobrassicin, although a total of 11 different GS could be distinguished. The panel rated broccoli cooked by microwaving for 19 flavour attributes, and there were significant differences between cultivars. The major determinants of broccoli flavour were ‘cooked corn-cabbage-green’, ‘bitter-musty-off-flavour’, ‘cooked-broccoli’ and sweetness factors. Interestingly, negative or no correlation between GS content and qualities such as sulphurous aromas and flavours, ‘cooked corn’ aroma and ‘green’ flavour suggested that they do not arise from GS breakdown. The ‘green’ flavour has been attributed to alcohols and aldehydes generated by lipid oxidation but others may be MCSO breakdown products (Baik et al., 2003). If this is indeed their source, strategies for greater partitioning of sulphur into GS at the expense of MCSO could yield both flavour and health benefits.

Investigation of the basis of sulphur and bitter flavours in cooked cauliflower showed that neoglucobrassicin and sinigrin were responsible for bitter flavours (Engel et al., 2002). In addition, dynamic headspace GC–olfactometry and mass spectrometry identified allyl isothiocyanate, dimethyl trisulphide, dimethyl sulphide and methanethiol as the key contributors to sulphur odours. The latter three originate from MCSO rather than GS. The levels of these volatiles differed amongst 13 cauliflower cultivars and corresponded to the main GS differences between the varieties. In a comparison of taste, GS and sugars in nine different coloured broccoli and cauliflower cultivars by a trained sensory panel and a consumer panel of women who regularly purchased broccoli or cauliflower, there was a definite preference for bright colours, low levels of bitter GS and a higher sucrose content (Schonhof et al., 2004). There was a significant correlation between levels of sinigrin, gluconapin, progoitrin, glucobrassicin and neoglucobrassicin and perception of a bitter taste by the panels. The total level of GS was substantially higher during a cooler growing season, although the differences detected by the sensory analysis were due to genotype rather than to environment. Fructose and glucose were the main sugars except in green pyramidal cauliflower where it was sucrose. The distinctly sweet flavour of this sugar may have contributed to the markedly different taste of this variety. The impression of a sweet taste was masked in cultivars with higher contents of bitter alk(en)yl and indole GS (Schonhof et al., 2004).

5.4.3 Biosynthesis and distribution of glucosinolates
GS are mobile within the plant, being taken up by a specific carrier system and transported by phloem in *A. thaliana* and *B. napus* (Brudenell et al., 1999; Chen and Andreasson, 2001). Myrosinase is found in myrosin cells in both the phloem parenchyma and, in the ground tissue in *B. napus* but is only in the phloem in *A. thaliana* (Andreasson et al., 2001). GS are also present in the myrosin cells.

The content of GS differs between plant parts. It is higher in broccoli shoots than roots (Aires et al., 2006). In cauliflower it is higher in the floret buds than the stalk. The upper leaves of curly kale contain substantially more than lower leaves and the level of many GS is higher in outer rather than in inner leaves of white...
cabbage. These locations within the reproductive organs and younger leaves match with the idea that GS are defence compounds. Storage reduced the amount of sucrose in white cabbage (Nilsson et al., 2006).

**5.4.4 Effects of environmental and cultivar factors**
Climate and genotype affect GS content. Comparing over two spring and autumn growing seasons the total GS, indole GS and glucoraphanin content differed between genotypes of broccoli, Brussels sprouts, cabbage, cauliflower and kale, and at harvest was lower after either increasing day length from transplant to harvest or increasing mean photosynthetic photon flux (Charron et al., 2005). The effect of irrigation during head development in cabbage, relative to other times, is beneficial in terms of weight and size and also affects flavour because it increases fructose and glucose and decreases sucrose and GS (Radovich et al., 2005). The GS content and composition of five groups of Brassicas (broccoli, Brussels sprouts, cabbage, cauliflower, kale) grown under the same conditions (Kushad et al., 1999) differed.

Sulphur and nitrogen fertilisation affect the level of GS in mature broccoli and did not benefit GS levels in young Brassica salad sprouts (Aires et al., 2006). Manipulating the sulphur fertilisation of kale grown in hydroponic culture showed that GS and MCSO decreased as S levels decreased (Kopsell et al., 2003).

**5.4.5 Methyl cysteine sulfoxide in Brassica flavour**
All Brassicas contain MCSO in addition to GS with Brussels sprouts containing higher levels than broccoli, cabbage or cauliflower (Marks et al., 1992). After tissue damage this is converted by C–S lyase to volatile sulphur compounds and pyruvate. Sulphides such as methanethiol, dimethyl disulphide and dimethyl trisulphide contribute to the flavour of cooked Brassicas (Engel et al., 2002). These have been implicated as the sources of sulphurous aromas and over-cooked ‘off’ flavours. If the C–S lyase is denatured by heating during boiling, blanching or other thermal processing, the flavour can be reduced. Activity of the C–S lyase from broccoli is not destroyed by freeze–thawing but it is reduced by the accompanying drop in pH, which also increases the adherence of dimethyl disulphide to the tissue surfaces (Tulio et al., 2002). MCSO can also undergo thermal degradation to yield volatile sulphur products.

**5.5 Allium flavour**

**5.5.1 Overview**
Onions and garlic, along with leeks, spring onions (*A. fistulosum*) and shallots (*A. cepa*), are used in cooking worldwide and onions are the second most important horticultural crop after tomatoes (Griffiths et al., 2002). The excellent storage properties of onion and garlic mean that they have been traded worldwide for
longer than other vegetables. In addition, health benefits from garlic sulphur compounds have been demonstrated with reduced incidence of some cancers and also cardiovascular disease (Griffiths et al., 2002; Collin, 2004). Selenium can substitute for sulphur and there have been suggestions that high-selenium onions could be a vehicle for increasing selenium levels within the human population since this would also provide health benefits (Goldman et al., 1999; Arnault and Auger, 2006). Onions also contain flavonoids from the anthocyanin (red, purple onions) and quercitins. These are not involved in flavour but contribute to health benefits (Griffiths et al., 2002).

5.5.2 Sugars in Allium flavour
Sweetness from the sugars that form a major part of the soluble solids in onion bulbs cannot be detected in strong onions unless the pungency is reduced through, for example, cooking. The monosaccharides glucose and fructose and disaccharide sucrose are present in addition to the storage carbohydrate fructan. Onions have traditionally been bred with a high dry matter content to obtain good storage qualities and accumulate fructans over the whole bulbing period although sweeter, low dry matter varieties also exist which rapidly cease accumulation of fructans as bulb development proceeds (Kahane et al., 2001). Levels of glucose appeared most closely correlated with genotype while fructose appears to be more affected by the environment in commercial long-day cultivars. However, analysis of mapping populations from crosses between high dry matter and sweet onions has shown a bimodal segregation of dry weight, reducing sugar and fructan content suggesting the effect of a major gene which may lie on chromosome 8 (McCallum et al., 2006). There is also substantial inter-bulb variation in sugar levels (Bedford, 1984; Hamilton et al., 1997).

5.5.3 Effects of environmental and cultivar factors
Sulphur supply has an impact on onion flavour and appears to affect MCSO, PCSO and PeCSO accumulation differentially (Randle et al., 2002). At low levels of sulphur there can be little correlation between levels of sulphur in the growth medium and flavour precursors within onion bulbs from many cultivars (Randle and Bussard, 1993a). At levels that cause symptoms of sulphur deficiency, the majority of available sulphur is channelled into CSOs and $\gamma$-glutamyl propenyl cysteine sulphoxide while at higher levels sulphur is used for additional compounds which may include sulphate (Randle et al., 1995, 1999).

CSO and $\gamma$-glutamyl peptides are also a sink for nitrogen and its supply has effects on biosynthesis (Coolong and Randle, 2003). Levels of $\gamma$-glutamyl propenyl cysteine sulphoxide are sensitive to low nitrogen availability, while the balance between MCSO and PeCSO alters so that MCSO is the major CSO at high nitrogen availability. Effects of sulphur and nitrogen nutrition have also been shown under field conditions (McCallum et al., 2005). Investigation of the genetic basis of these effects has indicated that expression of genes required for sulphate uptake and
assimilation alters in response to sulphur availability (McCallum et al., 2002). An additional soil factor that, perhaps unexpectedly, affects onion flavour is sodium chloride which onion tolerates at a higher level than most other vegetable crops. Glasshouse experiments showed that bulb pungency could be reduced significantly through exposure to NaCl at the early stages of bulbing (Chang and Randle, 2005).

Understanding changes in flavour during storage is important in onions and garlic which are stored for many months before use. During storage of garlic at low temperatures, there is marked conversion of the γ-glutamyl peptides to sulphoxides with consequences for flavour (Hughes et al., 2006; Ichikawa et al., 2006).

5.5.4 Sweet and mild onions
Low pungency onions, with less than 5 µmol pyruvate g fresh weight⁻¹ and a low dry matter content can command a price premium within a market sector for mild, sweet onions. At this level, sweetness from soluble solids within the bulb is no longer masked by the CSO cleavage products (Crowther et al., 2005). They are intended for use in salads, burgers and salsas where the fresh flavour of a mild onion should not overwhelm the other food flavours. This market has been established within the USA for several decades from locally grown onions in regions such as Vidalia (Georgia), Walla Walla (Washington) and Texas (e.g. ‘Vidalia’, www.vidaliaonion.org; others, www.sweetonionsource.com). Selection of appropriate varieties and generally low sulphur, well-irrigated soils is necessary to obtain consistent yields of low pungency onions (Randle, 1992). Sweet onions are also grown, particularly for export, in Chile (‘OSO Sweet’) and Hawaii (‘Maui’) and production is developing in Australia and New Zealand (e.g. www.sofresh.co.nz). However, probably due to the low CSO and higher water content, these onions frequently do not keep well, posing problems for export markets. Within the UK, a sweet onion that can be grown locally has been developed and marketed since 2003, with imports supplying the remaining part of the year (the ‘Supasweet’ onion, www.onions.org.uk/facts/supasweet.htm). The need for a rapid and reliable method of quality control for sweet onions has caused technical developments in pungency analysis (see Section 5.6.5 below).

5.6 The human dimension in vegetable flavour

5.6.1 An overview
The flavour and aroma of vegetables come from perception of a mixture of flavour compounds and textures. In cooked food the mixture will be even more complex, and is not considered here. Not all secondary metabolites contribute to flavour or aroma as perceived by humans; some may be below perception thresholds. This judgement can only be made using test panels through assessment of pure compounds complemented by evaluation of plant material to determine the con-
tribution of each component. This information will be needed to guide any efforts in plant breeding or genetic modification to improve flavour.

5.6.2 Human flavour perception

Genetics, physiology and psychology lead to our individual appreciation of the taste and aroma of food. The apparently simple problem of describing the flavour of vegetables is surrounded by the complexities of analytical chemistry and the psychology of taste perception. Variation between individual plants and people add to the difficulties. However, the plants selected for domestication as vegetables all have qualities that appeal to the palate of most people, indicating the flavours that we appreciate.

The development and launch expense of new commercial food products means that sensory and consumer evaluation are almost mandatory (Cooke et al., 2005). Taste panels can report on overall impressions and, in combination with analytical measures, can give leads to factors that can be manipulated to benefit both flavour and health qualities. However, the cost and time needed for trained taste panels or consumer research limits their use so that there are many more reports of evaluations of added-value processed foods than of raw vegetable materials. In addition, individuals differ in perception of flavours and aromas and in interpretation of the terms applied to their description. Factors such as the appearance of food, its newness or familiarity, the time of year, the surroundings, emotions and culture also play a part, allowing each person’s views on a flavour to be different on occasion (Köster, 2003). Members of taste panels, even after training, therefore continue to have fundamentally different experiences of tastes. To accommodate these human factors, multivariate statistical techniques, particularly principal component analysis (PCA) and generalised procrustes analysis (GPA) can be applied to estimate the contribution of each factor to flavour (e.g. Cooke et al., 2005; Seljäsen et al., 2001b). The development of objective analytical methods, validated against human assessments, is therefore the most practical way forward.

5.6.3 Objective methods of flavour assessment

There are several reasons for wanting objective measures of vegetable flavour and to identify the compounds that make the most significant contributions to flavour. Quality assurance within commercial production requires reproducible and rapid methods if flavour is to be part of the product specification. These are also needed to determine optimal storage and processing systems for flavour retention. If breeding programmes are to focus on flavour, high-throughput methods for analysis of breeding lines and the effects of environmental factors are needed. This has been realised for onions, where the determinants of flavour are simpler than in other vegetables. Several high-throughput systems have been described (Randle and Bussard, 1993b; Yoo and Pike, 1999). Sensitive and reproducible analysis of flavour compounds in garlic has also attracted considerable attention because of the pharmacological activity of several components and a commercial and regulatory need for quality assurance (e.g. Arnault et al., 2003).
In other vegetables, where contributions from several compounds which may be present at low concentrations are involved, determining the contribution of each to taste and the consequences of change is even more challenging. Static headspace analysis using gas chromatography can measure how volatile flavour compounds are partitioned between an aqueous solution and the gas phase above it. However, flavours change as each mouthful is savoured and this may be caused by mastication, saliva and or psychological effects. Dynamic analytical techniques can be used to monitor changes due to the vegetable material and eating process to give a time-intensity plot. Gas chromatography–olfactometry (GC–O) allows the human nose to detect and describe compounds above their olfactory threshold as they are separated by gas chromatography. For volatile flavours and aromas, mass spectrometry combined with soft ionisation techniques such as atmospheric pressure chemical ionisation (APCI) or proton transfer reactions have been developed to record rapid, subtle changes (Cooke et al., 2005). APCI–MS is popular because it causes little fragmentation of ions and gives simple spectra that are easy to interpret. One very interesting technical development is methodology to sample gases from within the nose, mouth or space above food to provide a rapid signal to match with perceptions from a trained flavour analyst (Taylor et al., 2000; Hodgson et al., 2003; www.flavometrix.co.uk).

### 5.6.4 Flavour analysis strategies

Most vegetables contain a large number of potential flavour compounds, both volatile and non-volatile, as well as sugars. The spectrum of tastes (bitter, salty, sour, sweet, umami) is much more limited than that of odours but the two combine in the gustatory experience. The levels of sugars in vegetables can be high, but sweetness is frequently masked by other flavours. It is very clear that humans prefer sweeter flavours, and development of vegetable varieties has been towards reduction in other flavours, especially bitterness (Drewnowski and Gomez-Carneros, 2000).

However, although measurement of individual flavour compounds has become increasingly sensitive, the compounds with the highest levels do not have to be the major contributors to flavour. Some are detected more sensitively by the human sensorium than by instruments, while the opposite is the case for others. Technical developments such as gas chromatography–mass spectrometry–olfactometry, in combination with suitable extraction and concentration methods, are providing an insight into human perception of the contribution of volatiles to flavour. Experimental designs to allow for differences in human perception and to optimise the performance of the sensory panel are an important part of these studies (e.g. Pollien et al., 1997). Development of an appropriate controlled vocabulary, training sessions for the panel members, and, ideally, control samples to anchor the panel’s perceptions, are also valuable for obtaining repeatable data (Engel et al., 2002).

Although strategies involving human perception are essential for exploring the relationship between flavour and vegetable chemistry, they are time consum-
ing and expensive. Faster, cheaper, in-field or in-store methods are needed based on validated perception–analysis relationships. In addition, there are quality assurance needs within the market for vegetables which may be solved by international acceptance of standard methods. There is interest in rapid methods to estimate GS. Near infra-red spectroscopy (NIRS) is used as a rapid and cost-effective analytical technique for many food products and has shown potential for measurement of both total and individual glucosinolates in cabbage leaves (Font et al., 2005). Biosensor technology is another rapid method, proven for in-home use in medical diagnostics for blood glucose, which also has considerable potential for the food industry (Terry et al., 2005). This method uses electrochemical principles with an integrated receptor-transducer incorporating a biological recognition element, such as an enzyme, to provide quantitative or semiquantitative information for a specific compound. There has been initial exploration of this technique for GS (Wu et al., 2005). However, the best examples of rapid approaches come from onions because of the value and international trade in this crop.

5.6.5 Flavour analysis of onion
The flavour volatiles in Alliums undergo further complex chemical transformations depending on atmospheric conditions after they are generated by the action of alliinase and LF synthase on ACSOs. Measurement of the lachrymatory factor thiopropanal-S-oxide using gas chromatography has been suggested (e.g. Kopsell et al., 2002; McCallum et al., 2005) but has not been widely adopted. The volatiles are not readily controlled and the focus therefore has been on the measurement of other, more stable, compounds as a proxy for flavour assessment. In onions, where products from cleavage of PrenCSO dominate, there is an international consensus that measurement of pyruvate is a reliable indicator of pungency, provided that appropriate quality control measures are taken (Randle, 1992; Havey et al., 2002). Small-scale comparisons between pyruvate levels and taste panels indicated a good correlation (Schwimmer and Gudagni, 1962; Bedford, 1984; Wall and Corgan, 1992) and a recent large-scale study has validated this approach over the full range of onion flavour (Crowther et al., 2005). The analytical method is generally based on the spectrophotometric Schwimmer and Weston (1961) protocol, although developments have included high-throughput strategies (Randle and Bussard, 1993b).

The commercial advantage of a rapid assay, especially for quality control of sweet onions, has led to development of a prototype disposable pyruvate biosensor (Abayomi et al., 2006). This used pyruvate oxidase mediated with meldolas blue and despite interference from other, unidentified, compounds in some cultivars, there was a strong correlation between the spectrophotometric and the biosensor responses using undiluted onion juice.
5.7 Future trends in vegetable flavour

5.7.1 Marketing and distribution
There are several important trends in the marketing of vegetables within the EU and USA. The decline of the wholesale market has been particularly conspicuous in the UK, so that the majority of sales are now directly between growers/packers/importers and the supermarkets or processors. A further trend involving small producers is the growth of ‘farmers’ markets’ with direct sales from producers to consumers. These closer relationships have led to new quality standards, especially if this generates a price premium. Flavour is difficult to appraise but the international adoption of pyruvate as a proxy for onion flavour is a good example of what can happen. This in turn creates a market for instrumentation to provide rapid, reliable and cost-effective quality control.

5.7.2 Organic vegetables
The organic vegetable market is a premium price sector that has grown by 20–30% annually within the last decade in both the EU and USA and is therefore an important trend. Organic production methods require that chemical herbicides, fungicides, insecticides and fertilisers are not used. This demands vegetable varieties with different performance characteristics from conventional agriculture. Although views are expressed that the flavour of vegetables from organic production systems is superior, many comparative studies of organic and conventional agriculture focusing on phytochemicals have flaws or limitations in their design (Zhao et al., 2006). Environmental factors affect secondary metabolite synthesis and organic methods may enhance their production since the vegetables are less protected from pathogens and stress. Indeed, it has been suggested that controlled post-harvest abiotic stresses could be used to improve vegetable quality (Cisneros-Zevallos, 2003). A study of nine prominent phenolics from lettuce, collards, and pac choi grown on adjacent organic and conventional plots only identified higher levels in organic pac choi which had been attacked by flea beetle (Young et al., 2005). This is an area where well-designed investigations of the effects of genetic and environmental factors on flavour would provide important information for both production systems.

5.7.3 Ready-prepared foods
The market for ready-prepared foods, both fresh, part and fully cooked, is a growth sector within western countries. Effects on flavour are particularly important in ready-prepared fresh products, since washing, peeling and chopping cause damage to the vegetables with accompanying changes to flavour and microbiological qualities. Increased knowledge of the ways low temperature storage, packing materials, modified atmospheres and antimicrobial treatments, as well as preparation methods, affect flavour would be valuable to optimise processing and storage strategies.
5.7.4 Public health initiatives
One current public health trend in the UK and USA is to encourage vegetable consumption because of health benefits through lower rates of cancer and coronary heart disease (e.g. WHO/FAO, 2002; Five-a-day, www.5aday.nhs.uk). Initiatives such as the European Nutrigenomics Organisation (NuGO) founded in 2004, aim to bring quantitation and systems biology to the measurement of human health in relation to nutrition, advancing from the observational approach of epidemiological studies and uncertainty of extrapolation from animal studies (www.nugo.org). These health benefits arise in part from secondary metabolites with anti-oxidant activity, although since the same benefits do not accrue from ingestion of tablets of antioxidants as from eating fruit and vegetables, the exact basis is complex (Melton, 2006). Clearer identification of individual, or mixtures, of plant compounds that provide health benefits is essential. Some beneficial compounds are flavourless but others contribute unwanted bitter flavours, notably phenolics and GS, producing a dilemma for the food industry (Drewnowski and Gomez-Carneros, 2000). If production systems aim to enhance the level of beneficial metabolites, this has to be done without compromising flavour, or the aim of increasing consumption by members of the public will not be achieved. Sensory testing must therefore accompany metabolite analysis to guide development of varieties or cultivation systems for products that are both palatable and may deliver health benefits.

5.7.5 Taste perception
A better idea of variability and sensitivity among consumers is important for setting realistic goals within both plant breeding and flavour measurement methodologies. Increased understanding of the genetics and receptor physiology of taste may give leads to new secondary metabolites or food additives that benefit flavour. An additive that masked bitter flavours could confer health benefits through making vegetables high in GS and phenolics more palatable.

5.7.6 Post-genomics and genetic manipulation
Current knowledge of the genetic basis of most flavour and aroma volatiles is limited and has been mostly conducted in model systems, fruits and flowers (see Chapter 13 this volume). Crop science will nevertheless make use of post-genomic technologies following insights into plant physiology from Arabidopsis thaliana and other model systems. The substantial recent progress in understanding GS metabolism and its control in A. thaliana have allowed engineering of new GS profiles and is an early indication of how flavour may eventually be manipulated (Grubb and Abel, 2006). Sweetness is a very important factor in the acceptability of all vegetables, and a greater understanding of the factors governing it will give targets for flavour improvements. The recent identification of a QTL relevant to onion sweetness is one example and a direct outcome of the onion gene map (McCallum et al., 2006).
Although aspects of secondary metabolism are species specific, insights from model systems, especially into the control of carbon flux will define principles that can be extended to crops. Application may be the most rapid within Brassicas where a Brassica genome project is under way (http://brassica.bbsrc.ac.uk/), but will also occur in other vegetable crops. Post-synthesis subcellular localisation and transport of all plant volatiles are neglected areas but important to flavour, where greater knowledge from model systems will eventually feed into crop improvement. Advances in metabolomics will provide the analytic technology to match secondary metabolite profiles to genomics.

In the short term, information is likely to be used to gain a clearer idea of the genetic basis of current quality traits, and for improvements through marker-assisted breeding and QTL analysis. This will probably be required in any case before genetic modification could be contemplated to complex traits like flavour. Technical difficulties with genetic modification in some species, as well as consumer resistance to genetically modified crops may slow the use of genetic modification in Europe and directed breeding may turn out to be the most effective approach. The increasing through-put and decreasing cost of genomic technology with its associated data analysis and curation will make approaches that are currently only feasible for model systems realistic for vegetable crops in the future.

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Fruit and vegetable flavour


6

Postharvest flavor deployment and degradation in fruits and vegetables

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6.1 Introduction

The development, decline and alteration of flavor during the postharvest life of fresh fruits and vegetables constitute a very broad topic, particularly if one includes the rapidly expanding area of fresh-cut or minimally processed produce. After beginning with a brief overview of postharvest physiology and technology and how they relate to changes in flavor, the remainder of this chapter is focused rather narrowly on the following three subjects: 1. flavor changes with fruit ripening, 2. the influence of storage conditions and prestorage treatments, and 3. the influence of ethylene and its recently discovered antagonist 1-methylcyclopropene (1-MCP) on flavor and texture. A large segment of the chapter is devoted to the first subject, and more specifically to gene expression and enzymes involved in aroma volatile production in ripening fruits. In recent years, use of molecular genetic tools has resulted in rapid and exciting progress in this area. Coverage of the second subject is centered on the effects of refrigeration, controlled or modified atmospheres, and prestorage treatments administered to retard ripening and senescence or to kill insect pests that restrict fruit exports. This section focuses mainly, but not exclusively, on the negative impact these technologies can have on flavor and aroma, a topic more thoroughly explored by Porat and Fallik in Chapter 8 (this volume). The final section deals briefly with the crucial roles of ethylene in postharvest ripening and senescence, and the rapidly increasing utilization of 1-MCP treatments to delay deterioration of fresh produce and extend storage life.
6.2  Postharvest physiology and technology: an overview

In a Utopia, fruits and vegetables would always be grown under optimal conditions, harvested at the peak of ripening or maturity, and consumed within a few days to ensure full flavor and nutritional value. Clearly such a scenario is not practical, particularly in light of the trend toward very large regional horticultural concerns and the requirement to ship fresh produce to transcontinental and, more recently, global markets. This reality has necessitated development of postharvest technologies to preserve the quality and salability of fruits and vegetables well beyond their normal shelf life. Over the years this research has focused largely on maintaining appearance and texture, with relatively little consideration of flavor and aroma. As a result, the storage and shelf life of many horticultural crops often exceeds their flavor life. An added dimension of this problem is that breeding programs have generally sought to enhance attributes required to endure shipping and long-term storage, or that favor visual appeal (e.g. bright color and large size), sometimes at the expense of flavor and aroma (Goff and Klee, 2006; Tieman et al., 2006). Finally, the advent of fresh-cut fruits and vegetables as a consumer-driven, multi-billion-dollar arm of the fresh produce industry has posed new challenges for food technologists and postharvest biologists to deliver safe, wholesome, flavorful products with maximum shelf life (Watada and Qi, 1999).

Harvested fruits and vegetables are living, actively respiring organs that have been removed from their source of water and nutrients. Inherently, these typically fleshy or leafy commodities are destined to deteriorate as a consequence of natural or stress-induced senescence, and/or decay by postharvest fungal or bacterial pathogens. The first line of defense against postharvest deterioration is to limit the rates of respiration and water loss. Generally this is accomplished by shipping and storage under conditions of low temperature and high humidity. Another commonly used strategy is to place the produce under controlled or modified atmospheres including low O₂ and, in some cases, elevated CO₂ concentrations. Although these technologies have long been employed as dependable postharvest measures to extend storage life, the specific conditions of temperature, humidity and levels of O₂ and CO₂ must be assessed for each commodity, and often for individual cultivars (such as with apples). The tolerances for low temperature, low O₂, and high CO₂ are highly variable (Wang, 1990; Beaudry, 1999). Many fruits and vegetables of tropical or subtropical origin are susceptible to chilling injury at temperatures ranging from roughly 0–15 °C, levels of O₂ below a certain threshold elicit anaerobic respiration, and elevated CO₂ can induce physiological disorders in sensitive commodities. The net result with each of these problems is accelerated deterioration of quality, including altered metabolism that leads to flavor loss or development of off flavor and aroma.

The rate of postharvest senescence is also quite variable among fruits and vegetables. For example, pome fruits can generally be stored for three months or more, whereas broccoli and asparagus last a few weeks under optimal conditions, and fresh-cut products typically have a shelf life of only seven to ten days. Aside from the negative impact of water loss, the gaseous hormone ethylene often plays
a central role in triggering and driving senescence of fresh and fresh-cut produce. Ethylene also has a key regulatory role in postharvest ripening of climacteric fruits (e.g. apple, banana, tomato), eliciting desirable changes in flavor and aroma via activation of the required metabolic pathways at the level of gene transcription and translation. Availability of the new inhibitor of ethylene action, 1-MCP, promises to be one of the most important developments in postharvest technology for years to come, and has already had a major impact on the fresh apple industry (Watkins, 2006). Although 1-MCP treatment is quite effective in extending the storage life and maintaining the firmness and overall quality of apples and other commodities, a common downside is marked suppression of aroma volatile production.

6.3 Changes with fruit ripening

6.3.1 Climacteric and non-climacteric fruits

Fruits are grouped in two categories, climacteric and non-climacteric, depending on their physiological regime during ripening. Climacteric fruits (e.g. banana, tomato, mango and apple) exhibit a distinct peak of respiratory activity during ripening, the respiratory climacteric, whereas non-climacteric fruit (e.g. strawberry, grape, orange and cherry) show a slow decline in respiration over the course of ripening. Interestingly, the melon species *Cucumis melo* includes both climacteric (e.g. cantaloupe) and non-climacteric (e.g. honeydew) fruit types. Sugars and organic acids are the principal respiratory substrates in fruit tissues, and accumulation of these classes of compounds in excess of what is required for postharvest respiration is also a major factor determining fruit flavor (see Chapter 10 this volume). The magnitude of the respiratory climacteric is highly variable among fruits and is generally inversely correlated with shelf life (Tucker, 1993). In non-climacteric fruits, despite the absence of a respiratory increase with ripening, a relatively high basal rate of respiration usually equates with a short shelf life. The transient rise in respiration in climacteric fruit is associated with a more or less concurrent burst of autocatalytic ethylene production, whereas ethylene levels remain low during ripening of non-climacteric fruits. Ethylene regulates many important processes and metabolic changes associated with climacteric ripening (Brady, 1987). In contrast, ethylene is not required for ripening of non-climacteric fruits, although it can influence certain late elements of the regime, such as degreening and carotenogenesis in citrus fruits (Stewart and Wheaton, 1972; Rodrigo and Zacarias, 2007).

With respect to postharvest handling and strategy, climacteric and non-climacteric fruits differ considerably, inasmuch as the former will complete ripening after harvest, particularly when treated with exogenous ethylene, whereas the latter will not ripen further when removed from the plant. For both types of fruit, it is generally advantageous to delay harvest as long as is feasible to optimize sugar content and color development (see Chapter 10 this volume). However, storage life of climacteric fruits is maximized by harvesting when fully mature but still in the
pre-climacteric state. Consequently, in the commercial situation many of the desirable changes in flavor and aroma occur after harvest. With non-climacteric fruits, on the other hand, care must be taken not to harvest when over-ripe or senescent. The postharvest objective is to maintain the fruit in a fresh-like state as long as possible, delaying flavor loss or development of off flavors and aroma.

6.3.2 Aroma development during fruit ripening

Many fruits produce in excess of 100 volatile aroma compounds, although generally a relatively small percentage of these are considered to be major contributors to the unique flavor of a particular fruit (Baldwin, 2004; Goff and Klee, 2006). As elaborated elsewhere in this volume (Chapters 4, 13 and 14), aroma volatiles in ripe fruit are derived primarily from metabolites of fatty acids, amino acids and carbohydrates, with usually minor but often important additional components coming from the shikimate, phenylpropanoid, and isopentenoid pathways. Increasingly during the past decade, the tools of molecular biology have enabled rapid progress in elucidating and characterizing a number of the key genes and enzymes involved in generation of fruit aroma volatiles. These include, but are certainly not limited to, lipoxygenases (LOXs), hydroperoxide lyases (HPLs), alcohol acyl-transferases (AATs), alcohol dehydrogenases (ADHs), terpene synthases (TPSs) and carotenoid cleavage dioxygenases (CCDs).

With varying degrees of substrate and product specificity, the sequential action of LOX and HPL on linoleic and linolenic acids yields C6 and/or C9 saturated or unsaturated aliphatic aldehydes (Feussner and Wasternack, 2002; Matsui, 2006). These can then be reduced by ADH to the corresponding alcohols, which along with many other alcohols and an equally broad array of organic acids serve as substrates for AAT in the formation of esters that confer characteristic fruity or floral notes (see Chapters 4 and 13 this volume). The TPSs of major interest with respect to fruit aroma are mono- and sesquiterpene synthases that yield 10- and 15-carbon isopentenoids, respectively, from the corresponding geranyldiphosphate (GDP) and farnesylphosphate (FDP) precursors (Aharoni et al., 2006). Finally, CCDs utilize different carotenoid substrates to generate (usually) C9 to C13 ketones or aldehydes, referred to as norisoprenoids or apocarotenoids, which can have a major impact on fruit flavor (Leffingwell, 2001; Simkin et al., 2004).

Lipoxygenase (LOX) and hydroperoxide lyase (HPL) metabolism of linoleic and linolenic acid

In-depth studies of LOX and HPL activities and localization during fruit ripening have been conducted with climacteric tomato (Riley et al., 1996; Riley and Thompson, 1997, 1998; Yilmaz et al., 2002) and non-climacteric strawberry (Pérez et al., 1999; Leone et al., 2006). As observed in numerous other investigations, cutting or maceration of the tissue greatly increased LOX/HPL-mediated generation of C6 volatiles. For both fruits it was shown that linolenic acid (18:3) was the preferred LOX substrate and HPL activity was rate-limiting in the generation of C6 aldehydes. LOX and HPL activities from strawberry were mostly...
membrane or lipid-protein particle associated, whereas tomato LOX was mainly soluble and HPL predominantly microsomal. Curiously, Leone et al. (2006) found that both LOX and HPL activities declined substantially with strawberry ripening, yet production of hexanal increased dramatically in ripe fruit (also shown by Pérez et al., 1999), suggesting the involvement of non-enzymatic lipid peroxidation. Bruising of red ripe strawberries resulted in rapid increases in LOX and HPL activity, and emission of (E)-2- plus (Z)-3-hexenal, followed by evolution of downstream alcohol and ester products of aldehydes from the LOX/HPL pathway (Hamilton-Kemp et al., 2003; Myung et al., 2006). In tomato fruit, Riley et al. (1996) found that microsomal LOX activity increased between the mature-green and breaker stages, declining thereafter, while HPL activity changed little during ripening. LOX and HPL activities were assayed in stored fruit of 12 tomato cultivars at the green, pink, and red stages by Yilmaz et al. (2001, 2002), who found that both enzyme activities peaked at the pink stage, were highly variable among cultivars, and were not influenced by the stage of ripening at harvest. As noted for strawberry, these results were not entirely in accord with C6 aldehyde production, which in tomato was about four- to five-fold greater in macerates from red compared with green fruit (Riley and Thompson, 1998).

An explanation for this apparent incongruity has come from work characterizing LOX genes and their expression in tomato fruit. It is now known that plants include families of LOX genes with a broad range of physiological functions (Feussner and Wasternack, 2002; Liavonchanka and Feussner, 2006), and there are five LOX genes, designated Tomlox(A–E), with varying patterns of expression in ripening tomatoes (Chen et al., 2004). TomloxC, one of two Tomlox genes with a chloroplast targeting sequence, was shown to be strongly up-regulated between the mature green and turning stages under the control of ethylene plus an unknown developmental factor (Griffiths et al., 1999). It was subsequently demonstrated in a study employing Tomlox antisense and sense transgenic tomato lines that the product of TomloxC is almost entirely responsible for generation of C6 aldehydes and alcohols in macerated fruit tissue, and is in fact localized in chloroplasts (Chen et al., 2004). Surprisingly, there has been little work on HPL gene expression in tomato fruit. One complete HPL cDNA was cloned using RNA from immature fruit and the recombinant cytochrome P450 enzyme was characterized, showing a substrate preference for 13S-hydroperoxylinolenic acid (13S-HPOD) over 13S-hydroperoxylinolenic acid (13S-HPOD), and little activity with 9S-HPOD or 9S-HPOD (Matsui et al., 2000). Expression of this HPL gene during fruit ripening was not examined, but the encoded enzyme is nearly identical to an HPL cloned using tomato leaf RNA (CYP74B3), which has been studied in greater detail. The bacterially expressed leaf HPL (LeHPL) also strongly preferred 13S-HPOD as substrate (Howe et al., 2000), and it was shown that, despite the lack of a chloroplast targeting sequence, native LeHPL was integrated into the outer chloroplast envelope membrane with most of the protein located on the inner membrane surface (Froehlich et al., 2001). Taken together, these studies support a proposed scheme wherein tomato fruit LOX and HPL isozymes co-localized in chloroplasts are provided with 18:3 and 18:2 substrates derived from lipolysis in...
thylakoid membranes during the ripening-associated transformation to chromoplasts. It is interesting to note that, in a study of red ripe tomatoes of three cultivars stored for up to 21 days at 20 °C (simulating market or household conditions), hexanal and several other important aroma volatiles uniformly increased postharvest, whereas (Z)-3-hexenal tended to decline (Krumbein et al., 2004). Moreover, hexanal, along with 2-isobutylthiazole, was related to the undesired attribute ‘mouldy’, which increased in intensity during storage.

Concerning the roles of LOX and HPL genes and enzymes in aroma volatile production in other fruits, there is currently little information. Complete LOX cDNAs have recently been reported for fruit of strawberry (GenBank accession number AJ578035), honeydew melon (DQ267934) and kiwifruit (DQ497792 and DQ497797). The only report pertaining to these showed that CmLOX1 is expressed at low levels in mesocarp tissues of ‘Honey Brew’ hybrid honeydew melons, attaining maximum transcript abundance at about the time of harvest (Whitaker and Lester, 2006). Although the amino acid sequence identifies the encoded CmLOX1 protein as a 13S-LOX, it is 93% identical to a cucumber root LOX that generates both 13S and 9S products, and also can act on phospholipid substrates. A 98-kDa LOX protein from olive fruit was recently purified to homogeneity and biochemically characterized (Lorenzi et al., 2006). The olive LOX was found to be more active with 18:2 than with 18:3 and yielded 13S-HPOD from 18:2, consistent with C6 aldehydes being important, desirable aroma volatiles in virgin olive oils (Angerosa et al., 2004). Hot water treatments of olive fruit to reduce oil bitterness altered the aroma volatile profile, reducing levels of C6 aldehydes apparently as a result of partial inhibition of LOX plus HPL activities (Pérez et al., 2003). HPL genes have been cloned from fruit of bell pepper (Matsui et al., 1996), guava (Tijet et al., 2000) and cantaloupe melon (Tijet et al., 2001). In each case the RNA used was isolated from immature or unripe fruit, and there was no analysis of gene expression over the course of fruit ripening. The recombinant HPL enzyme from bell pepper was membrane associated and cleaved 13S-HPOD releasing hexanal, whereas the guava HPL expressed in E. coli showed ten-fold higher activity with 13S-HPOT than with 13S-HPOD or with either 9S hydroperoxide, in accordance with the report of (Z)-3-hexenol and (Z)-3-hexenyl acetate as major aroma volatiles from guava fruit (Vernin et al., 1991). In contrast with the bell pepper and guava HPLs, the recombinant enzyme from cantaloupe melon showed a substrate preference of 9S-HPOT over 9S-HPOD, and also had moderate activity with the 13S hydroperoxides. An HPL purified from cucumber fruit also preferred 9S over 13S substrates, although it was somewhat more efficient with 9S-HPOD compared with 9S-HPOT (Hornostaj and Robinson, 1999). C9 aldehydes are important components of melon and cucumber fruit aroma (Schieberle et al., 1990), and a recent study showed that 9-HPL activity increases in cucumbers to full maturity, while 13-HPL activity declined throughout development and was 2.5-fold lower than that of 9-HPL in mature fruit (Matsui et al., 2006).
Interconversion of aldehydes and alcohols by alcohol dehydrogenase (ADH)

Alcohol dehydrogenases (ADHs) contribute to the biosynthesis of fruit aroma volatiles via interconversion of aldehydes and alcohols, the latter also serving as substrates for production of esters by alcohol acyltransferases (AATs). ADH genes have been cloned from fruit of apple (GenBank accession number Z48234), grape (AF194173, AF194174, AF194175), grapefruit (DQ083539), muskmelon (DQ288986, DQ288987), pear (AF031899, AF031900) and tomato (X77233), yet there are few reports of changes in ADH gene expression during fruit ripening and storage. Over a decade ago it was shown that the ADH2 gene is strongly up-regulated during tomato fruit ripening, particularly at the later stages, and the level of transcript increased a remarkable 100-fold within 8–16 hours of hypoxia under a 3% O₂ atmosphere (Chen and Chase, 1993; Longhurst et al., 1994). Suppression or over-expression of ADH2 in fruit of tomato plants transformed with constructs of the cDNA driven by the CaMV 35S or polygalacturonase promoter resulted in an appreciable decrease or increase, respectively, in the levels of hexanol and (Z)-3-hexenol, without affecting levels of the corresponding aldehydes (Speirs et al., 1998). In preliminary taste trials, transgenic fruit with increased ADH activity were deemed to have a more intense ripe fruit flavor. By contrast with tomato, in ‘Granny Smith’ apple fruit steady-state levels of ADH mRNA decreased during postharvest ripening (Reid et al., 1996). A less than twofold increase in ADH gene expression occurred during 0.5 °C storage in air, or with high CO₂ (20%) or ethanol vapor (1 mL L⁻¹), and a ninefold increase or less was induced by ultra-low oxygen (0.3%) and CO₂ (0.02%) CA storage. Recently, Defilippi et al. (2005a,b) determined that neither ADH gene expression nor production of alcohols in ripening apple fruit is regulated by ethylene. Similarly, Mita et al. (2006) found that an ADH gene (Pm38) was up-regulated during ripening of Japanese apricot (Prunus mume), but its expression was not altered in response to ethylene or wounding. Storage of ‘Bartlett’ pears under 0.25% O₂ CA at 20 °C increased the levels of ethanol and ADH activity, and the latter remained elevated during subsequent ripening in air (Ke et al., 1994). Comparison of ADH gene expression and enzyme activity in ‘Packham’s Triumph’ pears ripened after two months of −1 °C storage in air or 3% O₂ CA (hypoxia) showed that, although a post-storage increase in ADH expression was greater in air-stored fruit, ADH activity remained about twofold higher in CA-stored fruit, and there was little difference between air- and CA-stored pears in accumulation of ethanol, methanol and acetaldehyde (Chervin et al., 1999). Three full-length ADH cDNAs were cloned from grape (Vitis vinifera), and their patterns of expression during berry development were determined (Tesnière and Verriès, 2000). Transcript of VvAdh2 increased sharply after véraison and accounted for the bulk of the ADH activity in ripening fruit. Although grapes are non-climacteric, Tesnière et al. (2004) showed that treatment with the blocker of ethylene action 1-MCP inhibited the ripening-associated increase in ADH activity. Manríquez et al. (2006) recently demonstrated that fruit-specific expression of two highly divergent ADH genes (Cm-ADH1 and Cm-ADH2) is likely to play a key role in the regulation of aroma production in ‘Charentais’ cantaloupe melons. Maximum transcript levels of both genes coincided with the
peak of the ethylene and respiratory climacteric. Moreover, Cm-ADH1 and Cm-ADH2 expression was greatly reduced in ACO antisense-suppressed and 1-MCP-treated fruit. The yeast-expressed recombinant enzymes from both cDNAs showed much greater activity as reductases, with a marked preference for aliphatic aldehyde substrates, although Cm-ADH1 was also able to reduce several branched aldehydes.

Role of acyltransferase (AAT) in the formation of esters
Progress on the cloning and characterization of alcohol acyltransferases (AATs) from ripening fruit has been rapid since the first report of an AAT gene (SAAT) involved in biogenesis of strawberry flavor (Aharoni et al., 2000). SAAT expression was specific to the fruit receptacle, increasing sharply at the white stage and remaining high at the red and dark red stages of ripening. The bacterially expressed SAAT enzyme used a broad range of substrates and was capable of generating all major esters among strawberry fruit volatiles. Substrate availability and the temporal expression of SAAT are thought to regulate fruit ester biosynthesis. Subsequent to cloning of SAAT, studies of AAT gene expression in relation to fruit ripening and aroma production have been reported for cantaloupe melon (Yahyaoui et al., 2002; El-Sharkawy et al., 2005), apple (Defilippi et al., 2005b; Souleyre et al., 2005; Li et al., 2006), banana (Beekwilder et al., 2004), Concord grape (Wang and De Luca, 2005) and Japanese apricot (Mita et al., 2006). A family of four AAT genes was cloned from ‘Charentais’ melon, three of which (Cm-AAT1, Cm-AAT3 and Cm-AAT4) yielded active enzymes with distinct substrate specificities when expressed in yeast (Yahyaoui et al., 2002; El-Sharkawy et al., 2005). Activity of recombinant Cm-AAT2 was restored by mutating 268-alanine to 268-threonine and Cm-AAT1 was inactivated by mutating 268-T into 268-A. All four Cm-AAT genes were strongly up-regulated during ripening, beginning in the early climacteric phase. Experiments with ACO antisense suppressed melons and normal fruit treated with 1-MCP or ethylene showed that expression of the four genes is positively regulated by ethylene. The multiple, functionally diverse Cm-AAT enzymes in ‘Charentais’ melon can account for the great diversity of esters produced. In apple expression of AAT genes is not usually restricted to fruit (Souleyre et al., 2005; Park et al., 2006), but Li et al. (2006) recently reported that MdAAT2 from ‘Golden Delicious’ is fruit-specific, and MdAAT2 protein is localized mainly in the peel tissue. As well, the highest level of MpAAT1 transcript was in the skin of ripening ‘Royal Gala’ apples (Souleyre et al., 2005). Expression of AAT2 during postharvest ripening of ‘Greensleeves’ apples was tightly regulated by ethylene (Defilippi et al., 2005b). Production of aldehydes and alcohols in fruit of ACO-silenced transgenic ‘Greensleeves’ was close to normal, whereas ester biosynthesis was markedly reduced, indicating the requirement for ethylene-induced AAT2 expression. Similarly, transcription and translation of MdAAT2 were depressed by 1-MCP treatment of ‘Golden Delicious’ fruit, and ester production was inhibited (Li et al., 2006). In banana fruit as well, 1-MCP treatment substantially increased accumulation of alcohols and inhibited production of the related esters (Golding et al., 1999). Recombinant MpAAT1 from ‘Royal Gala’
apple could use a broad range of substrates, but preference varied with concentration suggesting that substrate levels could be a key factor determining the distinct aroma profile (Souleyre et al., 2005). ‘Golden Delicious’ tissue disk assays with added alcohols also indicated that apple ester production largely depends on available substrates (Li et al., 2006). Using differential display analysis of genes expressed in pericarp tissue of Japanese apricot, Mita et al. (2006) identified an AAT gene, Pm94, which was up-regulated during ripening and in response to ethylene or wounding. Wang and De Luca (2005) showed that an unusual AAT enzyme (AMAT) capable of using methanol and anthranilic acid as substrates accumulates to high levels in outer mesocarp tissue of ripening Concord grape (Vitis labrusca) berries. The AMAT gene was not expressed in V. vinifera fruit, which do not produce the methylantranilate ester characteristic of Concord grape aroma. AMAT transcript and protein peaked in Concord grape berries during the last stage of ripening, coincident with maximum accumulation of anthranilic acid and methylantranilate. Other studies with fruit AATs have indicated that substrate specificity does not usually determine which esters are produced. Bacterially expressed AATs from wild strawberry (Fragaria vesca) and banana (Musa sapientum) were tested for substrate specificity, and for neither enzyme did the results reflect the characteristic ester profile from ripening fruit (Beekwilder et al., 2004). Expression of SAAT in transgenic petunia did not alter the volatile profile and supplying exogenous isoamyl alcohol resulted in production of isoamyl acetate. It was concluded that the ester profile of fruits is determined to a large extent by the abundance of alcohol and acyl-CoA precursors.

Terpenoid volatiles derived from terpene synthases (TPSs) and carotenoid cleavage dioxygenases (CCDs)

Study of the genes and enzymes involved in biosynthesis of monoterpenes, sesquiterpenes and norisoprenoids (apocarotenoids) in ripening fruit is still more or less in its infancy, in part because of the great diversity and complexity of these compounds. Generally, mono- and sesquiterpene volatiles appear to be important contributors to the aroma profiles of non-climacteric rather than climacteric fruits, the most obvious example being citrus fruits, which owe much of their distinctive aromas to these terpenes (Lücker et al., 2002; Sharon-Asa et al., 2003). However, in terms of relevance to postharvest changes in flavor and aroma, there is evidence that synthesis of some impact compounds is stimulated by ethylene (Tomás et al., 1993; Sharon-Asa et al., 2003), terpenoids can be major contributors to the flavor of fruit juices and wines (Maccarone et al., 1998; Martin and Bohlmann, 2004), and mono- and sesquiterpene alcohols are often sequestered as glycosides that can release aroma volatiles when cleaved by endogenous or exogenous glycosidases (Hasegawa et al., 1989; Lund and Bohlmann, 2006). One climacteric fruit in which terpenes are dominant flavor and aroma compounds is mango (MacLeod and Pieris, 1984). Monoterpenes in combination with smaller amounts of sesquiterpenes composed 80% or more of the total volatiles produced by ripening ‘Kensington Pride’ mango fruit, and significant changes in the terpene profile occurred over the course of ripening (Lalel et al., 2003a). Peak evolution of most monoterpenes
coincided with the crest of the climacteric, whereas maximum production of the major monoterpane, α-terpinolene, as well as the major sesquiterpane, germacrene D, occurred three to four days later in fully ripe fruit.

Terpene synthase genes with a role in aroma production in ripening fruits have been cloned from ‘Valencia’ orange (Sharon-Asa et al., 2003), lemon (Lücker et al., 2002), wild and cultivated strawberry (Aharoni et al., 2004), grape (Lücker et al., 2004; Martin and Bohlmann, 2004), apple (Pechous and Whitaker, 2004) and pear (Gapper et al., 2006). The sesquiterpene synthase gene Cstsps1 from ‘Valencia’ orange was expressed in bacteria and the recombinant enzyme yielded only one product from FDP, the flavor impact compound valencene (Sharon-Asa et al., 2003). Cstsps1 transcript increased sharply during fruit maturation, coincident with valencene accumulation, and both valencene production and Cstsps1 expression were stimulated by ethylene. Aharoni et al. (2004) rigorously established the molecular genetic basis of the profoundly different terpene aroma profiles in ripe fruit of cultivated and wild strawberry species. Briefly stated, they found that the truncated FaNES gene is abundantly expressed during ripening of cultivated Fragaria ananassa but not wild F. vesca and accounts for production of the mono- and sesquiterpene alcohols linalool and nerolidol in the cultivated species. Conversely, FvPINS is abundantly expressed in ripening F. vesca fruit, whereas the counterpart in F. ananassa was knocked out by an insertional mutation. FvPINS encodes a monoterpane synthase that determines the terpene profile of wild strawberry fruit, which includes α-pinene and a few other monoterpenes, as well as the downstream products of α-pinene, myrtenol and myrtenylacetate. Flavor and aroma of some Vitis vinifera grape varieties includes volatile terpenes as dominant components. Monoterpenes contribute to grape and wine aroma and flavor both as free volatiles and as glycosides of monoterpene alcohols. Two V. vinifera cDNAs were identified as (–)-α-terpineol synthases (Martin and Bohlmann, 2004). Their recombinant enzymes produced mainly (–)-α-terpineol from geranyldiphosphate (GDP), but also a few other monoterpenes including (–)-α-pinene, (++)-α-pinene, and 1,8-cineole. Two sesquiterpene synthases have also been cloned from V. vinifera, including VvVal, a (+)-valencene synthase (Lücker et al., 2004). Recombinant VvVal enzyme yielded (+)-valencene and lesser amounts of (–)-7-epi-α-selinene from FDP. VvVal transcript increased sharply in the final stage of grape berry ripening. In the climacteric pome fruits apple and pear, many cultivars accumulate high concentrations of the sesquiterpene (E,E)-α-farnesene in the peel tissue during low temperature storage. AFS1, the gene encoding apple (E,E)-α-farnesene synthase, was cloned using peel tissue RNA of cold-stored ‘Law Rome’ apples, and the bacterially expressed enzyme yielded (E,E)-α-farnesene as the predominant product of FDP (Pechous and Whitaker, 2004). The analogous and nearly identical gene PcAFS1 was subsequently cloned using peel tissue RNA from ‘d’Anjou’ pears (Gapper et al., 2006). Both AFS1 and PcAFS1 are strongly up-regulated by ethylene during the first few weeks of cold storage, followed closely by accumulation of α-farnesene in the peel tissue (Pechous et al., 2005; Gapper et al., 2006). Pre-storage treatment with 1-MCP blocked the induction of AFS1 and PcAFS1 expression, and consequently the
accumulation of α-farnesene, for several months. The ability to genetically engineer monoterpenoid production and consequently alter fruit aroma in tomato was demonstrated by Lewinsohn et al. (2001). Specific addition of S-linalool and 8-hydroxylinalool to the aroma profile of ripening tomato fruit was accomplished by transforming tomato plants with a Clarkia breweri S-linalool synthase gene driven by the fruit-specific E8 promoter. The future potential for metabolic engineering of mono- and sesquiterpenoids in plants was the subject of a recent review by Aharoni et al. (2006).

Whereas mono- and sesquiterpenes are produced by an array of synthases and cyclases that use GDP and/or FDP as substrate, norisoprenoids are products of oxidative cleavage of carotenoids and consequently their cyclic or acyclic structures depend on the carotenoids from which they are derived, as well as the site of cleavage. Also called apocarotenoids, these terpenoids are important flavor and aroma constituents in numerous fruits and berries, including apricot, cantaloupe, carambola, grape, grapefruit, kiwi, mango, passionfruit, tomato, blackberry and raspberry (Leffingwell, 2001). It has long been known that oxidative cleavage of carotenoids yielding flavor compounds is at least partly an enzymatic process, but only recently have researchers begun to elucidate the carotenoid cleavage dioxygenase (CCD) genes and enzymes involved in generation of norisoprenoids (Lewinsohn et al., 2005a,b). CCD enzymes have been partially purified from peel tissue of climacteric quince (Cydonia oblonga) and non-climacteric carambola (Averrhoa carambola) fruits (Fleishmann et al., 2002, 2003). For both quince and carambola, CCD activity with β-carotene as substrate was detected in proteins isolated from ripe but not from unripe fruit, and there was evidence of multiple isozymes. Cleavage of β-carotene by the partially purified CCDs from carambola yielded β-ionone, a major norisoprenoid aroma impact volatile produced in vivo by ripened fruit (Fleishmann et al., 2003). Genes encoding CCDs have been cloned from three Citrus species (Kato et al., 2006), V. vinifera grape (Mathieu et al., 2005), tomato (Simkin et al., 2004), and melon (Cucumis melo) (Ibdah et al., 2006). Nearly identical CitCCD1 genes were isolated from ‘Satsuma’ mandarin (Citrus unshiu), ‘Valencia’ orange (C. sinensis), and lemon (C. limon), and their expression in flavedo and juice sac tissues was shown to increase during the late maturation of each fruit (Kato et al., 2006). The encoded CitCCD1 proteins did not include a plastid targeting sequence, and the recombinant enzymes showed broad substrate specificity for β,β-xanthophylls (e.g. zeaxanthin), performing symmetrical cleavage at the 9,10 (9',10') position. Mahattanatawee et al. (2005) identified four norisoprenoids, α-ionone, β-ionone, β-cyclocitral and α-damascenone, as aroma impact compounds in ‘Valencia’ orange juice, together accounting for close to 80% of the floral aroma. The putative carotenoid precursors α- and β-carotene, α- and β-cryptoxanthin and neoxanthin were also detected in the juice. A full-length CCD cDNA, VvCCD1, was cloned from V. vinifera and bacterially expressed (Mathieu et al., 2005). VvCCD1 recombinant enzyme cleaved zeaxanthin symmetrically, yielding 3-hydroxy-β-ionone and a C₁₄-dialdehyde. Induction of VvCCD1 expression occurred at véraison and increasing transcript levels paralleled accumulation of C₁₃-norisoprenoids from véraison to full maturity. As noted
for mono- and sesquiterpene alcohols, hydroxylated norisoprenoids often accumulate as glycoside conjugates during fruit maturation and ripening (Aubert et al., 2003a; Lund and Bohlmann, 2006). Two CCD genes, LeCCD1A and LeCCD1B, were cloned from tomato and it was shown that LeCCD1B transcript increased substantially during fruit ripening, reaching very high levels by the orange stage (Simkin et al., 2004). LeCCD1A and LeCCD1B exhibited broad substrate specificity in both in vivo assays, within E. coli cells engineered to accumulate specific carotenoids, and in vitro assays with recombinant enzymes. The reaction products indicated symmetrical cleavage yielding a C$_{14}$ dialdehyde and a C$_{13}$ cyclohexanone. Over-expression of an antisense LeCCD1B construct reduced mRNA of both genes by about 90% in leaves and fruits, with no effects on plant phenotype. Production of the norisoprenoids β-ionone and geranylacetone in fruit of the antisense transgenics was reduced by 50% or more, indicating a likely role of the LeCCD1 genes in formation of these flavor volatiles. Cloning and functional characterization of a single CCD gene from muskmelon (Cucumis melo), CmCCD1, was recently reported by Ibdah et al. (2006), and the results were quite similar to those with tomato LeCCD1A and LeCCD1B. Specifically, expression of CmCCD1 in E. coli cells engineered to produce single carotenoids showed 9,10 and 9',10' cleavage, yielding geranylacetone from phytoene, pseudoionone from lycopene, β-ionone from β-carotene, and α-ionone plus pseudoionone from δ-carotene. Expression of CmCCD1 in pale green-fleshed (var. inodorus) and orange-fleshed (var. reticulatus) fruit increased about four-fold and eight-fold, respectively, during full ripening, despite the fact that pale green (honeydew type) melons contain no β-carotene and consequently produce no β-ionone. The conclusion of Lewinsohn et al. (2005a,b) from studies with near-isogenic tomato lines altered in fruit carotenoid composition, as well as an analogous group of differently pigmented watermelon varieties, was that production of norisoprenoids is very much dependent on the available carotenoid substrates. As a result, genetic variation in carotenoid content can profoundly influence fruit flavor and aroma. For example, a two-fold increase in total carotenoids in transgenic tomato fruit expressing the bell pepper fibrillin gene resulted in a similar increase in production of the carotenoid-derived volatiles 6-methyl-5-hepten-2-one, geranylacetone, β-ionone, and β-cyclocitrinal (Simkin et al., 2007).

6.4 Influence of storage conditions and pre-storage treatments

6.4.1 Off flavors resulting from chilling injury and fermentative metabolism

Maintaining the cold chain during handling, shipping, storage and marketing is the first rule of good postharvest practice. The aim is to hold produce at the lowest possible non-injurious temperature to retard ripening, senescence and general metabolism. However, many horticultural crops, and in particular fruits and
vegetables of tropical or subtropical origin, are prone to chilling injury when stored at temperatures ranging from just above 0 °C to as high as 10–15 °C for some very sensitive commodities such as banana, mango, breadfruit and jicama (Wang, 1990). Injurious low temperature of sufficient duration profoundly alters the metabolism of chilling-sensitive produce, often resulting in loss of flavor or development of off flavor. For example, along with the much-studied loss of juice and wooly texture of chill-injured peaches and nectarines (Lurie and Crisosto, 2005), there is a distinct loss of sweetness and ‘peachy’ aroma derived from γ- and δ-decalactones (Brovelli et al., 1998). Off flavor development in non-melting flesh peach genotypes during storage at 8 °C was correlated with increases in ethanol, phenolics and polyphenol oxidase activity, and a coincident decline in sugars and soluble solids (Karakurt et al., 2000). In mango, chilling injury adversely altered aroma, causing a general decrease in production of all ripening-associated volatiles (Nair et al., 2004).

In combination with low temperature, low O2 and/or high CO2 controlled atmospheres (CA), or modified atmosphere packaging (MAP), are frequently used to limit respiration, reduce ethylene production and responses (induction of ripening and senescence) and inhibit growth of postharvest pathogens. As well, coatings of waxes and other substances are often applied to fruit prior to storage to reduce water loss and maintain a glossy appearance, and this can modify the fruit’s internal atmosphere. Although these technologies are generally effective in maintaining quality and extending storage or shelf life, under some circumstances there can be dire consequences, such as development of CO2-induced disorders (Watkins et al., 1997) or production of highly offensive odors (Forney et al., 1991). The response of an individual fruit or vegetable to particular low temperature plus CA or MAP conditions is quite variable, and depends on the cultivar, maturity at harvest and preharvest factors (Beaudry, 1999; Watkins and Pritts, 2001). The primary cause of off flavor development during postharvest storage or subsequent shelf life is accumulation of the fermentative metabolites acetaldehyde and ethanol as a result of various types of stress, including low or high O2 and/or high CO2 atmospheres, injurious chilling and hypoxia induced by coatings (Cameron, 2003; Pesis, 2005). Acetaldehyde production occurs with ripening in all fruits, but tissue levels are often greatly increased by use of coatings and CA or MAP storage. Postharvest treatment with acetaldehyde or ethanol can be used to promote synthesis of volatiles and improve aroma, but above threshold levels that are often quite low in tropical fruits, phytotoxicity can be a problem (Pesis, 2005).

Short-term low O2 CA induced off flavors in strawberry, papaya and mango. Ten days of ≤0.25% O2 at 0 or 5 °C induced off flavors in strawberry fruit that were correlated with high levels of ethanol, ethyl acetate and acetaldehyde (Ke et al., 1991). Insecticidal quarantine treatment of papaya for more than three days in 0.4% O2 resulted in off flavors correlated with increased activities of pyruvate decarboxylase (PDC) and lactate dehydrogenase but not ADH (Yahia et al., 1992). Lastly, fruit of two mango varieties developed off flavors due to ethanol accumulation after two to three weeks under 2 or 3% O2 CA at 12–15 °C (Bender et al., 2000). Contrary to these examples, certain tree fruits, including preclimacteric
apples and avocados, can tolerate and actually benefit from low or ultra-low O₂ CA storage. Initial low oxygen stress (0.25 or 0.4% O₂) for two weeks followed by several months of CA storage under 1 or 1.5% O₂ at about 1 °C effectively controlled superficial scald disorder and maintained overall quality in apple fruit (Wang and Dilley, 2000; Zanella, 2003). In ‘Haas’ avocado fruit, accumulation of acetaldehyde and ethanol, and associated increases in PDC and ADH activities, occurred after 4–5 days at 6 °C under ≤0.4% O₂ CA (Burdon et al., 2007). However, these fermentative metabolites and enzyme activities were rapidly restored to basal levels after increasing O₂ to ≥2%, indicating that avocado should be amenable to dynamic CA storage for maintenance of optimal quality.

Use of certain coatings on guava (McGuire and Hallman, 1995), mango (Baldwin et al., 1999) and mandarin (Hagenmaier, 2002) induced anaerobic respiration and elevated levels of ethanol and acetaldehyde. In the case of mandarin, application of the least permeable coatings resulted in poor flavor scores. A more recent study determined that increased ethanol and acetaldehyde concentrations in waxed and fungicide-treated fruit of two mandarin cultivars stored under 1, 3, or 5% O₂ CA at 5 °C did not significantly alter flavor, but there was no extension of storage life compared with air controls (Luengwilai et al., 2007). For fruit and vegetable products placed in MAP, the respiration rate of the product and the O₂/CO₂ transmission rates of the packaging material must be carefully considered, and proper low temperature must be maintained (Smyth et al., 1999; Cameron, 2003). Storage of cherries (Meheriuk et al., 1997; Petracek et al., 2002) and litchi fruit (Pesis et al., 2002) in plastic films (MAP) resulted in anaerobic conditions and production of unacceptably high levels of ethanol and acetaldehyde. Oddly enough, high O₂ CA storage of strawberries (40–100% O₂), tested as a treatment to control decay, also resulted in fermentative metabolism and greatly increased levels of acetaldehyde, ethanol and ethyl acetate (Wszelaki and Mitcham, 2000). In contrast, storage of fresh-cut bell pepper cubes at 5 °C under 50 or 80% O₂ plus 15% CO₂ for up to nine days inhibited growth of microbes while maintaining overall sensory quality (Conesa et al., 2007). An intriguing new GRAS chemical treatment of strawberries to slow decay and extend storage and flavor life without use of CA or MAP was recently reported by Mo and Sung (2007). As an outgrowth of studies on biocontrol of postharvest fungal pathogens, it was found that fumigation of fruit with 1 mM phenylethyl alcohol controlled decay, markedly reduced loss of water, sugars and organic acids, and preserved a fresh-like profile of aroma volatiles for up to 15 days at 4 °C in ventilated air storage.

Radio frequency (RF) heating is being developed as an alternative to methyl bromide for quarantine treatments to eradicate medfly larvae and other insect pests. A recent study found that, although RF heating of oranges induced much less accumulation of acetaldehyde and ethanol than conventional hot water heating, there were still substantial changes in levels of other constituents of the aroma profile, most notably hexanal and mono- and sesquiterpenes (Birla et al., 2005). Sensory panel evaluations will be required to determine if the RF heat-treated fruit are acceptable to consumers.
6.4.2 Influence of storage conditions on sensory quality of immature green vegetables

*Harvest-induced senescence*

Green vegetables such as broccoli florets and asparagus spears are young, actively growing tissues and consequently have high metabolic and respiratory rates when harvested. These crops are prone to harvest-induced senescence, which is initiated within a few hours after removal from the parent plant (King and O’Donoghue, 1995; Downs and Somerfield, 1997). The senescence program is promoted by ethylene or dehydration and inhibited by cytokinin or sucrose, and is characterized by rapid depletion of sugars and organic acids, proteolysis, lipid peroxidation, increases in free amino acids and ammonia, and alteration of gene expression (King and Morris, 1994; Zhuang et al., 1995; Coupe et al., 2003; McKenzie et al., 2004; Gapper et al., 2005). The changes in gene expression include differentially regulated induction of at least four cysteine proteases (Coupe et al., 2003) and a marked up-regulation of asparagine synthase as a consequence of sucrose depletion (Downs and Somerfield, 1997; Winichayakul et al., 2004). Even in the early stages, these dramatic changes result in loss of fresh flavor and coincident increase in undesirable flavor and aroma. The flavor acceptability of asparagus spears decreased significantly after two days at 1 °C, with an additional decline during the subsequent five days of storage (King et al., 1988). This loss of sensory quality was associated with off flavor development, decreased soluble carbohydrates and an increase in soluble protein.

*Benefits of low temperature, high humidity, and controlled atmosphere (CA) or modified atmosphere packaging (MAP) storage*

The best measures to retard harvest-induced senescence and maintain fresh-like sensory quality are rapid cooling with little delay after harvest and subsequent maintenance of low temperature and high humidity during storage (Toivonen and Forney, 2004; Jones et al., 2006). However, there also has been considerable success in devising suitable CA or MAP conditions for preservation of quality in broccoli florets up to twice as long as in air storage (Jacobsson et al., 2004; Toivonen and Forney, 2004; DeEll et al., 2006). Jacobsson et al. (2004) found that use of low-density polyethylene film, inclusion of an ethylene-absorbing sachet, and a package atmosphere of 5% O$_2$, 7% CO$_2$ for MAP storage gave the best fresh-like sensory quality of cooked broccoli. DeEll et al. (2006) showed that use of packets including sorbitol plus potassium permanganate markedly reduced accumulation of off odor fermentative volatiles in MAP broccoli during 29 days of storage at 0–1 °C. These good results notwithstanding, there is always the potential for production of malodorous volatile sulfur compounds, most notably methanethiol and dimethyl disulphide, when O$_2$ falls below 1% or CO$_2$ exceeds 10% (Forney et al., 1991; Hansen et al., 1992; Di Pentima et al., 1995). Quite recently, as a first step toward elucidation of the molecular mechanisms whereby CA delays the rapid onset of harvest-induced senescence in broccoli florets, Eason et al. (2007) used differential display analysis to identify a set of stress response genes induced during the first 24 hours under 10% CO$_2$, 5% O$_2$ CA.
Glucosinolate content and flavor in Brassica vegetables
Broccoli and other Brassicas such as cauliflower, cabbage and Brussels sprouts include indole and aliphatic groups of typically bitter compounds known as glucosinolates, which, despite their valued potential long-range health benefits as anticancer agents, could negatively impact the flavor of these vegetables (Jones et al., 2006). At present there seems to be no consensus as to whether the glucosinolates contribute substantially to Brassica flavors, and there are also widely disparate reports of changes in concentration during CA and MAP storage. No clear link between sensory attributes and glucosinolate content of broccoli was established by Hansen et al. (1995) and Baik et al. (2003) found that there was no correlation of bitterness with the principal glucosinolates in cooked samples of 19 broccoli cultivars. In contrast, Schonhof et al. (2004) showed a strong correlation between bitter taste and several major glucosinolates in broccoli and cauliflower, but the bitterness was much less perceptible in genotypes with high sugar content. One factor that could lead to different results in sensory analyses is whether the vegetable is cooked or raw, since cooking can cause substantial loss of glucosinolates (Jones et al., 2006). In addition, it was recently reported that variability in a particular taste receptor gene determines whether consumers perceive glucosinolates as bitter compounds (Sandell and Breslin, 2006). Concerning the influence of CA or MAP storage on glucosinolate content of Brassica vegetables, the general trend seems to be improved retention of these compounds in parallel with preservation of overall quality (Rangkadilok et al., 2002; Jones et al., 2006; Xu et al., 2006). However, Vallejo et al. (2003) reported a major loss of glucosinolates during MAP storage of broccoli for one week at 1 °C, Hansen et al. (1995) observed a greater increase in air-stored than in CA-stored (0.5% O₂, 20% CO₂) broccoli after one week at 10 °C, and in cauliflower heads held at 0 °C for up to 56 days, Hodges et al. (2006) showed a large increase specifically in glucobrassicin and gluconapin that was delayed in CA compared with air storage. These discrepancies may at least in part be explained by genotypic differences in Brassica varieties and cultivars, and different storage conditions.

6.4.3 Influence of controlled atmosphere storage and 1-methylcyclopropene (1-MCP) treatment on fruit aroma volatile production
Both CA storage, which usually suppresses ethylene production, and prestorage treatment with 1-methylcyclopropene (1-MCP), which blocks ethylene action, can have profound effects on aroma volatile production during ripening of climacteric fruits. Extensive literature exists pertaining to these two topics and only a brief summary is presented here. Plotto et al. (1999) conducted a thorough comparison of aroma and flavor in CA- and air-stored (RA) ‘Gala’ apples, including evaluation of whole and cut fruit by a trained sensory panel. After 10 weeks in CA storage, fruity and floral notes declined, whereas vegetative and citrus characters were retained. Soursness and astringency were higher and sweetness was lower in CA-stored apples, and a musty note was perceived in CA fruit after 20 weeks. Analysis of aroma and flavor compounds showed that fruity/floral esters were 15-fold lower...
Postharvest flavor deployment and degradation in fruits and vegetables

in CA than in RA fruit after 20 weeks. Malic acid declined much more in RA fruit, explaining the ‘sour’ perception in CA fruit, but there was no difference in soluble solids concentrations. Although the effects of CA storage on other apple cultivars and other climacteric fruits may not be as pronounced, the findings for ‘Gala’ apples are representative of the negative impact CA can have on fruit flavor and aroma. On the other hand, Tough and Hewitt (2001) reported that, despite a dramatic decline in production of esters such as butyl acetate during CA storage of ‘Pacific Rose’ apples, an untrained taste panel found that this had little effect on overall flavor, and the only perceived off flavor was in RA fruit, increasing with duration of storage and shelf life.

As mentioned in Section 6.3, 1-MCP treatment of apples, cantaloupes and other climacteric fruits strongly suppresses biosynthesis of aroma volatiles, particularly esters, at the level of gene transcription and translation (Defilippi et al., 2005b; El-Sharkawy et al., 2005). Kondo et al. (2005) found that production of volatile alcohols and esters remained very low in 1-MCP-treated ‘Delicious’ and ‘Golden Delicious’ apples stored for up to 28 days at 20 °C. A low-dose 1-MCP treatment (0.2 µL L⁻¹) reduced synthesis of acetate esters in ‘Packham’s Triumph’ pears ripened five days at 20 °C after two months of RA storage, but the ester biosynthetic capacity was recovered after four to six months of storage, and a consumer panel preferred 1-MCP-treated over untreated fruit stored for the longer durations (Moya-León et al., 2006). Similar results were obtained with multiple very low-dose 1-MCP treatments (50 nL L⁻¹) of ‘Conference’ pears (Rizzolo et al., 2005). Most notably, optimal sensory quality was reached after longer storage times and production of butanol and ethyl butanoate were suppressed. Mattheis et al. (2005) evaluated the effects of both 1-MCP and CA, in combination or separately, on the production of various classes of aroma volatiles by ‘Gala’ apples after up to 28 weeks in 1 °C storage plus one week at 20 °C. With or without 1-MCP treatment, ester synthesis declined continuously in CA-stored fruit, whereas apples treated with 1-MCP and stored in air began to produce all types of volatiles after 20–28 weeks. Exposure to ethylene for one week at 20 °C after storage for 12 or 28 weeks stimulated production of mainly esters and alcohols in both CA-stored and 1-MCP treated fruit, but did not increase the levels of aldehydes or acetic acid. Treatment of apples and pears with 1-MCP also inhibited production of the sesquiterpene α-farnesene during cold storage by blocking expression of the α-farnesene synthase gene AFS1 (apple) or PcAFS1 (pear) (Pechous et al., 2005; Gapper et al., 2006).

There are mixed reports of detriments or benefits of 1-MCP treatment in fruits other than apple and pear. In mature green mango fruit treated with 1-MCP at harvest, production of total aroma compounds, including mono- and sesquiterpenes, aldehydes, esters, alcohols and tetradecane, was reduced during ripening at 21 °C (Lalel et al., 2003b). As well, production of esters derived from the fatty acid and amino acid metabolic pathways that contribute to the strong, characteristic aroma of ripe mountain papaya was shown to be markedly reduced by treatment with 0.3 µL L⁻¹ 1-MCP (Balbontín et al., 2007). Conversely, treatment with the ethylene-generating compound ethephon stimulated production of aroma volatiles in both
mango (Lalel et al., 2003b) and mountain papaya (Balbontín et al., 2007). Botondi et al. (2003) found that, in the high aroma cultivar of apricot, Ceccona, 1-MCP treatment profoundly altered the volatile profile during postharvest ripening at 20 °C. Production of lactones characteristic of apricot and peach aromas was abolished, whereas (low level) generation of monoterpene alcohols increased over threefold. Effects of 1-MCP treatment on volatiles produced by ‘Big Top’ nectarines during postharvest ripening at 20 °C varied with both 1-MCP concentration (0.25 to 1.0 µL L⁻¹) and harvest maturity (Rizzolo et al., 2006). Total volatile production was decreased in fruit from the later harvest by 1-MCP at 0.25 and 1.0 µL L⁻¹, but increased by 1-MCP at 0.5 µL L⁻¹. The 0.25 and 1.0 µL L⁻¹ treatments reduced the proportion of lactones and thus altered the aroma of ripened fruit. This change was described as a reduction of the ‘over mature’ note and an enhancement of the ‘fresh’ note. In contrast with the effects of 1-MCP treatment, a comparison of tree-ripened nectarines with those harvested when mature and then artificially ripened showed that the latter produced equal or higher levels of both lactones and C₁₃ norisoprenoids (Aubert, 2003b). Interestingly, despite the substantially lower concentration of soluble solids and ratio of soluble solids to titratable acidity in artificially compared with tree-ripened fruit, sensory analysis by a taste panel detected no significant difference in sweetness or sourness.

6.5 Roles of ethylene and the future of 1-methylcyclopropene

The chapter has already dealt at some length with the roles of ethylene in postharvest ripening and senescence, and the effects of 1-MCP treatment on aroma volatile production in fruits. Nevertheless, a few additional comments are in order. As stated in Section 6.3.1, the gaseous hormone ethylene regulates many of the processes associated with ripening in climacteric fruits. These include cell wall modification resulting in softening, conversion of starch to sugars, dramatic changes in pigmentation often associated with plastid transformation, and typically a large increase in biosynthesis of aroma compounds as well as a shift in the types of volatiles produced. Coordination and stimulation of these events by ethylene enables harvest of climacteric fruits at the mature, unripe stage followed by ethylene treatment postharvest to promote uniform ripening, a commercial practice used with bananas, tomatoes and other fruits. Despite the insensitivity to ethylene in non-climacteric fruits, the same processes and metabolic changes are no less profound during ripening on the parent plant. In some instances it is clear that other hormones replace ethylene in the control of ripening-related events. For example, it is well documented that auxin produced by the achenes on developing strawberry fruit inhibits the onset of ripening, and removal of the achenes triggers transcription and translation of ripening-associated genes (Manning, 1994). In addition, as mentioned earlier in the chapter, it appears that expression of some genes and enzymes involved in flavor generation in non-climacteric fruits late in the ripening regime are induced by low levels of ethylene, e.g. ADH in grape
berries (Tesnière et al., 2004), valencene synthase in Valencia orange (Sharon-Asa et al., 2003), and a cassette of carotenogenic genes in Navelate orange (Rodrigo and Zacarias, 2007).

Ethylene biosynthesis, perception, and signal transduction in plants are highly complex processes involving an array of genes and their encoded receptors and enzymes (for recent reviews see: Guo and Ecker, 2004; Klee, 2004). In the two committed steps of ethylene synthesis, the enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS) generates the precursor ACC and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) performs the reaction yielding ethylene from ACC. ACS and ACO belong to multigene families, with distinct patterns of expression among the individual isogenes. Two families of ethylene receptors (generically ETR and ESR) are responsible for ethylene perception, and it is thought that the mode of action of 1-MCP entails irreversible binding to these receptors (Blankenship and Dole, 2003). Not surprisingly, there is evidence of differential regulation of these multiple receptors in tissues of Arabidopsis and tomato (Klee, 2002). All receptors appear to interact with CTR1, a Raf-like kinase, as the next step in the signal transduction pathway, and gene knockout of CTR1 results in an always on ethylene response phenotype.

Much of what is known about regulation of ethylene synthesis and perception in relation to fruit ripening has been determined in studies using tomato as a model system (Nakatsuoka et al., 1998; Adams-Phillips et al., 2004; Giovannoni, 2007), although recent studies have begun to examine other fruits such as apple (Wiersma et al., 2007). In addition to the promise of practical commercial benefits with respect to storage life and control of physiological disorders, studies with ACS and/or ACO antisense transgenic lines, and on the effects of 1-MCP treatment, have provided a great deal of information on ethylene’s regulation of texture changes and flavor development during fruit ripening (e.g. Fan et al., 1999a,b; Yahyaoui et al., 2002; Defillippi et al., 2004, 2005a,b; Li et al., 2006; Manriquez et al., 2006; Nuñez-Palenius et al., 2007). The tools of molecular biology have also been used to investigate the role of ethylene in harvest-induced senescence of broccoli florets (Gapper et al., 2005). As well, a recent study of the pectin esterase gene family in strawberry provided the first evidence that ethylene plays a part in the undesirable textural changes during senescence of this non-climacteric fruit via repression of FaPE1 (Castillejo et al., 2004).

On the applied side, effects of 1-MCP treatment on many aspects of textural and sensory quality are currently being evaluated in a broad range of fruit and vegetable crops. Examples include extended storage life and reduced decay of papaya (Manenoi et al., 2007), attenuated ripening and softening of tomatoes (Guilién et al., 2007), reduced softening of plums (Khan and Singh, 2007), retention of firmness, juiciness and acidity in summer apples (Pre-Aymard et al., 2005), decreased sprout growth and reduced loss of sugars in onion bulbs (Chope et al., 2007), reduced rates of softening and browning in fresh-cut kiwifruit, mango, and persimmon slices (Vilas-Boas and Kader, 2007), and prevention of ethylene-mediated quality loss in fresh-cut watermelon slices (Saftner et al., 2007). It is anticipated that this list will continue to grow at a rapid pace as 1-MCP
becomes registered for use on additional commodities and the technology for low
dose and/or repeated applications is perfected (Watkins, 2006).

6.6 Summary and conclusions

Having progressed from the era of genomics to proteomics and now metabolomics,
knowledge of the genes and enzymes involved in production of fruit and vegetable
flavor compounds is increasing rapidly. The dramatic increase in, and changing
profile of, aroma volatiles during the late stages of fruit ripening is a major focus
of this chapter. The scope is limited to products of fatty acid and isopentenoid
metabolism. Specifically, production of \( C_6 \) and \( C_9 \) saturated and monoenoic
aldehydes from linoleic and linolenic acid by the combined action of LOX and
HPL, reduction of aldehydes to alcohols by ADH, formation of esters from
carboxylic acids and alcohols by AAT, production of mono- and sesquiterpenes by
terpene synthases, and generation of norisoprenoids from carotenoids by CCD are
addressed. Of particular interest is the extent to which these genes and enzymes are
regulated by ethylene, the gaseous hormone required for ripening of climacteric
fruits. As a general rule, expression of AAT genes in climacteric fruits is ethylene
dependent, and consequently ethylene perception and responsiveness are required
for volatile ester production. ADH genes, on the other hand, may or may not be up-
regulated by ethylene, as in climacteric melon and apple fruit, respectively.
Postharvest treatment with the blocker of ethylene action, 1-MCP, and storage in
low \( O_2 \) CA, which suppresses ethylene synthesis, are both effective means of
maintaining overall quality and extending storage life. A downside of these
technologies is that inhibition of ethylene perception or synthesis also blocks
production of esters and usually other aroma volatiles, resulting in a marked
decrease in overall aroma and shift in the aroma volatile profile.

CA storage, low temperature and MAP are widely used, effective measures for
preservation of fruit and vegetable quality after harvest. However, exceeding low
\( O_2 \)/high \( CO_2 \) threshold limits for a given commodity, or temperature abuse (low or
high) can result in production of fermentative metabolites (acetaldehyde, ethanol
and ethyl acetate) or development of other off flavors and aromas. Harvest-
induced senescence of metabolically active green vegetables such as broccoli and
asparagus, which includes rapid loss of sugars and other flavor components, is
another important area of postharvest research. Basic studies at the molecular level
are aimed at elucidation of how ethylene, dehydration and sugar depletion promote
senescence, and how CA storage delays it. On the applied side, good progress has
been made in establishing CA and MAP systems to maintain fresh-like quality, but
there is still the risk of generating intense off odors if conditions exceed the
fermentative threshold.

Molecular genetic manipulation of specific genes or groups of genes to increase
or modify aroma and flavor generation in fruits and vegetables promises to be a
highly active area of future research. Part of this work will undoubtedly focus on
ways to maintain the benefits of inhibiting ethylene action and/or production
without seriously compromising flavor and, more particularly, aroma. This pursuit encompasses further research on the use of 1-MCP to extend the storage life of fresh and fresh-cut fruit and vegetable products.

6.7 References


Fruit and vegetable flavour


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7

Importance of texture in fruit and its interaction with flavour

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7.1 Introduction

It might seem that a chapter on texture is out of place in a book on fruit and vegetable flavour. However, this is not the case. Food texture is conventionally divided into three classes of sensory attributes: mechanical properties, geometric properties associated with mouthfeel and another class that includes water content (Szczesniak, 1963). It is the mechanical properties of fruit and vegetables and how these properties influence the way products break down during chewing that is of fundamental importance if we are to understand the release of sugars, acids, proteins and volatile compounds that stimulate human perception of flavour.

Some sensory attributes associated with the texture of fruit are provided in Table 7.1. In fruit, much research has focused on the mechanical properties and how they change during maturation, storage and ripening (Section 7.3.3). The changes in the mechanical properties of fruit have been followed using a wide range of fundamental, imitative and empirical instrumental measurements as described by Harker and coworkers (1997a). The emphasis in the literature on the mechanical properties of fruit is driven by the need to understand the biological processes associated with softening of fruit, in order to optimise the duration for which fruit can be stored commercially. However, there is also substantial literature on mechanical properties that have a direct influence on consumers’ perception of the quality of produce. For example, mechanical properties associated with hard and crisp apples are critical to acceptance of quality by consumers (Liu and King, 1978).
### Table 7.1  Examples of sensory texture attributes and definitions used to describe fruit by trained analytical panels

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardness/Firmness</td>
<td>The force required to compress the sample with the back teeth</td>
<td>Harker <em>et al.</em>, 1997a;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jaeger <em>et al.</em>, 2003a</td>
</tr>
<tr>
<td>Crispness</td>
<td>The amount and pitch of sound generated when the sample is first bitten with the front teeth</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Crunchiness</td>
<td>The amount of noise generated when the sample is chewed at a fast rate with the back teeth</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Fracturability</td>
<td>The force with which the sample breaks</td>
<td>Paoletti <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>Deformation degree before material fractures</td>
<td>Paoletti <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>Ease of breakdown</td>
<td>The amount of chewing required to break down the sample so it can be swallowed</td>
<td>Harker <em>et al.</em>, 1997a; Wismer <em>et al.</em>, 2005</td>
</tr>
<tr>
<td>Melting</td>
<td>The degree to which the sample disintegrates evenly in the mouth, often without chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td><strong>Geometric/Mouthfeel</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrousness</td>
<td>The amount of readily separated filaments present</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td></td>
<td>The degree of fibrousness of the flesh</td>
<td>Wismer <em>et al.</em>, 2005</td>
</tr>
<tr>
<td>Flouriness</td>
<td>The amount of dry, fine, powdery particles that count the mouth during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Graininess</td>
<td>The presence of small firm particles detected during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Grittiness</td>
<td>The presence of small hard sharp particle detected during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Mealiness</td>
<td>The amount of small lumpy particles that becomes apparent during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td></td>
<td>Crumbling during mastication</td>
<td>Paoletti <em>et al.</em>, 1993</td>
</tr>
<tr>
<td>Pastiness</td>
<td>The amount of soft, smooth mass that doesn’t release moisture during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Pulpiness</td>
<td>The amount of wet, web-like material that develops during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Starchiness</td>
<td>The amount of fine particles that coat the mouth during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td>Gelatinous</td>
<td>The presence of gelatinous flesh texture</td>
<td>Wismer <em>et al.</em>, 2005</td>
</tr>
<tr>
<td><strong>Water content</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juiciness</td>
<td>The amount of free fluid released from the sample during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
<tr>
<td></td>
<td>The amount of liquid perceived in the mouth after three chews of the fruit</td>
<td>Wismer <em>et al.</em>, 2005; Paoletti <em>et al.</em>, 1993</td>
</tr>
<tr>
<td></td>
<td>The amount of moisture released during chewing</td>
<td>Harker <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Flouriness</td>
<td>The amount of dry, fine, powdery particles that coat the mouth during chewing</td>
<td>Harker <em>et al.</em>, 1997a</td>
</tr>
</tbody>
</table>
The geometric or mouthfeel properties of fruit are often related to the presence of sclerified and lignified structures in the flesh, as well as with the cell wall residues that are left in the mouth after the juice is released during chewing (Table 7.1). A typical example of the former is the grittiness associated with stone cells in pear tissue or the fibrous texture associated with the presence of lignified vascular bundles. Generally, fruit breeding programmes have sought to eliminate these from commercial varieties (Reeve, 1970; Bell and Janick 1990). Cell wall residues are often described as being pasty or pulpy (Table 7.1).

Water content is the defining texture attribute in many fruit. For example, consider the intensity of perceived juiciness that is experienced when a consumer eats a watermelon. While some fruit such as banana and avocado are low in perceived juiciness (Harker et al., 1997b), generally no other food type (e.g. cereals, meat) approaches the levels of extreme juiciness that is experienced when eating fruit (Harker et al., 2003). It is the release of the juice containing dissolved sugars, acids, and volatiles that defines many of the links between texture and flavour. In this chapter we will review human perception of fruit texture as it relates to flavour, the way tissue breaks down in the mouth and how this influences taste and flavour, how ripening influences all these processes, and the genetic factors that influence texture–flavour interactions.

Another topic addressed in this chapter is the close biological interactions between texture and flavour and how they jointly contribute to the eating experience. Texture changes during fruit softening and the associated development of flavour are bound together by the metabolic processes that direct fruit ripening. This tightly interlinked and often inescapable tripartite interaction between ripening, texture change and flavour development means that it is often difficult to attribute consumer preferences specifically to either texture or flavour. For example, the change in texture that occurs as a fruit moves from an unripe to ripe state is often tracked using a simple puncture measurement (Harker et al., 1996). Usually consumers prefer softer fruit, yet this does not mean that texture is driving these preferences. Rather their preference will be associated with multiple changes in texture and flavour that occurred during ripening. Thus it is important to standardise ripeness/texture when comparing flavours of fruit (Jaeger et al., 2003b) and preferably to use a range of matched ripeness/texture bands (Jaeger et al., 2003a).

### 7.2 Texture and the consumer

The influence of fruit texture on consumer choice of produce can be split into two main areas. First, texture is as important as taste and flavour in driving consumer preferences and choices of many fruit. Second, the texture of fruit is among the primary attributes that determine how convenient fruit are to handle and eat. It is important to realise that food convenience is a key trend that has driven product development in the food and beverage sector.
7.2.1 Preferences for texture attributes
Consumers often have diverse preferences for foods. What is liked by one consumer is disliked by another consumer. For this reason, a methodology known as consumer preference mapping is sometimes used to interpret consumer preference in terms of defined sensory texture and flavour attributes. Increasingly, preference mapping has been used to explore preferences for fruit (Daillant-Spinnler et al., 1996; Jaeger et al., 1998) and, in particular to identify new product opportunities for fruit breeding (Jaeger et al., 2003a,b; Wismer et al., 2005). Examination of many of these preference maps for fruit establishes that it is unwise to view texture and flavour as separate unconnected food characteristics. Rather, flavour and texture attributes need to be integrated to provide a holistic view of product quality. For example, Daillant-Spinnler and co-workers (1996) concluded from their research on apples that consumers were segmented into groups according to whether they preferred a sweet, hard apple or a juicy, acidic apple. In the same fashion, preferences for pears and kiwifruit are driven by combinations of texture, taste and odour attributes (Jaeger et al., 2003a,b).

Bourne (1979b) separated fruit into two texture categories: those that soften greatly as they ripen (peach, strawberry) and those that soften moderately as they ripen (apple, cranberry). These are convenient groupings because they broadly define the different textures preferred by consumers. Apples are characteristically fruit that soften moderately or not at all during ripening. Consumers prefer their apples with mechanical properties that confer hardness, crispness and crunchiness. Measurement of hardness, whether by instrumental or sensory testing, is a good predictor of consumer acceptability. This was well demonstrated in the research undertaken by Liu and King (1978), and confirmed in many more recent studies (e.g. Hoehn et al., 2003; Kupferman et al., 2005). The robust nature of the relationship between instrument measurements of hardness and human responses allowed Harker and co-workers to examine the ability of people to detect differences in quality from day to day (Harker et al., 2002a). Using this knowledge, they developed predictions that indicated whether or not a consumer would detect differences in quality of different lines of apples based on instrumental measurements of puncture force (a measurement of fruit hardness) that are widely used by industry (Harker et al., 2006b). Most types of fruit, however, fit into Bourne’s first fruit texture category – those that soften greatly as they ripen. Consumers prefer these types of fruit (e.g. peaches, strawberries) to be soft when eaten.

Relatively few studies have sought to identify formally the appropriate firmness at which fruit that ‘soften greatly as they ripen’ should be eaten. For kiwifruit, the firmness should be about 4 to 10 N (measurement made with a standard penetrometer fitted with a 7.9 mm Effegi probe) (Stec et al., 1989). Firmness values in this range are also representative of ready-to-eat ripeness across a number of types of fruit (e.g. European pears – Jaeger et al., 2003a; avocado – White et al., 2005). Common sense dictates that most types of fruits should be soft and juicy when eaten. However, the decision as to what firmness/ripeness can have a profound impact on results in studies of volatiles and human perception. To optimise characteristic flavour it is important to use fruit that are fully ripe. Stec and coworkers (1989)
found that kiwifruit firmness had a significant influence on perception of sweetness and acidity in kiwifruit. They concluded that ‘sensory assessment of treatment effects on kiwifruit should be approached with care and with strict control on fruit firmness’. This would seem to be best practice for most other types of fruit that fall within Bourne’s first fruit texture category – those that soften greatly as they ripen.

7.2.2 Texture and mastication of food in the mouth

The physical structure of the fruit flesh is generally composed of relatively large parenchyma cells with large vacuoles. The size of these parenchyma cells can be diverse and it is not unusual for them to reach diameters up to 700 µm in fruit such as watermelon (Harker et al., 1997b) The large size of cells is often suggested as one of the reasons why some fruit are juicier than others (Szczesniak and Ilker, 1988; Harker et al., 1997b). In citrus, the juice is held in a 10 mm long × 2 mm wide vessel with a multicellular ‘skin’ known as the juice sac, which Harker et al. (1997a) suggest is perhaps analogous to a super-sized cell. The perception of juiciness most obviously occurs as a result of damage and bursting of cells and the associated release of free liquid into the mouth during chewing of fruit such as apples (Harker and Hallett, 1992; Harker et al., 2006a). However, for other types of fruit this might not be the only mechanical/biological phenomenon associated with perception of juiciness. Examination of fractures that occur during mechanical testing and during biting and chewing of soft fruit such as pears and peaches suggest that a relatively small number of cells are broken open (Harker and Sutherland, 1993; Harker and Hallett, 1992; De Belie et al., 2000). Indeed, examination of flesh from ripe European pears that have been chewed up to 50 times indicates that many individual cells and clumps of cells retain their cellular integrity as determined by exclusion of the dye Trypan Blue (I. C. Hallett, pers. comm.). It is possible that the surface of cell walls, particularly those cell walls that hydrate and expand up to ten-fold in thickness during ripening (Redgwell et al., 1997), have a critical role in the expression of juiciness and transfer of tastants from fruit tissue bolus to the oral mucosa in the mouth.

As in any plant cell, the mechanical strength is a function of the properties of the cell wall, the turgidity or internal pressure within each cell and the strength of bonding between neighbouring cells (Harker et al., 1997a). In fruit, the weakening of cell walls and bonding between cells that occurs during ripening is important if the fruit is to become edible (De Belie et al., 2000). At the point of consumption, the flesh can be considered as ‘a living structure within which juice and tastants are encapsulated within individual cells’ (Harker et al., 2006a). The way in which this flesh structure and individual cells break down during mastication can have a profound influence on the flavour release. Good evidence for this interaction between texture and taste was provided by a trained sensory panel in a study of kiwifruit (Table 7.2). Maceration of the flesh resulted in a pulp in which the influence of cell and tissue structure on flavour was minimised. Sensory evaluation of the pulp and the corresponding undamaged half fruit showed that the texture of kiwifruit was suppressing perception of acid taste, suppressing perception of many
Table 7.2  Sensory analysis of taste, flavour and odour attributes in kiwifruit before and after maceration of flesh. An eight-member trained panel assessed sensory attributes of fruit cut in half along the longitudinal axis. Intact half fruit were tasted and the remaining half was macerated (~10 s) into a pulp immediately before presentation to panellists. Each panellist assessed intact flesh and corresponding pulp from six individual kiwifruit over three days. Definitions for sensory attributes, training and testing facilities are as described by Jaeger et al. (2003) and Wismer et al. (2005). Means with the same letter within a row are not significantly different from one another ($P > 0.05$).

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Intensity (150 mm Linescale)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact fruit</td>
</tr>
<tr>
<td>Taste</td>
<td></td>
</tr>
<tr>
<td>Sweet</td>
<td>73.46 a</td>
</tr>
<tr>
<td>Acid</td>
<td>43.04 a</td>
</tr>
<tr>
<td>Flavour (retronasal odour)</td>
<td></td>
</tr>
<tr>
<td>Vomit flavour</td>
<td>21.26 a</td>
</tr>
<tr>
<td>Lolly/fruit candy flavour</td>
<td>32.44 a</td>
</tr>
<tr>
<td>Grassyl flavour</td>
<td>24.18 a</td>
</tr>
<tr>
<td>Sweet vomit/candy flavour</td>
<td>34.28 a</td>
</tr>
<tr>
<td>Feijoa flavour</td>
<td>10.27 a</td>
</tr>
<tr>
<td>Lemon/lime flavour</td>
<td>29.59 a</td>
</tr>
<tr>
<td>Green apple flavour</td>
<td>28.03 a</td>
</tr>
<tr>
<td>Fresh banana flavour</td>
<td>25.84 a</td>
</tr>
<tr>
<td>Odour</td>
<td></td>
</tr>
<tr>
<td>Vomit odour</td>
<td>22.63 a</td>
</tr>
<tr>
<td>Lolly/fruit candy odour</td>
<td>16.43 a</td>
</tr>
<tr>
<td>Grassyl odour</td>
<td>38.21 a</td>
</tr>
<tr>
<td>Sweet vomit/candy odour</td>
<td>27.10 a</td>
</tr>
<tr>
<td>Feijoa odour</td>
<td>8.70 a</td>
</tr>
<tr>
<td>Woody/earthy odour</td>
<td>28.39 a</td>
</tr>
<tr>
<td>Green apple odour</td>
<td>33.52 a</td>
</tr>
<tr>
<td>Sweet green odour</td>
<td>24.35 a</td>
</tr>
<tr>
<td>Fresh banana odour</td>
<td>19.85 a</td>
</tr>
<tr>
<td>Lemon/lime odour</td>
<td>28.86 a</td>
</tr>
</tbody>
</table>

aromatic flavour and odour components, and enhancing perception of sweet taste (Table 7.2). The latter may reflect that acids released in the macerated pulp were suppressing perception of sweet taste (Rossiter et al., 2000; Marsh et al., 2006).

The influence of texture on taste raises the possibility that the loss or reduction of flavour that occurs during prolonged storage of fruit may be partly due to changes in texture. In particular this may be a problem for stonefruit and some cultivars of apple that develop dry and mealy textures during storage (Harker and Hallett, 1992; Lurie and Crisosto, 2005). There has been only one attempt to characterise such an interaction between texture and taste. Harker and co-workers (2006a) explored the relationship between juiciness and sweet taste in ‘Braeburn’ and ‘Cox’s Orange Pippin’ apples, of which the latter is well known for its propensity to become mealy during storage. They found that sweetness was not influenced by juice release, and concluded that the apples were probably not
sufficiently mealy for taste to have been affected (Harker et al., 2006a). But more than this, the research raised the complication that only a small amount of juice may need to be released from the flesh during mastication in order for sugars and acids to saturate taste receptors in the mouth. These types of relationship between texture and taste are likely to be complicated. A more direct, although undemonstrated, influence of texture on flavour may arise via the influence of cell wall pectins on juice viscosity. The solubilisation of cell wall pectin during fruit ripening (Schroder and Atkinson, 2006; Brummell, 2006) may increase viscosity of juice released during chewing. It has been shown that increases in solution viscosity reduce flavour perception (Lethuaut et al., 2003; Walker and Prescott, 2000) and this is generally thought to be because of reduced diffusion from the solution across the oral mucosa of the mouth (de Roos, 2003).

Research demonstrating the role of texture in the release of volatile flavour compounds has recently become possible with the development of technologies that allow breath-by-breath analysis of volatiles released during chewing (Taylor et al., 2000; Friel and MacRae, 2007). Earlier approaches for measuring of fruit volatiles often involved collecting the headspace samples from semi-intact pieces of tissue over prolonged durations, and provided no information on the time-intensity relationship between volatile release and mastication (Friel et al., 2007), which are unrealistic compared with human mastication. MS Nose™ is a technology that uses atmospheric pressure ionisation processes and detects compounds that fragment to give the same mass without needing a chromatography step (Taylor et al., 2000). As such, MS Nose™ can be used for real time analysis of volatiles sampled from the nose in what is known as breath-by-breath analysis. Studies using fruit have demonstrated that there are temporal differences in the appearance of different volatiles in the nose (Brauss et al., 1998; Friel and MacRae, 2007), temporal differences and fruit-to-fruit variability in release of volatiles during maceration (Boukobza et al., 2001) and that some of the fruit-to-fruit variability in release of volatiles during maceration may be related to firmness (Friel and MacRae, 2007).

7.2.3 Role of texture in determining convenience
The search for convenience is one of the megatrends driving product development in the food and beverage sector (Jaeger, 2003). Many fruit and vegetable industries are responding to this megatrend by providing fresh-cut and pre-washed products; retailers are tending to sell ready-to-eat whole fruit in preference to unripe fruit; and some breeding programmes are focusing on fruit attributes that add convenience. For example, kiwifruit is considered by some consumers to be inconvenient to handle and eat (Harker et al., 2007). However, a kiwifruit breeding programme has released the more convenient kiwiberry, which is the size of a grape and has an edible skin (Jaeger, 2003).

Fruit convenience is multidimensional and for consumers can mean: (a) no preparation or clean-up; (b) ease of handling; (c) a variety of uses; (d) suitability for the entire family; and (e) consistent and high availability (Jaeger, 2003). Texture
and softening characteristics of fruit can have an impact on three out of these five convenience factors: preparation and clean-up, ease of handling and availability. Fruit such as European pears and peaches that are soft and juicy when ripe are often messy to eat. For some consumers this may restrict the numbers of situations where they are willing to eat these fruit, for example where a plate and knife is available to minimise the mess, where they can clean themselves up afterwards, and where they don’t embarrass themselves in front of strangers. Breeding of pears and peaches that have firmer textures when at eating ripeness is one of the ways that the convenience of these fruit can be improved (White and Brewer, 2002; Jackman et al., 2003).

The softening characteristics of fruit are often key determinants of availability during the year and handling in the home. Fruit such as apples can be stored for up to a year (Kupferman, 1997), and are therefore consistently available to consumers even if out-of-season supply from the opposite hemisphere is not considered. Some fruit soften rapidly during storage and are only seasonally available.

Identification of how consumers handle fruit in the home that is a key to understanding convenience. Yet, few studies have considered this issue. In an online survey of 234 New Zealand consumers who regularly purchase fruit about half of the participants indicated that they threw away fruit every two weeks or more frequently (Amos, 2005). Interviews in homes suggest the most thrown-away fruit are bananas, followed by kiwifruit (Amos, 2005). In focus groups, consumers have mentioned that kiwifruit were usually sold quite firm and needed to be left in the fruit bowl to ripen for up to a week: ‘I buy them hard, then forget about them, then they turn overripe’ (Harker et al., 2007). A recent study from Turkey has estimated fruit wastage to be 370.6 g per household per day (Pekcan et al., 2006).

The shelf-life of fruit in the home has the potential to affect consumer perceptions of reliability of the product. The concept of shelf-life in the home is perhaps more intuitively redefined as the edible period: the time from when the fruit first becomes edible to the point where it has deteriorated so much that a consumer no longer wants to eat it. Fruit that deliver consistent flavour and texture without deteriorating over long periods in the home potentially offer better value to consumers because there is less wastage. Softness, colour change and aroma are often the cues that consumers use to decide that fruit is ready to eat. In our online survey of 234 New Zealand consumers (Amos, 2005), 77% of participants disposed of bananas on the basis of skin colour and presence of rots; 58% disposed of kiwifruit on the basis of firmness and presence of rots; 63% disposed of citrus on the basis of rots and shrivel; 71% disposed of apples on the basis of firmness and rots. What was more interesting was the low number of consumers that reported that they disposed of fruit on the basis of the length of time fruit had been in the home, or on the basis of the taste of the previous fruit consumed. This perhaps suggests that consumers focus on individual fruit, recognising high levels of fruit-to-fruit variability, and rely on appearance and squeezing as cues to quality. The latter may reflect the relatively high value of the fruit and an associated reluctance to waste food.
7.3 Ripening as the universal driver of changes in texture, taste and odour

7.3.1 Role of ethylene in ripening
The gaseous plant hormone ethylene is an important regulator of ripening for many fruits, particularly those that continue to ripen after harvest such as apples, stonefruit, avocado, kiwifruit and pears (Kader, 1999). These fruit types produce moderate-to-large quantities of ethylene, and technologies that extend storage life often revolve around the suppression of ethylene biosynthesis and action. Ethylene treatment can be used to hasten ripening, and is an essential component of supply chains for fruits such as bananas and avocados, to ensure the delivery of fruit to retail outlets at a uniform and suitably advanced stage of ripeness. Other fruit types such as citrus and berries do not continue to ripen independently after harvest and have low ethylene production (Kader, 1999). These fruit are often harvested tree-ripe to achieve full flavour.

Ripening is a complex process that requires the simultaneous induction of pathways involved in softening, colour change, volatiles biosynthesis, conversion of starch to sugars, reduced acidity, and removal of compounds in unripe fruit that may cause bitterness, astringency and toxicity. While ethylene is required to induce some of these ripening pathways, others may progress independently of ethylene (Guise et al., 1997). Thus, postharvest technologies that perturb ethylene biosynthesis and action may be effective at retarding the ethylene-dependent pathways (e.g. softening, loss of acidity, biosynthesis of volatiles), while ethylene-independent pathways (e.g. conversion of starch to sugars) will continue to progress (Fan et al., 1999). This loss of synchronicity can have implications for the texture and flavour profile of fruit, as the ripening pathways that contribute to each component may be differentially affected.

7.3.2 The ripening behaviour of melting and non-melting fruits
There is considerable variation amongst different types of fruit in the magnitude by which texture and flavour changes during ripening. Fruit that soften appreciably (80–97%) during ripening are described as having a melting texture, while those that soften moderately (<50%) are described as non-melting (Bourne, 1979b). These two classes of fruit differ appreciably in their ripening behaviour, and the manner by which the tissue breaks down during mastication, both of which can have significant impacts on the texture and flavour profiles at the time of consumption.

Apples, Asian pears, crisp-fleshed peaches, cranberries and quinces are examples of non-melting fruits, while kiwifruit, avocados, European pears and stonefruit (except for crisp-fleshed cultivars) are examples of melting fruits (Bourne, 1979b; Karakurt et al., 2000; White and Brewer, 2002). Non-melting fruits are generally consumed while firm, and characteristically have a crisp, fracturable and juicy texture (Bourne, 1979b). This contrasts with melting fruits, which require softening before consumption and characteristically have a texture that is soft or creamy,
Importance of texture in fruit

not crisp, not fracturable, juicy and/or pulpy (Bourne, 1979b). While softening is generally considered essential for the consumption of melting fruits, softening can reduce the acceptability of non-melting fruits such as apples through reduced crispness, firmness and juiciness (Daillant-Spinnler et al., 1996; Liu and King, 1978; Wills et al., 1980).

7.3.3 Harvest maturity and storage technologies: implications for texture and flavour

Commercially grown fruit are usually harvested unripe and at an early stage of maturity to maximise storage life and to reduce losses from physical damage (e.g. bruising). As the fruit matures on the tree, there are a number of complex physiological changes that influence the ripening behaviour after harvest. The maturation of fruits while attached to the plant is important for flavour development in many fruit types (Fallman et al., 2003; Kader, 1999; Kader et al., 1977; Shewfelt et al., 1987), as fruit harvested at an immature stage of development usually lack the competence to ripen and fail to develop the flavour and textural characteristics expected by consumers. For example, the harvesting of immature avocados results in uneven ripening, rubbery texture, and green or watery flavour (Harding, 1954; Lee et al., 1983). The harvesting of fruit at an over-mature stage of development can also be detrimental for texture and flavour. For example, apples lose the potential for retaining texture and become more susceptible to textural disorders when harvested over-mature (Harker and Hallett, 1992; Johnston et al., 2002), while avocados may develop rancid off flavours (Erickson et al., 1970-71; Hofman et al., 2000). Harvest maturity also affects the predisposition of many fruits to develop physiological disorders and rots during ripening, with susceptibility often higher in immature and over-mature fruit (Kader, 1999). Thus, commercial harvest guidelines often have to consider many quality factors, and flavour may be unintentionally compromised to satisfy other aspects of quality. The lack of emphasis on flavour is probably a reflection of the limited understanding of the biological drivers for flavour in fruit, and how flavour influences purchasing behaviour relative to other quality factors such as appearance and texture. There is also a lack of objective and reliable instrumentation that fruit industries can use to measure flavour in a commercial setting.

Fruit industries use low temperatures, controlled atmospheres and ripening inhibitors to slow ripening and to improve the storability of fruits that would otherwise deteriorate rapidly at ambient temperatures and atmospheres. For apples, softening, loss of acidity, accumulation of sugars and biosynthesis of flavour volatiles are all slowed by these postharvest technologies (Bai et al., 2005; Defilippi et al., 2004; Magness and Diehl, 1924; Tough and Hewett, 2001). Sensory studies on apples have shown that texture and flavour are closely related during ripening, but that the maximum ratings for texture and flavour occur at different stages of ripeness and are dependent on the storage atmosphere (Plocharski and Konopacka, 2001). For peaches, low temperature storage affects the synchronicity of softening relative to development of flavour, where low tempera-
tures inhibit colour change and the development of flavour, yet softening still proceeds (Shewfelt et al., 1987).

The importance of a crisp and juicy texture for the sensory acceptability of apples often means that storage technologies are optimised to reduce softening, rather than to maintain volatile production. The importance of odour components for acceptability of apples remains largely unresolved, but may explain variation in consumer preferences not explained by texture and taste (Harker et al., unpublished data). A lack of understanding of the importance of volatiles for acceptability of apples is probably due to insufficient studies that compare fruit with differing volatile composition, but similar texture and taste attributes. Most studies to date have compared the volatile composition and sensory responses of apples from different storage treatments (Lurie et al., 2002; Pre-Aymard et al., 2005; Tough and Hewett, 2001), treatments which also have significant impacts on texture and taste. The sensory responses for acceptability in these studies are probably overwhelmed by the large textural differences between storage treatments, making it difficult to assess the relative importance of volatiles.

While one of the objectives of storage technologies is to retain quality after harvest, these technologies also influence the ripening competence of fruits, particularly those that require extensive ripening before consumption. For European pears, low temperature storage is not only essential for maintenance of fruit quality, but is also a physiological prerequisite to induce ripening (Agat et al., 2000). Improvements in the storage life of European pears can be achieved by controlled atmospheres and ripening inhibitors, although these technologies can affect the texture and odour profile once ripe (Bai et al., 2006; Lara et al., 2003; Rizzolo et al., 2005). In some circumstances these storage technologies can result in loss of ripening competence, although this problem is often alleviated by optimising the inhibitor dosage and by implementing post-storage conditioning treatments (Bai et al., 2006). Thus, while storage technologies are essential in many fruit industries to maintain quality, the duration and choice of storage technology can have significant impacts on the synchronicity of ripening pathways for texture and flavour.

7.4 Genetic factors important for defining texture–flavour interactions

Texture, flavour and storability are recognised as important traits in many fruit breeding programmes (Alston, 1988; Brown et al., 2004; Causse et al., 2003; Lecomte et al., 2004; Volz et al., 2004; Wismer et al., 2005). These programmes exploit biodiversity amongst wild and cultivated genotypes to produce progeny with a diverse range of textural and flavour characteristics. Seedlings with promising traits are then evaluated for commercial potential, and/or are incorporated in crosses for further genetic improvement.

Apples bred for superior eating quality tend to be screened for enhanced crispness, juiciness and taste, and the propensity to retain these attributes after
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harvest (Alston, 1988; Brown et al., 2004; Volz et al., 2004). ‘SciFresh’ and ‘HoneyCrisp’ are examples of apple cultivars bred for enhanced textural characteristics that have shown early promise in the marketplace (Brown et al., 2004; Volz et al., 2004). The attainment of plant variety rights for ‘SciFresh’ also means that the licence holder can exclusively supply this cultivar into key markets, so that supply and demand can be more effectively managed to achieve price premiums. Exclusivity of supply also occurs for ‘Cripps Pink’ apples and ‘Hort16A’ kiwifruit, and is the likely model for commercial release of new cultivars in the future.

The use of genetically diverse germplasm in breeding programmes has produced new genotypes that can be difficult to classify according to conventional descriptors for non-melting and melting fruit (Section 7.3.2). An example of this is pears, where the crossing of Asian pears (non-melting texture, simple flavour traits) with European pears (melting texture, complex flavour traits) has produced progeny with a diverse range of textural and flavour combinations (White and Brewer, 2002). Some of these progeny have flavour characteristics normally associated with melting genotypes, but they don’t soften to the extent expected for a fruit with these flavour characteristics. The commercial release of these genotypes may challenge the consumer’s expectations for how a firm or soft fruit should taste. This concept has been explored for crisp-fleshed peaches, where consumers more familiar with melting peaches found the concept of crisp-fleshed peaches unappealing before tasting, but were more amenable to the concept after tasting (Jackman et al., 2003).

The evaluation of new cultivars for consumer acceptability often requires a combination of instrumental and sensory techniques (Crisosto et al., 2006; Hampson et al., 2000; Ulrich et al., 2006; Vaysse et al., 2005; Wang and Kays, 2003; Wismer et al., 2005). Instrumental assessments are preferred over sensory panels, as they tend to be more rapid, inexpensive, objective, and require fewer personnel. However, instrumental techniques often aren’t sufficient to predict complex sensory responses (Harker et al., 2002b). Techniques for instrumental evaluation of fruit texture have been reviewed extensively (Bourne, 1979a; Harker et al., 1997a; Jackman and Stanley, 1995), and many studies have characterised the relationship between instrumental measures and sensory responses during ripening (Abbott et al., 1984; Harker et al., 2002b,c; Liu and King, 1978; Wills et al., 1980). For apples, the puncture test (the current industry standard) tends to be a reliable predictor of ripening related changes in texture within any one cultivar, but less is known about the effectiveness of this technique for predicting sensory responses across cultivars. Preliminary studies in this area suggest that the puncture test has limitations for evaluating texture across genotypes, as apple cultivars with similar puncture firmness can be perceived by sensory panels as having different textural ratings (Johnston et al., unpublished data). This concept is supported by results that show that the minimum firmness for sensory acceptability of apples differs across cultivars and storage technologies (Hoehn et al., 2003; Plocharski and Konopacka, 2001). Ongoing research is required to develop instrumental techniques for breeders and marketers that more accurately predict sensory responses across genotypes.
The texture and flavour diversity amongst seedling populations provides a valuable resource for fundamental studies aiming to understand the genetic and biophysical basis of these traits (King et al., 2000, 2001; Seymour et al., 2002). For apples, the analysis of seedling populations segregating for texture resulted in the identification of quantitative trait loci (QTL) associated with sensory and instrumental aspects of texture, such as crispness, juiciness, puncture firmness and acoustic stiffness (King et al., 2000, 2001). For peaches, breeding populations have been used to understand the biological processes associated with melting versus non-melting and stony hard phenotypes, and for freestone versus clingstone phenotypes (Haji et al., 2005, Peace et al., 2005). For tomatoes, QTLs associated with flavour traits have been identified (Lecomte et al., 2004). The markers derived from such studies have the potential to be used to understand the heritability of textural traits, the interaction between genotype and environment, and to accelerate the selection and commercialisation process for new genotypes with novel textural characteristics. Seedling populations may also provide the means to determine the biophysical basis of texture, where segregating textural traits could be related to morphological and structural characteristics (e.g. cell size, number and adhesion; cell wall composition and architecture; calcium content). This is important for understanding how these cellular components contribute to texture before and after harvest, and may give new insights as to why the maximum firmness during development varies for fruit from different orchards, regions and years.

### 7.5 Future trends

The trend for improved convenience is influencing the fruit industry in the way that it has influenced the vegetable industry in the past decade or so. Consumers increasingly expect fruit to be ready-to-eat at the time they are purchased and there is an increasing array of fresh-cut fruit products being sold by retailers. Both these opportunities for further product development provide considerable challenges. There remains a need to control ripening in a manner that delivers high quality produce without compromising either texture or flavour. But the ranges of product specifications in terms of acceptable levels of ripeness are becoming much tighter than they ever were before.

Consumers’ demands for convenience are also driving changes in product targets in plant breeding programmes. Flavour intensity and flavour diversity remains the focus of many fruit breeding programmes. Alongside these flavour targets is the increasing recognition that new products also have to deliver increased convenience, whether it is a new grape-sized kiwifruit with an edible skin, or pears and peaches with crisp textures that are less messy to eat as well as delivering a longer period in which they remain edible in the home.
7.6 Conclusions

There are two ways in which texture has an impact on the flavour of fruit and vegetables. First, the cellular and tissue structure of the flesh and the way the structure breaks down during chewing plays a critical role in the release of flavour compounds. While the knowledge of these types of interaction between flavour and texture is far from complete, there are examples showing how release of juice from fruit flesh can be inhibited during the development of some texture disorders. Second, the temporal links between softening and flavour development in fruit are the natural consequence of ripening processes. Therefore, care should be taken when attributing sensory perception and/or consumer preference to texture or flavour when both may be changing. Texture measurements are sometimes used as convenient measurements to standardise ‘ripeness’ when comparing flavour of different fruit treatments. However, research on fruit flavour also needs to take into account the potential for harvest maturity as well as storage conditions and durations to influence physiology of the produce. Knowledge of flavour–texture interactions is likely to expand as researchers exploit diverse germplasm and access to crosses such as those between Asian and European pears.

7.7 References


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Production of off-flavours in fruit and vegetables under fermentative conditions

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8.1 Introduction

One of the major problems in postharvest storage and handling of fruit and vegetables is the development of off-flavours and loss of authenticity. The appearance and sensation of off-flavours are highly associated with the accumulation of the ethanol fermentation products acetaldehyde (AA) and ethanol (Cohen et al., 1990; Hagenmaier, 2002). During aerobic respiration plant cells generate chemical energy in the form of ATP, via three distinctive biochemical pathways: glycolysis, the citric acid cycle and the electron transport oxidative phosphorylation chain (Fig. 8.1). However, in the case of anoxia (low oxygen conditions), the citric acid cycle and the electron transport oxidative phosphorylation chain in the mitochondria are inhibited, and the glycolysis pathway operating in the cytosol remains the only means of ATP synthesis (Geigenberger, 2003; Tadege et al., 1999). Under anaerobic conditions, pyruvate, the end product of the glycolysis pathway, is routed towards the production of lactate or ethanol (Perata and Alpi, 1993). Ethanol fermentation is a two-step process in which pyruvate is first carboxylated to acetaldehyde (AA) by pyruvate decarboxylase (PDC) and AA is subsequently converted to ethanol by alcohol dehydrogenase (ADH) (Fig. 8.1). Activation of ADH also involves regeneration of NAD⁺ from NADH. This regeneration is needed to keep the glycolysis process going (Megenigal et al., 2003). For each type of fruit or vegetable the O₂ level at which fermentation metabolism starts and ethanol tends to accumulate was previously termed the
Fig. 8.1  Schematic diagram of the glycolysis, citric acid cycle, electron transport oxidative phosphorylation and ethanol fermentation pathways. Glycolysis and ethanol fermentation take place in the cytosol, whereas the citric acid cycle and electron transport oxidative phosphorylation occur in the mitochondria. A list of factors that enhance ethanol fermentative metabolism in the cytosol or inhibit oxidative respiration in the mitochondria is boxed.

Pasteur point (Fidler and North, 1971), but more recently has been referred to as the lower oxygen limit (LOL), the anaerobic compensation point (ACP), the fermentation induction point (FIP) or the fermentation threshold (Boersig et al., 1988; Beaudry, 1993; Yearsley et al., 1996; Petracek et al., 2002).

The off-flavour volatiles AA and ethanol normally accumulate at low levels during fruit maturation and ripening, and thereby play an important role in the biosynthesis of fruit aroma volatiles. However, following exposure of the fruit to
anoxic conditions, the accumulation of the ethanol fermentation metabolites, AA and ethanol, may be enhanced to extreme levels, which leads to the development of off-flavours. Unfortunately, such conditions may occur at certain points during post-harvest processing and along the marketing chain. Examples of such points include: following application of wax coatings, which restrict gas exchange through the peel layer; during storage under controlled or modified atmospheres that contain low O$_2$ or high CO$_2$ levels; following implementation of quarantine treatments that involve exposure to anaerobic atmospheres, if the produce is exposed to inappropriate storage conditions, such as high temperatures, prolonged storage, and inadequate ventilation that causes a reduction in O$_2$ levels; and after exposure to ethylene and stresses.

In addition to environmental factors, the occurrence of anaerobic conditions in the internal atmospheres of fruit and vegetables, and their tendency to develop off-flavours also depend on their anatomical structure and morphology, which influence their permeability to gases and, therefore, availability of oxygen. For example, in bulky storage organs, such as apples, bananas, potatoes and legume seed pods, the internal O$_2$ concentrations may fall to very low levels imposing anaerobic stress conditions (Magness, 1920; Banks, 1983; Geigenberger et al., 2000; Rolletscheck et al., 2002).

Overall, fruit and vegetables may become exposed to anoxic conditions during their postharvest handling and along the storage and marketing chain, and this may lead to induction of fermentative metabolism and accumulation of off-flavours. In this chapter, we will describe the effects of ripening and postharvest management on ethanol fermentation metabolism, and will shed light on the processes leading to the development of off-flavours and possible ways to manipulate and perhaps reduce them.

### 8.2 Accumulation of off-flavours during ripening

AA and ethanol are precursors for biosynthesis of natural aroma compounds, and they accumulate at low levels during fruit ripening, even under aerobic conditions. In fruit tissues, AA may be converted to ethanol by the enzyme ADH, or to acetyl coenzyme A (CoA) by the enzyme aldehyde dehydrogenase (ALDH). The alcohols produced serve as precursors for the formation of volatile esters, via the activity of alcohol acetyltransferase (AAT) (Knee and Hatfield, 1981; Perez et al., 1996), and acetyl CoA serves as the precursor for the formation of acetate esters (Gilliver and Nursten, 1976).

Many studies have found a gradual increase in the production of AA and ethanol during fruit ripening on the tree, and that their accumulation is correlated with parallel increases in PDC and ADH activities. For example, in strawberry, the expression of *Fapdc1*, one of two distinct PDC genes, increased during fruit ripening and was hypothesized to play an important role in aroma biogenesis (Moyano et al., 2003). During grape berry development, PDC gene expression was rather constitutive, but the expression of *VvAdh2*, one of three different ADH
genes, significantly increased, concurrently with ADH enzyme activity during ripening (Or et al., 2000; Tesniere and Verries, 2000). The main aroma volatiles detected by measurements during on-tree ripening of peaches and nectarines were ethyl acetate, propyl acetate, butyl acetate, ethanol, propanol and butanol (Lavilla et al., 2001). Furthermore, in the light of their evaluation of the differences between the behaviour of early- and late-season cultivars of peaches and nectarines, Zerbini et al. (2001) even suggested that accumulation of certain levels of AA and ethanol could be the criteria for indicating the optimal harvest time of each cultivar. Similar increases in AA and ethanol accumulation during natural ripening on the tree have been reported for pear, litchi, banana and orange (Hyodo et al., 1983; Bruemmer and Roe, 1985; Chervin et al., 1999; Pesis et al., 2002).

Unlike the low levels of AA and ethanol that accumulate during normal ripening, enhanced levels of these compounds are associated with over-maturation and development of off-flavours. For example, ethanol is present in relatively small amounts in most apple cultivars, but accumulates to high levels in over-ripe and senescent fruit (Nicholas and Patterson, 1987). Similarly, in citrus fruit, the levels of AA and ethanol rapidly increased in juice of fruit that remained on the tree for long periods and, consequently, developed off-flavours (Davis, 1970).

Furthermore, it was reported that late-harvested fruit produced more AA and ethanol and were more prone to develop off-flavours after postharvest storage than early- and mid-season fruit. Hagenmaier (2001) reported that ‘Murcott’ mandarins harvested periodically from January until April developed about 20 ppm more ethanol for each additional week on the tree, during subsequent storage periods of seven days at 21 °C. In pears, it was reported that, after storage, postclimacteric (after onset of ripening) fruits produced much greater amounts of ethanol fermentation volatiles than preclimacteric ones (Ke et al., 1994b). Similarly, ‘Haden’ mangoes that entered a two-week storage period at the onset of the climacteric peak produced ten times more ethanol than preclimacteric mangoes of the same cultivar (Bender et al., 2000). In tomato, it was reported that pink fruits produced much more AA and ethanol after harvest than mature green or breaker-stage fruit (Ratanachinakorn et al., 1997). And finally, late-harvested litchi fruits produced much more AA and ethanol during postharvest storage under modified atmospheres than early-harvested ones (Pesis et al., 2002).

In summary, it can be seen that ethanol fermentation metabolites, AA and ethanol, accumulate at low levels during maturation and ripening and play an important role in aroma biogenesis. However, enhanced accumulation of ethanol fermentation products in over-ripe fruit and during postharvest storage may have deleterious effects and lead to the development of off-flavours (Pesis, 2005).

### 8.3 Postharvest factors governing the accumulation of off-flavours

The severity of off-flavours and loss of authenticity largely depends on how the produce has been treated after harvest. In particular, any treatment that reduced the
availability of O$_2$ to the produce or restricted gas exchange through its peel layer would have the potential to enhance anaerobic respiration and thus to induce fermentative metabolism and accumulation of off-flavours. On the other hand, appropriate postharvest management that reduces respiration and consumption of O$_2$, such as maintaining the cold chain during storage, transport and marketing and maintaining a sufficient supply of O$_2$ by adequate ventilation, would reduce the opportunities for the produce to develop off-flavours. In the following, we will discuss the effects of the main postharvest operations and handling procedures on the potential of the commodity to develop off-flavours after harvest.

8.3.1 Application of wax coatings

Coating with edible waxes is a standard commercial postharvest operation that is applied to many fruits, such as apples and citrus, since it imparts a high gloss and a shiny appearance that makes the produce more attractive to buyers and also retains firmness and reduces shrinkage and weight loss. However, application of wax coatings also inserts an additional mechanical barrier that restricts gas exchange between the fruit and its surrounding atmosphere, so that the internal atmosphere of the fruit is modified, with enhanced CO$_2$ and reduced O$_2$ levels (Davis et al., 1967; Hagenmaier and Baker, 1993). The build-up of anaerobic conditions in waxed fruits leads to enhanced anaerobic respiration and increased production of the off-flavour volatiles AA and ethanol (Davis and Hofmann, 1973; Cohen et al., 1990; Hagenmaier and Shaw, 2002).

In apples and in many other fruits, coating with waxes results in lower O$_2$ and higher CO$_2$ levels in the internal atmospheres, and concomitant accumulation of ethanol and off-flavours (Bai et al., 2003). However, large differences have been observed between the responses of different cultivars to wax coatings: in some apple cultivars, such as ‘Delicious’, the coated fruit produced only trace amounts of ethanol and remained firmer and tastier than unwaxed fruit after storage, whereas other cultivars, especially ‘Granny Smith’, were very sensitive to wax coatings, and accumulated large amounts of ethanol and developed off-flavours (Bai et al., 2003). Among citrus fruits, it is known that mandarins are most sensitive to waxes, and suffer from enhanced accumulation of AA and ethanol, and development of off-flavours (Hagenmaier, 2002; Hagenmaier and Shaw, 2002). In a recent study, we found that mandarins were indeed much more sensitive to anaerobic stress conditions than other citrus fruits, such as grapefruit, and responded to application of wax coatings with enhanced anaerobic respiration and production of off-flavour volatiles (Shi et al., 2005).

In various studies, the effects of different types of waxes on gas permeability and, consequently, on the buildup of anaerobic conditions were investigated, and it was concluded that wax-based coatings, and especially those based on polyethylene, were much more permeable to gases than shellac and wood rosin coatings (Hagenmaier and Shaw, 1992, 2002; Mannheim and Soffer, 1996; Bai et al., 2003). Recently, we recommended that ‘Mor’ mandarins, which suffer from development of off-flavours, be coated with a modified wax formulation contain-
ing only half the amount of shellac present in commercial wax formulations, since the proposed combination provided enough gloss and reduced shrinkage, while still reducing the build-up of anaerobic conditions in the internal atmosphere of the fruit, and the consequent development of off-flavours (Porat et al., 2005).

As well as apples and citrus, it was reported that tropical fruits are especially sensitive to the application of wax coatings, which may severely impair their internal fruit quality. In mango cv. ‘Tommy Atkins’ the levels of AA and ethanol were much higher in fruits coated with NutraSeal than in those coated with carnauba wax (Baldwin et al., 1999). Moreover, guava fruits appeared to be extremely sensitive to waxes: coating with carnauba wax resulted in build-up of anaerobic conditions that interfered with normal ripening (McGuire and Hallman, 1995). In Galia-type melon fruits, Fallik et al. (2005) recommended use of a commercial polyethylene-based wax that contained no or very little shellac, which restricts gas exchange through the peel layer.

8.3.2 Controlled atmosphere and modified atmosphere storage

Controlled atmosphere (CA) and modified atmosphere (MA) storage involves keeping the commodity in atmospheres whose O₂, CO₂ and ethylene concentrations are different from those found in ordinary air (Kader et al., 1989). Usually, CA and MA environments feature low O₂ and high CO₂ levels, and are applied in order to reduce respiration and ethylene production, to delay ripening and senescence, and to retard the growth of decay-causing organisms (Kader et al., 1989; Kader, 2003). CA storage necessitates precise control of the gas composition and is usually applied in large storage rooms and in shipping containers. In contrast, MA is usually created by packing the commodity in polymeric films that create a microenvironment with low O₂ and high CO₂ levels inside the package.

Optimal gas compositions for CA and MA storage vary according to the cultivar, its maturity or ripeness stage, the temperature, and the duration of exposure. In any case, for each cultivar the O₂ concentration should never fall below a certain threshold level that might enhance fermentation metabolism. However, in commercial practice, individual lots of produce are often handled for differing times and there may also be fluctuations in the temperature during storage, transportation and retail display. These sudden increases in temperature may accelerate respiration, and result in fermentation damage (Brecht et al., 2003). The changes in the ambient temperatures during marketing create a special problem in MA storage, since the packages are normally designed for specific constant temperatures, on the assumption of constant permeabilities and porosities of the film. Therefore, a film that produces a favourable atmosphere at the optimal storage temperature may cause excessive accumulation of CO₂ and/or depletion of O₂ at higher temperatures; a situation that could lead to anaerobic respiration and induction of ethanol fermentation (Beaudry et al., 1992; Joles et al., 1994).

Many studies on the commercial application of CA treatments have concerned apples, which are relatively tolerant to low O₂ levels and can be stored for very long periods under CA. Nevertheless, a recent study that evaluated consumer
acceptability of ‘Fuji’ apples during extended shelf life at 20 °C after seven months of storage under CA (2% O₂, 2% CO₂) revealed that loss of quality was mainly associated with an increase in ripe taste and accumulation of alcoholic taste and odour (Varela et al., 2005). There is also a lot of interest in adopting CA and MA technologies to extend the shelf life of strawberries: low O₂ levels (0.25–1%) retarded ripening and maintained firmness, and high CO₂ (15–20%) reduced decay development (Ke et al., 1991). Unfortunately, such extreme conditions also led to the development of off-flavours that were correlated with the accumulation of ethanol, ethyl acetate and AA (Ke et al., 1991; Larsen and Watkins, 1995; Zhang and Watkins, 2005). In fact, Sanz et al. (1999) in Spain showed that ‘Camarosa’ strawberries packed in perforated polypropylene films retained their quality during three days of storage at 2 °C but developed off-flavours upon exposure of the fruit to shelf life conditions at 20 °C.

Traditionally, most studies on CA were related to apples and pears. However, in recent years, thanks to a remarkable increase in global trading and marketing, there has been more interest in applying CA and MA technologies to the extension of the shelf lives of tropical and subtropical fruits too. A detailed study on the response of fermentative metabolism in avocado fruits to very low O₂ (0.25%) or high CO₂ (80%) levels revealed a complex network of interactions involving modifications of PDC, ADH, lactate dehydrogenase (LDH) and pyruvate dehydrogenase (PDH) enzyme activities, and metabolic control of their functions, through changes in pH, ATP, pyruvate, NAD and NADH levels (Ke et al., 1995). In mango fruits, it was found that optimal gas compositions of 6% CO₂ and 3–5% O₂ improved postharvest storage at 13 °C; however, exposure to lower O₂ levels of 1.5–2% caused significant increases in ethanol fermentation and accumulation of off-flavours, which were attributed mainly to up-regulation of ADH activity (Bender et al., 2000; Lalel and Singh, 2006). In the case of persimmons, it was reported that a combination of a treatment with gibberellins followed by MA storage at −1 °C could extend shelf life to seven months after harvest, but the limiting factor for the extension of storage duration under these conditions was the accumulation of AA and ethanol, which caused development of off-flavours (Ben-Arie et al., 1991). For grape berries, high-CO₂ atmospheres may be applied instead of chemical treatments with SO₂, in order to reduce botrytis rot development. However, storage for 16 weeks under CO₂ levels above 10 or 15% resulted in perceptible off-flavours (Cristoto et al., 2002).

Vegetables may also benefit from CA or MA storage; most such commodities require a minimum level of 1 to 3% O₂ to prevent a shift towards anaerobic metabolism (Imahori et al., 2002). Excessive CO₂ levels may also cause damage: for example, high CO₂ atmospheres stimulated ethanol fermentation and development of off-flavours in tomato fruits, sweet potato roots and intact or cut lettuce (Chang et al., 1983; Pesis and Marinansky, 1993; Mateos et al., 1993).

8.3.3 Quarantine treatments
International trading in agricultural produce necessitates the application of approved
quarantine treatments for security against infestation by insect pests such as fruit flies, moths, mites, leaf miners and thrips. In the past, the most commonly used quarantine procedure was fumigation with methyl bromide. However, because of its toxicity, methyl bromide has been phased out, and alternative quarantine treatments have been developed including ionisation, radiation, cold, heat (hot air, hot water, vapour heat), and exposure to anaerobic atmospheres. Ionisation and radiation are not often used in postharvest handling of fresh produce (fruits and vegetables), and cold quarantine treatments require long exposures of up to two to three weeks which complicates their implementation. Many fruits and vegetables, such as citrus, guava, mango, papaya and banana that require approved disinfection treatments are also chilling sensitive, so that exposure to low temperatures might cause chilling injuries. Therefore, most quarantine approaches nowadays are focused on exposure to heat and/or to anoxic conditions.

However, one of the major problems with commercial implementation of heat and CA treatments for quarantine pest control is that they may enhance anaerobic respiration and thereby promote the accumulation of AA and ethanol, with the consequent development of off-flavours. For example, Obenland et al. (1999) reported that exposure of oranges to a 48.5 °C forced-air heating treatment for more than 200 min enhanced ethanol build-up to about 1200 ppm, which caused perceptible off-flavours. Short (up to 20 min) exposure of oranges to a newly developed radio frequency heat treatment against the Mediterranean fruit fly also caused considerably enhanced ethanol levels (Birla et al., 2005). In mango fruit, hot forced air treatments at 46 °C and 48 °C for three to five hours resulted in enhanced accumulation of AA and ethanol (Mitcham and McDonald, 1993). Similarly, insecticidal CA treatments that were given alone and sufficient for insect eradication and which usually involved exposure to anaerobic conditions of ~0.25–0.5% O₂ and 35–80% CO₂ for two to five days, also induced high levels of AA and ethanol (Ke et al., 1994a, b; Ahumada et al., 1996).

In the light of these determinable effects of heat and insecticidal CA treatments on fruit taste and quality, newly developed quarantine technologies involve the combination of thermal treatments with oxygen-poor atmospheres. The integration of high-temperature and low-O₂ treatments enables considerable reduction of the time needed for pest eradication and, therefore, is much less harmful to fruit taste and quality (Shellie et al., 1997, 2001). Nevertheless, special care must still be taken to avoid induction of fermentative metabolism and development of off-flavours during commercial implementation of postharvest quarantine treatments.

8.3.4 Storage conditions
Accumulation of the fermentative metabolites AA and ethanol largely depends on the postharvest storage conditions applied to the commodity, especially storage temperature and duration. Generally, the higher the storage temperature, the greater is the production of AA and ethanol. For example, muskmelon fruits produced more AA and ethanol when kept at room temperature than at 5 or 13 °C (Choi et al., 2001). As storage temperatures increased from 0 to 25 °C, sweet
cherries stored in polyethylene packages produced more AA and ethanol, which resulted in a gradual increase in their respiratory quotient (the ratio of CO₂ production to O₂ consumption) and reduction in their shelf life (Petracek et al., 2002). Furthermore, various fruits and vegetables produced much more AA and ethanol after transfer from cold storage to shelf life at ambient temperatures (Davis et al., 1974; Choi et al., 2001; Pesis et al., 2002). In addition to higher temperatures, extended storage also resulted in gradual increases in AA and ethanol concentrations (Davis et al., 1974; Cohen et al., 1990).

Finally, another factor affecting the accumulation of ethanol fermentation metabolites is ventilation. For example, studies with citrus fruit have shown that the ventilation rate, i.e. the rate of air replacement in the storage room affects CO₂ concentrations in the external and internal atmospheres of the fruit, and that under commercial storage conditions inadequate ventilation often leads to the accumulation of ethanol and the development of off-flavours (Waks et al., 1985). Accordingly, it was recommended that the air volume in the storage room or shipping container be totally changed every hour during storage and transport (Waks et al., 1985).

### 8.3.5 Ethylene and 1-methylcyclopropene

It is generally accepted that the plant hormone ethylene is involved in some way in the response to anaerobic stress. First, exposure to anaerobic conditions increases the biosynthesis of ethylene (English, 1995) and, second, exogenous application of ethylene induces ADH gene expression and production of ethanol (Tesniere et al., 2004). In contrast, 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, reduced ethanol fermentation (Sisler and Serek, 1997). Overall, in various fruit species, such as citrus, it was noted that exposure to ethylene increased the production of AA and ethanol during post-harvest storage (Porat et al., 1999), whereas removal of ethylene from storage rooms reduce the accumulation of AA and ethanol and development of off-flavours after harvest (McGlasson and Eaks, 1972; Testoni et al., 1992).

### 8.3.6 Exposure to stresses

Besides low oxygen, exposure to various stresses, including chilling, freezing, water deficiency, and ozone also induce the production of the fermentative metabolites AA and ethanol (Kimmerer and Kozlowski, 1982). Thus, induction of ethanol fermentation seems to be an ancient and common stress response mechanism in plants. The exact function of the induction of the ethanol fermentation pathway under stress conditions is not yet clearly understood. Nevertheless, since the intricate mitochondrial ATP-generating machinery is liable to be disturbed and damaged under stress conditions, it may be that robust ethanol fermentation could provide an alternative source required to maintain glycolysis and to continue the generation of the ATP needed to cope with emergency situations (Tadege et al., 1999).

One of the most common stress responses imposed during postharvest storage
of fruit and vegetables is the development of chilling damage in chilling-sensitive commodities. Indeed, it is known that exposure to low temperatures induces ADH gene expression and enzyme activity in various plant species (Christie et al., 1991). Furthermore, it has been reported that prolonged storage of chilling sensitive commodities at low temperatures resulted in both development of chilling injuries and enhanced accumulation of AA and ethanol. For example, in chilling-sensitive ‘Oroblanco’ citrus fruit, prolonged storage for up to four months at 2 °C remarkably increased the levels of AA and ethanol. However, intermittent warming of the fruit by applying storage cycles of three weeks at 2 °C followed by one week at 13 °C reduced chilling injuries and improved the taste and quality of the fruit, a phenomenon that was related to the reduced AA and ethanol levels (Porat et al., 2003).

8.4 Anatomical factors

Another important factor governing the degree of postharvest activation of ethanol fermentation and development of off-flavours comprises the anatomical structure and morphology of the commodity, which may influence its permeability to gases and the availability of the O₂ needed for respiration. For example, in bulky and dense storage organs, such as apples, bananas, potatoes and legume seed pods, the internal O₂ concentration may fall to very low levels of 8–10% in their periphery, and even down to 2–5% in their centres – conditions that may enhance anaerobic respiration and accumulation of AA and ethanol (Magness, 1920; Banks, 1983; Geigenberger et al., 2000; Rolletscheck et al., 2002).

By testing the responses of apple cultivars to different wax coatings, it was found that some cultivars, especially ‘Granny Smith’, were very sensitive and produced high levels of ethanol after being coated with low-permeability waxes, whereas other cultivars, such as ‘Delicious’, produced hardly any ethanol (Bai et al., 2003). Further studies regarding the various behaviours of these cultivars revealed that ‘Granny Smith’ apples had relatively very few pores on their peel surface and, therefore, suffered from low rates of gas exchange and tended to develop anaerobic conditions in their internal atmospheres, which led to anaerobic respiration and production of ethanol. In contrast, ‘Delicious’ apples had many pores on their peel surface, and thus retained sufficient gas exchange even when coated with low-permeability waxes and did not accumulate ethanol or develop off-flavours (Bai et al., 2003). Therefore, the anatomical structure and the porosity of the peel of each apple cultivar determine its tendency to develop anaerobic conditions in its internal atmosphere and to accumulate fermentation metabolites.

In the case of citrus, we recently showed that mandarins are much more sensitive than grapefruit to anaerobic conditions and, accordingly, tend to develop off-flavours after harvest (Shi et al., 2005). Furthermore, we found that one of the main reasons for this observed difference in the behaviour of these cultivars was that although the total thickness of the peel is greater in grapefruit, the cells in the mandarin peel are rather more dense and, accordingly, gas diffusion tests revealed
that the mandarin peel was less permeable to gases than that of grapefruit. This difference resulted in build-up of anaerobic conditions and accumulation of AA and ethanol in the internal atmospheres of the former fruit (Shi et al., 2007).

8.5 Future trends and conclusions

If fruits and vegetables are exposed to anaerobic conditions as they pass along the postharvest handling and marketing chain, they may accumulate ethanol fermentation metabolites and develop off-flavours. In general, adaptation of plants to anoxia requires considerable expenditure of stored carbohydrates in order to release energy, through glycolysis and fermentative metabolism. Therefore, whole plants can survive only transient exposures to anaerobiosis, because of the limited carbohydrate availability (Fukao and Bailey-Serres, 2004). However, mature fruit tissues contain abundant amounts of sucrose and glucose, and these can sustain ethanol fermentation for long periods, as a result of which off-flavours may develop long before exhaustion of substrates leads to cell death.

Enhanced fermentative metabolism imposes serious difficulties during post-harvest storage, since it leads to internal deterioration and loss of fruit quality. The main factors that may enhance fermentative metabolism and postharvest accumulation of off-flavours include harvesting of over-mature fruit, application of low-permeability wax coatings, inappropriate CA or MA conditions, exposure to high temperatures, prolonged storage, inadequate ventilation and exposure to ethylene and stresses.

In the future, one possible way to reduce fermentative metabolism rates and the consequent accumulation of AA and ethanol might be to reduce the activity of PDC and ADH through genetic manipulation. Alternatively, as an integral part of plant breeding programmes, it might be possible to develop new cultivars of fruits and vegetables that would have higher peel gas permeability and would, therefore, be more tolerant of anaerobic stress.

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8.7 References


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Part III

Flavour management
9

Fruit and vegetable flavour improvement by selection and breeding: possibilities and limitations

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9.1 Introduction

Since the Neolithic, about 10 000 years ago, humans have been domesticating animals and plants. This was a radical intervention of mankind in evolution and the basis of the modern society. The domestication and breeding of plants is still an ongoing process. Beginning with collecting, growing and selecting of plant types with profitable attributes, plant breeding today has developed into a complex process of anthropocentric system optimisation. Besides yield and a guaranteed yield level, flavour of a food plant must have been an important trait for selection at very early stages. This fact is not scientifically verifiable. But whoever tasted a wild relative of carrot, apple or cucumber cultivar will find out that the flavour of wild genotypes is sometimes awful. Like other quality traits also flavour was one of the optimisation parameters during the long-lasting domestication process.

In developed societies food is produced mainly from cultivated plants, the portion of wild species in nutrition is marginal. For thousands of years humans carried out a more or less target-orientated selection for plant types that corresponded with their specific needs. Nowadays the supply of fruit and vegetables around the year is taken for granted due to modern cultivars as well as cultivation, harvesting and processing technology. Nevertheless constant criticism by consumers especially regarding the flavour of the traded commodities is pervasive.

The contradiction between long-lasting breeding efforts (sometimes for
millennia) on the one hand and increasing criticism by consumers about sensory quality nowadays on the other is a result of the complexity of the trait flavour. There are three main problems regarding flavour in plant breeding:

- First, in the majority of cases flavour, taste and aroma are multigenic traits. The aroma of plant foods is mostly the result of a complex interaction of a high number of volatiles and non-volatiles. Therefore, the inheritance of taste and odour is widely unknown.
- Second, the specialities of the plant breeding process itself like: (a) processing of individual plants (limited sample size); (b) use of populations with high plant numbers to handle the statistical effects of inheritance (sometimes several ten thousands of samples per season); and (c) the demand for non-destructive analysis methods (propagation of plants). These boundary conditions are very unfavourable for the application of sophisticated analyses and especially for human sensory analysis.
- Last but not least, industrialisation of agriculture influences breeding aims. For decades, traits like yield, harvest time, texture, shelf life and so on have been dominating the efforts of breeders instead of flavour.

The totality of the sensory characteristics (appearance, smell, aroma, taste and mouthfeel) of food influences the decision to buy fruit and vegetables to a greater or lesser extent. There is a good deal of evidence that the sensory characteristics ‘taste’ and ‘aroma’ have a very specific effect on the consumers’ food choice. Therefore, high sensory quality is not only a question of enjoyment value but also an important aspect of healthy nutrition. Flavour, like many other quality attributes of fresh and processed fruit and vegetables, is affected by the cultivation of the plant material. Cultivation, in this case, refers to the whole process of cultivar selection, production and postharvest processes that affects the physiology of the plant (Beaudry, 2000). However, the basis for high sensory quality is given by the breeder. The genetic background of a cultivar plays the decisive role for the reaction of the plant to environment. What is not fixed in the genes cannot be improved even by best sophisticated technologies. The above-mentioned contradiction can only be solved by the plant breeder using innovative analytical methods.

### 9.2 From wild genotypes to cultivars

Strawberry is one of the most popular fruits worldwide. Today we know that the cultivated strawberry derives from spontaneous hybridisation and subsequent breeding programmes, which started at the end of the eighteenth century. In Europe at that time, the so-called Chilean strawberry (*Fragaria chiloensis* (L.) Mill.) and the Scarlet strawberry (*Fragaria virginiana* Mill.) from North America hybridised spontaneously (Darrow, 1966). The resulting cultivar *Fragaria × ananassa* Duch. obtained from its parent lines several positive traits like big fruits...
from the Chilean strawberry, red colour from *F. virginiana* and pleasant aroma from both of the parents. Up to now, breeders have created an abundance of cultivars from this combination of genotypes. Around 1000 cultivars are preserved in germplasm collections worldwide. The aroma of cultivated strawberries has been studied profoundly (Latrasse, 1991). So far, 360 volatiles have been identified. Very early Drawert et al. (1973) and Staudt et al. (1975) differentiated the aroma patterns of the wild species *F. virginiana*, *F. chiloensis*, *F. vesca*, *F. moschata* and *F. nilgerrensis* Schltdl. ex J. Gay in comparison with the cultivated strawberry *F. × ananassa* cv. ‘Revata’ by gas chromatography–mass spectrometry (GC–MS). At that time, gas chromatographic separation was conducted with steel columns. Nevertheless, more than 50 volatile compounds were identified. The comparison of genotypes based on about 40 compounds. The aroma patterns of the three species varied qualitatively and quantitatively (Staudt et al., 1975). A remarkable result was that the sum of quantities of all detected volatile compounds in the wild types outreaches that of the cultivated one. The extract of *F. virginiana* contains a concentration of volatiles which is about 15 times higher than that of the level of *F. × ananassa*. A lack of this early research is that obviously it was not possible to conduct a profound sensory test of the wild accessions. Unfortunately, reliable sensory characteristics of wild strawberry genotypes have not been available in literature until now.

Recently Ulrich et al. (2007) published sensory characteristics of wild *Fragaria* accessions. The aim of the sensory assessment was to collect terms for the overall description of the sensory quality of the four chosen accessions. Taste and aroma were characterised with the impressions summarised in Table 9.1. In general all investigated wild types have a higher aroma intensity than the cultivated one. The flavour quality differed significantly. The wild species *F. vesca* ‘Geising’, *F. vesca f. alba* and *F. moschata* ‘Cotta’ comprise sensations which normally were not associated with cultivated strawberries, whereas the aroma of *F. × ananassa* ‘Elsanta’ and *F. virginiana* ‘W9’ is described as green-fruity and fresh-fruity. Especially the so-called flowery notes (e.g. like the flowers of violet, acacia . . . ) are outstanding. The notes of *F. vesca* ‘Geising’ and *F. vesca f. alba* are very intense and sometimes result in negative statements like soapy and perfume-like. Of special interest is the pleasant lactone-like note (like milk) in *F. moschata* ‘Cotta’. Noteworthy is also the astringent mouthfeeling and sometimes bitter taste of all wild types. Only the cultivated strawberry was characterised as pleasant sugar-acid balanced.

Table 9.2 contains a compilation of the main compounds which were identified by a MS library search at least in one of the genotypes (relative amounts in relation to an internal standard). The first line of Table 9.2 represents the sum of all identified volatiles. Additionally, the sums of two important substance classes are given. The lowest volatile content was found in *F. × ananassa* ‘Elsanta’ (23.40) and the highest in *F. moschata* ‘Cotta’ (166.93). In *F. virginiana* ‘W9’, the volatile content is about twofold that of the tested cultivar ‘Elsanta’. Esters are known as the most important key compounds of the strawberry aroma (Fischer and Hammerschmidt, 1992; Latrasse, 1991; Ulrich et al., 1997) which are responsible
<table>
<thead>
<tr>
<th>Genotype</th>
<th><em>F. × ananassa</em> A</th>
<th><em>F. virginiana</em> B</th>
<th><em>F. vesca</em> C</th>
<th><em>F. vesca f. alba</em> D</th>
<th><em>F. moschata</em> E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>Sweet</td>
<td>Medium sweet</td>
<td>Sweet</td>
<td>Low sugar</td>
<td>Sweet</td>
</tr>
<tr>
<td></td>
<td>Harmonically</td>
<td>Sour</td>
<td>Low acid</td>
<td>Stale</td>
<td>Astringent</td>
</tr>
<tr>
<td></td>
<td>sugar/acid</td>
<td>Astringent</td>
<td>Bitter</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>balanced</td>
<td></td>
<td>Astringent</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mealy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pronasal and retronasal smell</td>
<td>Medium aroma</td>
<td>Intensive aroma</td>
<td>Intensive</td>
<td>Very intensive aroma</td>
<td>Very intensive aroma</td>
</tr>
<tr>
<td></td>
<td>Fruity</td>
<td>Fresh-fruity</td>
<td>aroma</td>
<td>Heavy sweet</td>
<td>Sweet-flowery like</td>
</tr>
<tr>
<td></td>
<td>Green</td>
<td>Sweet</td>
<td>Sweet-flowery</td>
<td>Flowery-like</td>
<td>like melon and raspberry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>like violet</td>
<td>Jasmine</td>
<td>Green</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tart</td>
<td>Fresh-green</td>
<td>Animal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caramel</td>
<td>Red currant</td>
<td>Cheesy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slightly soapy</td>
<td>Perfume-like</td>
<td>Mouldy like milk</td>
</tr>
</tbody>
</table>

Table 9.1 Inventory of flavour impressions of wild strawberry species
Table 9.2  Result of substance identification and semi-quantification in strawberry extract by GC–MS and library search

<table>
<thead>
<tr>
<th>Substance group</th>
<th>Relative concentration(^1/)genotype(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Total of 119 volatiles</td>
<td>23.40</td>
</tr>
<tr>
<td>Sum of esters</td>
<td>5.63</td>
</tr>
<tr>
<td>Sum of terpenes</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Notes:
\(^1\) Results represent means of a three-fold replication calculated as relative concentrations related to an internal standard (0.1 ppm v/v).

for the fresh-fruity impressions. In addition, ester and terpene contents follow the trend found for the overall volatiles – low in *F. × ananassa* ‘Elsanta’ and higher in the wild types with a maximum in the investigated *F. moschata*.

With a total production of approximately 24 million tons (22 millions tonnes) carrot is world-wide one of the most important kinds of vegetable (Habegger and Schnitzler, 2005). Carrot consumption is of eminent economic importance. Additionally, carrots are of health interest to the consumer because they are the major vegetable source of provitamin A. An increase in consumption might be supported by a desirable flavour. The sensory quality of carrots is influenced by the texture, juiciness, sugar content, bitter compounds and volatile pattern. The majority of volatile compounds emitted from raw carrots are mono- and sesquiterpenes, which can comprise up to 97% of the total amount. More than 100 aroma compounds have been identified in the raw vegetable (Simon, 1985; Kjeldsen et al., 2001). The origin of wild carrot is widespread; wild genotypes grow in Europe and Asia. Today’s carrot cultivars are the product of a long cultivation process, which is not known in detail. By investigation of more than 40 carrot cultivars of different provenances using human sensory and instrumental analytics, those parameters for the sensory profile as well as the volatile and non-volatile patterns were estimated which were responsible for carrot types with high consumer preference (Hoberg et al., 2006). According to this, consumers like carrots with high values of the sensory parameters ‘sweet taste’ and ‘carrot-typical’, ‘nutty’ aroma. Parameters like ‘herbaceous’, ‘harsh’ and ‘astringent’ cause low preference ratings. High content of the terpenoid compounds α-humulene, caryophyllene and β-myrcene correlate with low preference and that is the reason why these compounds act as off-flavours. In Fig. 9.1 the gas chromatograms of extracts from a carrot cultivar and a wild type are compared. Obviously the wild type comprises qualitatively and quantitatively a manifold of terpenoid compounds in comparison to the cultivar. Also, the sugar content and the sensory perception of ‘sweet’ of the wild carrot is low. Compared with today’s expectations and experiences in carrot flavour the wild type is nearly inedible. Obviously in the beginning of domestication wild carrots were used more as a medicinal plant than a vegetable (Banga, 1957).
Human infants are born with a sweet preference and a bitter aversion (Liem et al., 2004; Reed, 2006). Humans eat almost anything, but there are some types of foods, and their associated taste qualities, that are preferred by large groups of people regardless of culture or experience. When many choices are available, humans choose foods that taste good, i.e. create pleasing sensations in the mouth and nose. The concept of good taste for most people encompasses both flavour and texture of food, and these perceptions merge with taste properly to form the concept of goodness (Reed et al., 2006). As can be seen by the examples of strawberry and carrot, the changing direction of sensory traits from wild types of fruit and vegetables to cultivars during domestication may have followed this behaviour concept of humans. Cultivars with pleasant sensory features were created from wild genotypes by selection and target-oriented breeding. The modifications of the genetic background and the metabolites profiles connected with the mentioned process are under examination (Aharoni et al., 2004) but still uninvestigated.
9.3 Plant breeding and genetic erosion

Today hundreds and thousands of registered cultivars of fruit and vegetables are available as a result of long-lasting and intense plant breeding. Nevertheless Alston (1992) stated in 1992: ‘Most of the flavours appreciated today in plant products were recognized many years ago and are often best represented in old varieties unsuited to large scale commercial production.’

After investigations of more than 70 strawberry cultivars and wild genotypes genetically manifested differences in the aroma patterns between old varieties and modern high yielding cultivars were found (Ulrich et al., 1997). Figure 9.2 shows the aroma patterns of two cultivars based on 19 character impact compounds. The cultivar ‘Mieze Schindler’ was created by Schindler in Dresden in the mid-1920s (Olbricht and Ulrich, 2006). This cultivar survived the rush of modern, high yielding cultivars in German house gardens in spite of a lot of unfavourable properties (low yield, soft berries, female flowers …). This cultivar can be generally considered as the standard for excellent strawberry flavour. Between the
aroma patterns of ‘Mieze Schindler’ and the modern cultivar ‘Elsanta’ significant
differences exist in the content of short chain fruit esters and the medium boiling
ester methyl anthranilate (Fig. 9.2). The fruit esters induce fruity, fresh aroma
impressions whereas the odour of methyl anthranilate is intensively sweetish-
flowery like the typical aroma of the woodland strawberry (*F. vesca* L.).

These metabolomic differences represent one facet of genetic erosion or the so-
called genetic funnel effect (Ladzinski, 1998; Gur and Zamir, 2004; Enigl and
Koller, 2003). It is well known that an erosion of important genetic characters like
resistance and a narrowing of the gene pool may be caused by the displacement of
traditional cultivars (local cultivars, land-races…). This alteration also affects
aroma pattern and thus sensorical traits. To overcome the funnel effect, strawberry
breeders use genetic resources by crossing with wild species. By the implementa-
tion of the Asian wild species *Fragaria mandschurica* STAUDT in a breeding
programme, clones were created whose berries possess a new type of aroma
pattern with the fivefold content of aroma compounds compared to the cultivars
(Olbricht *et al.*, 2006). But until now no knowledge exists about the inheritance
rules of aroma compounds and the work of the breeder has a coincidental mode.

**9.4 Modern breeding strategies for enhancing sensory traits**

In general, plant breeding is a very complex process, which demands a lot of
intuition from the breeder as well as scientific knowledge. For example, strawberry
breeders try to optimise up to 70 different parameters in the target cultivars. The
basic aim of plant breeding depends on, and changes according to, social develop-
ments. For decades yield was the predominating breeding aim. Later on, aspects
like resistance against diseases and quality aspects were considered. Generally
sensory quality is considered as an extremely difficult feature to handle in plant
breeding. Therefore in practical breeding this trait is included more or less
coincidentally and in very late stages of the selection process. To implement
complex quality traits like flavour in modern breeding strategies besides the
traditional parameters (yield, resistance…) innovative selection tools are required.
Plant breeding is a long-lasting interplay of creating and limiting diversity. This is
done in two steps: first, creating diversity by crossing, chemical and physical
treatments (mutation breeding) or genetic engineering (gene transfer) and, second,
selecting distinct plants with interesting traits from a multiplicity of offspring. For
the analyst these topics are very similar to those in basic research such as functional
genomics, metabolomics or metabolite profiling. The metabolite diversity, which
is created in the first step, is limiting the application of common strategies using so-
called targeted analyses particularly. In breeding it is of special advantage to use
unbiased or non-targeted strategies because the creation of diversity is the first and
essential step of the process. Using traditional strategies of targeted analyses which
include the steps of separation, compound identification and creation of calibration
tables, the possible metabolic differences caused by the diversity of the offspring
may then be overlooked. Non-targeted analysis strategies were developed to
overcome these limitations and make the metabolic data analysis unbiased. All non-targeted strategies are based on a fully automated alignment of metabolic profiles without prior assignment to the individual metabolites (Roessner et al., 2002; Tikunov et al., 2005).

In Table 9.3 examples of non-targeted strategies are summarised whereas only the first methodology, using HS–SPME–GC with flame ionisation detector (FID), has been used in practical breeding until now. The simplest way to perform a non-targeted analysis is to use gas chromatography with an FID. At the Federal Centre for Breeding Research on Cultivated Plants, rapid methods of aroma analysis were developed and applied as a selection tool. The aroma patterns of strawberry, apple, grape, carrot and parsley were determined effectively by rapid, non-targeted analyses (Schulz et al., 2003; Ulrich et al., 2005, 2006; Olbricht 2007). The developed method is a combination of effective sample preparation and non-targeted data processing. It consists of automated headspace solid phase microextraction (HS-SPME), gas chromatography (with FID or MS detector) and data processing by pattern recognition. SPME as sample preparation is a well-established method for isolation of volatiles (Pawliszyn, 1997). This technique fulfils the requirements for a rapid analysis of hundreds of samples also of small sample size, if necessary. Instead of using a calibration table, the chromatograms are cut into time slices by specially designed software (Chromstat 2.6 by Analyt, Müllheim, Germany). In Fig. 9.3 the creation of time slices on the basis of a set of 400 chromatograms is shown. By this method in principle the area of all peaks of a chromatogram set above a threshold are detectable. The method is fast and

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**Table 9.3** Examples of non-targeted metabolite profiling methodologies

<table>
<thead>
<tr>
<th>Object</th>
<th>Sample preparation</th>
<th>Separation</th>
<th>Detection</th>
<th>Data processing</th>
<th>Information</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry¹,</td>
<td>Homogenate, GC</td>
<td></td>
<td>FID</td>
<td>Pattern</td>
<td>200 peaks</td>
<td>Ulrich et al., 2005 and 2006;</td>
</tr>
<tr>
<td>apple¹, carrot,</td>
<td>HS-SPME</td>
<td></td>
<td></td>
<td>recognition</td>
<td></td>
<td>Schulz et al., 2003</td>
</tr>
<tr>
<td>parsley</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>Frozen powder,</td>
<td>GC</td>
<td>MS</td>
<td>HCA, PCA,</td>
<td>&gt; 20 000 mass</td>
<td>Tikunov et al., 2005</td>
</tr>
<tr>
<td></td>
<td>rehydrated</td>
<td></td>
<td></td>
<td>MMSR</td>
<td>fragments, 322</td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>Whole apple no</td>
<td>no</td>
<td>PTR-MS</td>
<td>–</td>
<td>Mass fragments</td>
<td>Zini et al., 2005</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>Liquid extraction</td>
<td>no</td>
<td>FTMS</td>
<td>PCA</td>
<td>Mass fragments</td>
<td>Gray and Heath, 2005</td>
</tr>
</tbody>
</table>

GC – gas chromatography; MS – mass spectrometry; FID – flame ionisation detector; HCA – hierarchical cluster analysis; PCA – principal component analysis; MMSR – multivariate mass spectra reconstruction; PTR-MS – transfer reaction-mass spectrometry; FTMS – Fourier Transform Ion Cyclotron mass spectrometry.

¹Method is introduced as selection tool in practical breeding programmes.
Time area skeleton with 87 time slices. The estimation of time slices is based on the analysis of altogether 400 chromatograms. In inactive areas no peaks (above a defined threshold) occur in all of the 400 chromatograms.

ensures that new or unexpected peaks which may occur as a result of diversity in aroma patterns are included in data processing. The results of data analysis by pattern recognition allow a fast and unbiased comparative multivariate analysis of the volatile metabolite composition.

The mass fragment pattern as an additional dimension of information is available by using a mass spectrometric detector instead of an FID (Krumbein and Ulrich, 2003; Fernie, 2003; Tikunov et al., 2006; Keurentjes et al., 2006). Tikunov et al. (2006) analysed the intensity patterns of more than 20,000 individual molecular fragments. A total of 322 different compounds could be distinguished using multivariate mass spectral reconstruction (reconvolution). Combining this kind of metabolite profiling for instance with further non-destructive spectroscopic analyses, a complex characterisation of plants is possible. Figure 9.4 demonstrates
a strategy for screening single carrots from an F2 population by molecular, spectroscopic, texture and aroma analyses (Ulrich et al., 2006).

But in general it has to be considered that any method to be used in practical plant breeding must be characterised by rapid sample preparation, robustness and stability over a long period because of the tediousness of breeding programmes. Using rapid methods including non-targeted strategies, heritability studies for flavour compounds were also enabled (Ulrich et al., 2005, 2006, 2007; Olbricht et al., 2006). Inheritance analyses of several important aroma compounds help breeders to select suitable crossing partners with high flavour potential, using the genetic variation in the plant kingdom, and thus assist the selection process. The possibility of analysing the flavour status in populations is the prerequisite to complete genomic maps with flavour data and for a marker assisted selection (Ulrich et al., 2006). This combination of molecular techniques and innovative metabolite profiling with traditional plant breeding methods is called ‘smart breeding’ (Gur and Zamir, 2004; McCouch, 2004).

9.5 Outlook: What can we control? To what should we aspire?

Because sensory traits are genetically determined, the basis for high sensory quality of plants is provided by breeding. Plant breeding is a long-lasting and never-ending process due to the fact that environment and social conditions are changing. The genetic architecture combined with the metabolic profiles are subject to a dramatic change by the process of domestication and cultivar breeding. As a result of domestication, cultivars with pleasant sensorial quality were created and often best represented in old varieties and land-races unsuited to large-scale commercial production. Plant evolution under domestication has led to increased productivity, but at the same time it has narrowed the genetic basis of crop species (Gur and Zamir, 2004; Ladizinsky, 1998) including traits like aroma. Today’s task for the breeder is to create good-tasting fruit and vegetables. But new cultivars which are placed on the market have to compete in every agronomical and quality aspect with the existing ones. Flavour is one of the most important aspects in this process. Extraordinary flavour may contribute to selling added values like bioactivity. Therefore a major objective in modern breeding is to return to those ancestors of crop plants which possess excellent sensory quality. It is necessary to employ some of the diversity that was lost during domestication and improvement of agricultural yields (Gur and Zamir, 2004). Excellent tasting fruit and vegetables play not only an important part in providing essential nutrients but they are also essential for our well-being and thus for human health. To implement a sophisticated trait like flavour in already complex breeding programmes, innovative and robust analytical tools as discussed above have to be used by cooperation between breeders, analysts and computer scientists.
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Role of maturity for improved flavour
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‘…but this [a fruit’s] beauty serves merely as a guide to birds and beasts in order that the fruit may be devoured and the manured seeds disseminated.’
Charles Darwin

10.1 Introduction
In this chapter the role of maturity and of harvest in determining fruit quality, and especially flavour, is reviewed. Flavour of fruits is characterized by sweet and sour taste, with typical aroma which is often very specific and allows identification of the species or cultivar. Texture is required to be such (crisp and juicy or melting) as to favour the release of the flavour in the mouth. These characteristics are not permanent, but are developed in the course of fruit growth, and especially during maturation and ripening. The main biochemical and physiological changes occurring in fruit maturation and ripening regard colour, composition and structure. Pigment breakdown and formation, hydrolysis of starch, sugar and acid metabolism, biosynthesis of volatiles, cell wall breakdown, respiration and ethylene production, all have an effect on flavour. The ripening syndrome is a transient process, ending with fruit senescence. For human consumption, optimal harvest of fruit should allow the development of all positive attributes of ripening fruit, while having the time for handling, storing and marketing the product. Maturation does not occur simultaneously in all fruit of a tree, giving rise to variability. An optimal harvest stage has to be determined so as to maximize quality and yield, while minimizing fruit loss. Maturity indices have been traditionally developed to define minimum maturity for harvest in different fruit species. Recently new non-
destructive methods are emerging as an innovation for quality assessment and control. The criteria for determining optimum harvest date are discussed, together with possibilities of maturity management.

10.2 Changes occurring in fruit with maturation and ripening

10.2.1 What is maturation and ripening?
During development, horticultural crops undergo a series of processes which comprise growth, maturation and ripening, ending with senescence. Each species has its own particular development, which is related to the physiological and biochemical changes as well as to the usage of the crop. The different developmental stages have been defined in a general way by Watada et al. (1984). Maturation is there defined as the stage of development leading to the attainment of physiological or horticultural maturity. Physiological maturity is the stage of development when a plant or plant part will continue ontogeny even if detached. Horticultural maturity is the stage when it possesses the prerequisites for utilization by consumers for a particular purpose. Ripening is the composite of the processes that occur from the latter stages of growth and development through the early stages of senescence and that results in characteristic food quality, as evidenced by changes in composition, color, texture, or other sensory attributes (Watada et al., 1984).

During maturation and ripening, which in many fruits corresponds to the last period on the tree, several biochemical and physiological changes occur in fruit. Some of them are readily recognizable at sight, like the increase of fruit mass and the change in colour, while others occur inside the flesh and cannot be seen.

10.2.2 Ethylene production
As regards ripening, traditionally fruits are divided into climacteric and non-climacteric. In the latter there is a gradual transition between mature and ripe fruit, while climacteric fruit are characterized by a sharp increase in ethylene production which controls the initiation of the ripening process (Alexander and Grierson, 2002). However, ripening processes differ greatly between species, and ethylene-dependent as well as ethylene-independent mechanisms co-exist to coordinate the process in climacteric and non climacteric fruit (Lelièvre et al., 1997). Recent discoveries in genomics of ethylene-independent signalling suggest that common regulatory cascades may operate in all fruits (White, 2002). Even in climacteric fruits, different feedback regulation systems of ethylene biosynthesis and different ethylene-dependent manners of ripening related gene expression operate in different kinds of fruits. Ripening phenomena in non-climacteric fruits are not different from those in climacteric fruits with respect to all events such as sugar accumulation, acid decrease, colour development, aroma production and flesh softening (Inaba, 2007). From a physiological point of view the climacteric fruit are characterized by the autocatalytic production of System 2 ethylene which results in an exponential increase of the ripening hormone with a peak. Senescence follows
the decrease of ethylene biosynthesis. The peak of ethylene may be accompanied by a peak of respiration, such as in apples, but in some species, such as peach and tomato, an increase in respiration is not always required (Saltveit, 1993). In peaches, ethylene production occurs earlier and is higher in fruit fed by a larger number of leaves (Souty et al., 1999).

In apples, ethylene production and respiration occurs differently according to the tissue: autocatalytic ethylene production in the core (carpellary) tissue of pre- and post-climacteric fruit preceded and generally was greater than within other tissues, while respiration was always higher in the skin, in which the climacteric rise was more drastic, suggesting a ripening initiation signal originating and/or transduced through the carpels to the rest of the fruit (Rudell et al., 2000). Thus, internal ethylene concentration (IEC) is suggested as a more accurate indicator of the climacteric ripening onset, as compared to evolved headspace ethylene (Fellman et al., 2003).

Many ripening processes are under ethylene regulation. Some regulate directly the biosynthesis or degradation of flavour compounds, such as the sugar (mainly sucrose and fructose) and organic acids metabolism and ester accumulation (Defilippi et al., 2004), the enhancement of colour (mostly carotenoid pigment biosynthesis), the loss of chlorophyll, cell-wall degradation, increases in pH and soluble sugars, and enhanced biosynthesis of aroma compounds (Alexander and Grierson, 2002).

10.2.3 Fruit mass and shape
In the last period on the tree the fruit mass increases: in peaches average fruit mass can increase by 2–10 g/day (Bassi et al., 1995), in Comice pears by 4 g/day (Eccher Zerbini et al., 1996a). Individual fruit mass is difficult to follow on the tree, whereas fruit diameter can be easily measured. In nectarines the increase of fruit diameter in the last 2–4 weeks before harvest was linear in time until the fruit was fully mature; the diameter increase was not affected by background colour, but was only dependent on initial diameter, i.e. the increase was larger in larger fruit (Eccher Zerbini et al., 1996b). So the smaller fruit are not likely to reach the same size of larger fruit, even if their harvest is somewhat delayed.

Some change in shape can also occur in the last stage of growth, such as a more rounded cross-section in banana, or a full ‘cheek’ in mango and stone fruits. Fruit size has also an impact on flavour, as larger fruit have generally a higher sugar content (Jacob et al., 2006), but, in the case of fruit for long storage such as apples, large size fruit may be more sensitive to storage disorders. Within a tree, the relative size of fruit can be traced back to flower bud formation (Gillaspy et al., 1993) and is related to the number of cells at anthesis, while for the relative size of fruit between trees, the contribution of cell volume due to cell expansion is more important (Jackson and Coombe, 1966). Resource availability during cell division influences final cell number: earlier fruits have a competitive advantage for assimilates in the period of cell division, so a larger number of cells (Jullien et al., 2001). While maturation and ripening appear to occur in the same way in all fruit
independently of size (Eccher Zerbini et al., 2006; Tijskens et al., 2006), within a tree the larger fruit are generally the earlier maturing ones (Jackson and Coombe, 1966; Génard and Gouble, 2005).

10.2.4 Fruit colour: chlorophyll, carotenoids and anthocyanins
The green colour typical of immature fruit is due to chlorophyll. Other pigments, which contribute to fruit colour, are anthocyanins and carotenoids. During maturation and ripening, chlorophyll in fruit skin and flesh is progressively broken down. Carotenoids may be masked by chlorophyll and become visible when chlorophyll is broken down. Carotenoids are associated to the turning from green to yellow (β-carotene) or red (lycopene, in tomato) as chloroplasts are transformed into chromoplasts. A similar change in colour occurs also in the mesocarp of some fruits (e.g. peaches, apricots and plums). The regulation of carotenoid biosynthesis and gene expression is complex and, at least partially, dependent on ethylene (Marty et al., 2005). Important fruit aroma volatiles (monoterpenes and norisoprenoid volatiles) are derived from the degradation of carotenoid pigments; so marked taste and aroma differences may be detected among fruits of different colours (Lewinsohn et al., 2005).

Anthocyanins provide the red colour which, as a blush, may mask other pigments in many fruits (apples, peaches, strawberries). In apples, anthocyanins are accumulated in the vacuoles of hypodermal and sometimes epidermal cell tissue (Fellman et al., 2000). Anthocyanins share precursors with acetate esters, which contribute to apple flavour. Among Red Delicious apple strains, higher-colouring strains have lower aroma content: increased synthesis and sequestration of acetate moieties in the anthocyanin molecules deposited in peel cell vacuoles appears to reduce the capacity for acetate ester synthesis by limiting substrate availability (Fellman et al., 2000).

Light is required for anthocyanin biosynthesis. In old varieties of peaches and apples a red blush is an indication of mature fruit well exposed to sunlight on the tree, thus of high quality. Breeders have favoured the selection of new cultivars with an early development of red blush already in the immature fruit, which is attractive for consumers but which often masks the green–yellow background colour, making it difficult to assess the maturity stage and the real quality of fruit.

10.2.5 Starch
Starch accumulates in many climacteric fruits, such as apples, pears, mangoes, kiwifruit, banana, etc. It does not accumulate in stone fruit (peaches and nectarines, apricots, plums, cherries). Starch hydrolysis usually begins in the later stages of fruit growth, so increasing sugar content (Knee, 1993). Typical patterns of starch hydrolysis, proceeding from the carpels to the skin during maturation, are evidenced by the iodine test and can be used as a maturity index in pome fruit. With starch hydrolysis, fruit flavour improves during ripening, due to the increase in sugar content.
10.2.6 Sugars and acids
Sugars are the product of photosynthesis, mainly of leaves which supply assimilates to fruit, but also of the fruit itself: for example, peach fruit can contribute up to 9% of the total carbohydrates (Pavel and DeJong, 1993). Total sugars increase during maturation. Different sugars can be present in different amounts depending on species and variety. In peaches, during the maturation of fruit on the tree, sucrose is accumulated in the fruit to become dominant fruit sugar (Kobashi et al., 1999; Moriguchi et al., 1990; Jacob et al., 2006). In pome fruit such as pears the main sugar is fructose (54–63%), sorbitol accounts for 22–31%, glucose for 11–15% and sucrose for 4–5% (Eccher Zerbini, 2002). During ripening, sucrose increases and sorbitol decreases without changing the Brix (Drake and Eisele, 1999).

In ‘La France’ pears more than 80% of each sugar was found in vacuoles, with no difference between sucrose, fructose, glucose and sorbitol. Sugars located in free space increase with fruit maturation, and also during ripening (Yamaki et al., 1993). The increase of sugar concentration in free space may be due to the enhancement of permeability of sugars across the tonoplast and plasma membranes, thus giving the fruit a sweet taste. The sugars (fructose) are precursors of furanones, which are key compounds of strawberry flavour (Bood and Zabetakis, 2002).

Different organic acids are present in fruits. The composition of organic acids may vary in different species and even in varieties within the same species. In general malic and citric acids are prevalent, while other organic acids (quinic, succinic, shikimic) are present in lower amount. During maturation and ripening, organic acids decrease as they are used as a substrate for respiration. In peaches, citric acid decreases and malic acid increases during ripening (Vanoli et al., 1993; Ventura et al., 1995; Jacob et al., 2006). The relative proportions of citric to malic acid may be reversed with increasing maturity. This may affect remarkably the fruit flavour, as it has been reported that citric acid masks the perception of sucrose (Schifferstein and Fritjers, 1990) and fructose (Pangborn, 1963), while, on the contrary, malate seems to enhance sucrose perception (Fabian and Blum, 1943).

10.2.7 Softening
During maturation and ripening, cell wall material changes its structure. Pectins and hemicellulose are progressively solubilized and depolymerized to lower molecular weights. The bonds between different constituents of the cell wall are weakened, decreasing its mechanical strength, leading to softening. Firmness measured by penetrometer is a standard measurement for maturity of many fruits. The ordered action of different enzymes on cell walls during maturation and ripening is largely, but not completely, coordinated by ethylene (Alexander and Grierson, 2002).

The effect of softening on flavour is mainly due to the texture which allows or not the release of the cellular content. When cell walls are easily fractured while cells are still adherent to one other, cellular content can be easily released, producing a juicy texture. If cells are easily separated while individual cell walls
remain intact, a mealy, dry texture is perceived. The modifications of both pectin and hemicellulose are essential for the development of a melting texture in pears (Hiwasa et al., 2004).

Softening may not occur homogeneously in the fruit: in mango it begins near the seed extending towards the peel, while in peach it begins below the peel extending to seed. Large differences of firmness are also found between different positions in the same fruit by as much as 400% (Tijskens et al., 2007). Some effects of softening are also related to metabolism. In tomato it was suggested that softening could induce activity of the alcohol dehydrogenase (ADH) enzyme, which is involved in several aspects of the flavour development in the ripening tomato fruit (Speirs et al., 2002).

10.2.8 Volatiles
The production of volatiles changes both in quantity and in quality with advancement of maturity and during ripening. In general aldehydes are high in immature fruit and decrease with ripening, while esters and specific character impact compounds increase and are high in ripe fruit. In immature peaches and nectarines, C6 aldehydes and alcohols are the major components. During fruit maturation, the concentrations of these compounds decrease, and those of lactones with long side chain (γ-decalactone, δ-dodecalactone), benzaldehyde and linalool increase, reaching the highest amounts in mature fruit (Engel et al., 1988; Horvat and Chapman, 1990; Robertson et al., 1990; Rizzolo et al., 1998; Visai and Vanoli, 1997). Both in peaches and in nectarines, lactones, especially γ- and δ-decalactones and γ- and δ-dodecalactones, are the character impact compounds, while the other contributive volatiles, such as aldehydes, alcohols and terpenoids, are responsible for the different bouquet of the various cultivars (Spencer et al., 1978; Rizzolo et al., 1995).

In muskmelon fruit, the main flavour components are esters which increase dramatically at ripening, in correspondence to the ethylene peak (Senesi et al., 2005). In strawberries the concentration of green aroma components such as hexanal, trans-2-hexenol and cis-3-hexenyl acetate progressively decreased during maturation, while total volatiles, mainly esters followed by aldehydes and alcohols, rapidly increased near to maturity (Azodanlou et al., 2004). In ‘Kensington Pride’ mango, the maturity stage at harvest affected the composition of fatty acids. Ripe fruit harvested at the sprung mature green stage exhibited higher total amounts of the aroma volatiles, monoterpenes, sesquiterpenes and aromatics. Fruit harvested at the fully ripe stage resulted in a high concentration of esters, alkene and norisoprenoids (Lalel et al., 2003a). Most of the fatty acids increased during postharvest ripening. Monoterpenes were the most abundant volatile compounds but decreased as ripening progressed; sesquiterpenes were intensively synthesized in the early part of the ripening process. The production of esters increased sharply with ripening. It appeared that production of terpenes was parallel with production of ethylene, while production of esters appeared to be associated with production of fatty acids (Lalel et al., 2003b).
Aldehydes are high in preclimacteric apples then decrease, while alcohols and esters increase with advancing harvest date (De Pooter et al., 1987; Rizzolo et al., 1988; Mattheis et al., 1991; Fellman et al., 1993). Volatiles are produced by apples during the postharvest ripening, but the onset of volatile production is delayed if apples are picked early and the production is lower compared with later picked apples (Dirinck et al., 1989; Vanoli et al., 1995). Too early a harvest may result in pronounced lack of flavour development (Mattheis et al., 1991). Late-harvested fruit may undergo rapid firmness loss during storage, losing the desired crisp texture, while early-harvested fruit have longer storage life. In apples, production of flavour volatiles, which is dependent on ethylene, occurs mainly in the skin tissue, apparently because of an abundance of fatty acid substrates resulting from modified metabolic processes and enhanced enzymatic activity (Fellman et al., 2000). Precursor availability is a more significant factor than enzyme activity for the development of aroma during on-tree maturation of Fuji apples (Echeverria et al., 2004a). At harvest, in multiple-harvest experiments with ‘Redchief Delicious’ apples, overall flavour perception by untrained panellists, and perceived fruit ripeness begin to increase at the onset of the climacteric. Total volatile production by stored fruit increased with harvest date (Fellman et al., 2003).

When considering the impact of volatile compounds on flavour, besides the actual amount of volatile compounds produced by fruit, their odour detection threshold concentration should also be considered, above which the compound can be detected by human olfaction, in order to evaluate their flavour quality (Rizzolo et al., 1997, 2006; Echeverria et al., 2004b).

10.3 The effect of harvest

Harvest is the single operation which most affects the quality of fruit. Harvest maturity is of remarkable importance for eating quality of fruit, for storage quality, for susceptibility to storage disorders and the ability to reach good quality after storage. With harvest, the input of water and sugars to the fruit is interrupted, while all the vital processes (fruit metabolism, respiration, transpiration) continue. Provided that a minimum level of maturity is reached by the fruit, biochemical changes due to maturation and ripening may occur both on and off the tree.

Tomatoes are generally harvested at the mature green stage and ripened off the vine during transit and marketing. Ripening off the vine reduces time to harvest, increases turnover, allows longer time for transportation and distribution, and increases shelf life. Many researchers have compared the quality of tomatoes ripened on and off the vine. In the literature reported by Arias et al. (2000), at sensory evaluation, field ripened tomatoes were judged sweeter and better in flavour and overall quality, while the differences in fruit composition were scarce and generally explained by the different light exposure of fruit on and off the vine. When a comparison was made in the same environmental conditions, tomatoes ripened on the vine had significantly more lycopene, β-carotene, soluble and total solids, higher a* and lower L*, and were firmer. However, the chemical and
physical differences were mostly not large enough to influence the perception of a 100-judge panel, who rated only the colour and the overall liking of the vine-ripened tomatoes as more intense than the fruit ripened off the vine (Arias et al., 2000). In a similar experiment Wold et al. (2004) found no significant difference as regards L*, a*, TSS, titratable acidity (TA), vitamin C and FRAP value (a measure of antioxidant capacity), while the only significant difference was found in b*-value and dry matter.

In strawberries harvested at four stages of colour development ranging from colour break to full red a comparison was made between changes occurring during development in the field and those during storage (eight days at 1 °C). Strawberries harvested at three-quarters coloured and full red stages continued their development and ripening during storage, while fruit harvested at the colour-break and half-coloured stages did not develop like those ripened in the field. Strawberries harvested at the three-quarters coloured stage showed after storage the same fruit composition (pH, acidity, TSS, ascorbic acid, total phenolics content) and quality parameters (colour, firmness) as the full red stage fruit at the time of harvest (Nunes et al., 2006). The amount of total volatiles compounds rapidly increases after the three-quarters red stage (Azodanlou et al., 2004).

Apples harvested long before harvest maturity produce far less flavour imparting volatiles than those harvested at optimum maturity (Song and Bangerth, 1996). In apples, during on-tree ripening, both chlorophyll and carotenoid content decreased synchronously; after fruit detachment a sharp increase in carotenoid content was found, with the carotenoid to chlorophyll ratio strongly correlated to the chlorophyll content at harvest (Solovchenko et al., 2005). In general a fruit may ripen off the tree but if it is harvested immature it will be of poor quality, while a mature fruit will attain good quality (Crisosto, 1994).

### 10.4 Maturity indices

Maturity indices have been developed to define maturity for harvest in different fruit species. A minimum maturity is generally indicated as the stage of development giving minimum acceptable quality to the ultimate consumer. The minimum level depends on the destination of the product: for nearby or far markets, for immediate consumption or for storage, for short-term storage or for controlled atmosphere long-term storage. The maturity may be more advanced for immediate consumption, so reaching a better quality in terms of sugars and volatiles, while for storage the fruit should not be full ripe, otherwise it can be subject to storage disorders and decay. A maximum maturity index was also developed for stone fruit cultivars, based on critical bruising thresholds, because with advancing maturity the tolerance to mechanical injury decreases (Crisosto et al., 2004a).

Many of the characteristics discussed in the Section 10.2, on changes occurring with maturation and ripening, have been used as maturity indices. The background colour of skin, when it is not covered by blush, is the most reliable maturity index in peaches and nectarines (Delwiche and Baumgardner, 1985; Eccher Zerbini et al.,
Fruit colour is currently used as an index for harvesting strawberries and has been recognized as a good maturity index also for guava (Mercado-Silva et al., 1998). The colour of the flesh is recommended for clingstone peaches (Kader et al., 1982) and may be used also for dark plums (Testoni et al., 1993). Starch hydrolysis is a maturity index for apples and pears. It can be evaluated, after reaction with iodine solution, by comparison with photographs, which reproduce the typical patterns occurring in fruit during maturation (Planton, 1995).

Total soluble solids (TSS) content is used to define a minimum level of maturity in some starch-accumulating fruits, like kiwifruit and mango. On the contrary, in stone fruits like peaches and plums, soluble solids content is very variable among seasons and varieties, and is more a quality index than a maturity index.

Firmness is a standard measurement for maturity of pears, apples, peaches and other fruits, despite the fact that it can only be measured on representative samples, being destructive. Internal ethylene production can be used for some apple varieties, like Gloster and Elstar, to define the maturity in relation to the beginning of the climacteric, while for other varieties, like Golden Delicious and Granny Smith, which do not produce substantial amounts of ethylene at harvest, it is less useful.

A better picture of fruit maturity can be obtained if many of the indices are combined together. The Streif index = Firmness/(TSS*Starch hydrolysis) helps in defining the harvest maturity of apples and pears (Streif, 1996). Recently new non-destructive methods are emerging as an innovation for quality assessment and control, which have also been used to evaluate maturity. Electronic noses have been used as non-destructive tools for classifying maturity of apple by discriminant analysis (Pathange et al., 2006) and to evaluate the optimum harvest date of apples, based on preclimacteric volatiles; the model was dependent on cultivar and year (Saevels et al., 2003). A non-destructive impact firmness sensor gave good agreement with current inspection method in categorizing clingstone peaches into firm and soft classes (Slaughter et al., 2006).

Optical methods are widely used, both as colorimetric and spectrophotometric methods. Spectral and colour measurements were used to detect immature tomatoes which will never turn red (Hahn, 2002). A portable miniaturized spectrophotometric module has been developed for use in the orchard for apple fruit maturity monitoring on the tree, by spectral measurement of partial light transmittance through the fruit in the wavelength range 500–1000 nm (Herold et al., 2005). The optimum harvest date of apples was predicted by a calibration model using visible and near infrared (VIS-NIR) spectroscopy; the error of prediction was of the same order as the variability of maturity in individual fruit (Peirs et al., 2005). In mango, NIR spectroscopy was used to develop calibration equations to determine dry matter and starch content as harvesting indices in green mango; the non-destructive measurement of dry matter and starch was correlated to TSS in ripe mango fruit (Saranwong et al., 2004). Also in mango, a model with Lab* colour values was found which predicts TSS (Jha et al., 2007).

While NIR spectroscopy can very well estimate TSS and dry matter, its results to predict firmness are not satisfactory. A different approach to predict firmness is
the measurement of scattering in spatially resolved diffuse reflectance by using multi-spectral (Lu, 2004) and hyperspectral imaging (Lu and Peng, 2006): soft fruit tend to have a broader scattering profile than firmer fruit. A new optical laser-based method, time-resolved reflectance spectroscopy (TRS), can measure both absorption and scattering properties within the fruit flesh, at a depth of about 2 cm, without being affected by the skin. The absorption coefficient at 670 nm, $\mu_a$, can be related to the chlorophyll content in the fruit flesh and hence to maturity (Eccher Zerbini et al., 2003). In nectarines, by using an appropriate model, $\mu_a$ can be considered as an index of the biological age of the fruit. Irrespective of the maturity or the size at harvest, the decay of $\mu_a$ follows the same curve in all fruit when expressed in biological time (i.e. the time relative to the biological age), indicating that the mechanism of decay is the same both on and off the tree, and is not affected by fruit size (Tijskens et al., 2006). A kinetic model was developed linking firmness decrease during ripening to the absorption coefficient $\mu_a$ measured at harvest: the results suggest that interestingly in nectarines the degreening of fruit flesh, expressed as $\mu_a$, is synchronized with the softening, so that, knowing the $\mu_a$ at harvest, the individual fruit softening can be predicted (Tijskens et al., 2007).

### 10.5 Optimal harvest stage

On a tree, fruits are heterogeneous, because of hormonal and growing factors: different position along the shoot, different number of leaves per fruit, different exposition to light and shade, different times of flowering and fruit set, etc. Fruit heterogeneity as regards size, colour, chemical composition as well as their physiological state, i.e. their maturity level. In one tree, at the same moment immature, mature and overmature fruit can be found. From a biological standpoint, fruits have evolved to protect and disperse seeds, so asynchronous maturity and ripening can be considered an effective strategy to escape adverse conditions and to increase the probability of seed dispersal by animals, but it is a challenge for growers who want to deliver fruit with consistent eating quality. An optimal harvest stage has to be determined so as to maximize quality and yield, while minimizing fruit loss. The choice of the optimal harvest thus regards both the right time for picking each fruit during its maturation, and the choice of which fruit to pick at each time. The total dessert quality of fruit at harvest and after storage is greatly influenced by the stage of fruit maturity at the time of picking. Fruits are often harvested before the stage of ripeness for optimal eating quality is attained, to improve storability and transportability.

Optimum harvest date for apples, i.e. the one associated with the longest storage life and lowest incidence of storage disorders, occurs just prior to the onset of respiratory climacteric (Luton, 1996). The onset of ripening is associated with the hydrolysis of starch into sugar. As ripening progresses, flavour potential increases, but storage potential decreases. Volatile production is highly maturity-dependent and closely related to the changes in the respiratory rate and ethylene production. Fruit maturity at harvest influences the timing of the onset of changes in CO$_2$,
ethylene and aroma production similarly: fruits picked very immature initiate a respiratory climacteric and an increased aroma production only very late, if at all, during storage (Song and Bangerth, 1996). Optimum harvest date is important to achieve good eating quality. Acceptance by consumers can be predicted by TSS, TA and firmness in some apple cultivars (Golden Delicious and Elstar). However, for Gala and Elstar, consumer acceptance was more dependent on aroma quality (related to volatiles) and juiciness (Hoehn et al., 2003). In plums, late harvested fruit are susceptible to flesh translucency, whereas early harvested fruit are prone to develop flesh bleeding/browning (Crisosto et al., 2004b). Consumer acceptance was higher for fruit with TSS>12°Bx, regardless of TA. TA played a significant role in consumer acceptance if TSS were in the range 10–12°Bx: high TA plums were more disliked than low TA plums. Ripening plums before consumption decreased TA, which may increase the acceptability of plums which otherwise would be unacceptable (Crisosto et al., 2004b).

A compromise must be reached between the prospective length of storage- and shelf-life on one side, and the eating quality on the other side. The choice of the optimum harvest date in a given orchard is based on monitoring the maturity indices and on the historical records of the outcomes of previous years. Due to differences from season to season, each year the optimum harvest date may be different and has to be confirmed.

10.6 Managing maturity and ripening in the fruit production chain

Ripe, soft fruit are easily bruised and sensitive to decay, so for transport and handling firm fruit is preferred. However, excessive firmness is often an obstacle to consumption of stone fruit like peaches and nectarines. Excessive firmness means that the fruit have not developed the typical flavour, and in extreme cases they may not ripen at all (Tijskens et al., 2007). Aware of this fact, some marketing chains or shippers in France have begun to commercialize fruit that will be ready to eat at the time of purchase. This involves careful picking at the proper maturity stage and controlled ripening of fruit before it is displayed to consumers. The process consists of continuing the natural maturation that begins on the tree under controlled temperature at 21–25 °C, and requires strict control on fruit management in the chain (Chapon et al., 2000). Following the ripening treatment, the fruit is cooled to 4–8 °C for transport within four days from harvest. A similar protocol for a preconditioning treatment has also been developed in California: a 24–48 hours cooling delay at 20 °C prevents chilling injury while making available ready-to-eat fruit (Crisosto et al., 2004c). Such fruit requires careful handling and packaging to avoid bruises, but is highly appreciated by consumers (Crisosto and Valero, 2006).

An alternative approach may be the use of a combination of non-invasive measuring techniques and process orientated modelling. Selecting fruit at harvest based on TRS absorption at 670 nm and applying the kinetic model parameters
linking \( \mu \) to softening, individual fruit can be graded at harvest into classes of usability. Selecting fruit with different age for different market segments and predicting their softening time, thereby guarantees sufficient firmness to transport the fruit and sufficient ripening potential to reach good eating quality upon arrival in the receiving regions or countries (Tijskens et al., 2007). This would be an improvement of fruit quality for the consumers, who would also experience a lesser variability from fruit to fruit. Consumers therefore will be more satisfied, encouraging them to repeated purchase. Retailers and wholesalers will be more satisfied since fruit unfit for sale no longer needs to be transported.

10.7 Future trends

Future trends of investigation as regards the role of maturity in flavour development can be envisaged in different directions: investigation on individual fruit basis by non-destructive means, modelling fruit and flavour development, and management of fruit variability. These aspects are also related to each other. Most of the studies on maturity in the past have been carried out by comparing some properties, which could not be measured by non-destructive means, in fruit harvested at different times. It may be expected that the continuous development of non-destructive methods will aid in studying the evolution of maturity in the same individual fruit, therefore avoiding the errors that are due to fruit-to-fruit variation.

The knowledge that has been and is being accumulated on physiology, biochemistry and molecular biology of fruit maturity, needs to be organized in some usable way. In recent years fruit crop models have been developed that go beyond fruit dry mass accumulation and include fruit quality: quality determining behaviour of fruits is described based on available knowledge of physiological processes, using kinetic models. Several models were developed to describe the main processes involved in the build-up of peach fruit quality during fruit growth and maturation, like changes in sugar composition (Génard and Souty, 1996; Génard et al., 2003) and in acids (Lobit et al., 2003, 2006). The autocatalytic burst of ethylene was described in function of ethylene biosynthesis, diffusion and dilution during fruit growth (Génard and Gouble, 2005). Some of these models are combined in a virtual peach fruit model simulating changes in fruit quality during the final stage of fruit growth considering fruit size, dry matter and sugar content (Lescourret and Génard, 2005). This model provides a systematic framework to describe the effects of the environment and fruit-bearing stem, process linkages to generate simple and general laws based on relationships between quality traits and physiological variables, and emerging properties describing complex behaviour, not intentionally included in the initial model design (Struik et al., 2005). The complexity and the challenges of this process-based modelling approach are discussed in Génard et al. (2007). This approach also has a potential use for other fruits, to improve our understanding of the key processes which determine fruit quality, also in view of application in orchard management for fruit production.
While the latter approach can describe average fruit quality behaviour based on fundamental physiological mechanisms, it does not tackle the biological variation which is always found in a batch of fruit. During growth, all kinds of small local differences in growing condition (position of fruit on the tree relative to leaves and other fruit, microclimate, hormonal and nutritional effects) integrate into a considerable variation in quality at the moment of harvest. Dealing with biological variation has become a major topic in recent literature (Schouten et al., 1997; Hertog, 2002; Tijskens et al., 2003; Lammertyn et al., 2003; Schouten et al., 2004; Hertog et al., 2004; De Ketelaere et al., 2006; Tijskens et al., 2006). Assuming that all fruit in a batch go through the same ripening process, variation in quality at harvest can be assumed to be due largely to a different stage of maturity, i.e. a different biological age. If the kinetic process describing ripening is combined with the stochastic theory to predict quality variation, then the biological variance can be modelled by a random variable using the concept of biological age (biological shift factor) and be incorporated into the model (Schouten et al., 2004; Hertog et al., 2004). The most interesting property of the biological age is its distribution, which is normal, regardless of the time of harvest (Schouten et al., 2004; Hertog et al., 2004; Tijskens et al., 2006), which greatly enhances its usability. On the contrary, other parameters taken as maturity indices, like colour, firmness or absorption coefficient $\mu_a$, have distribution which changes depending on the time of harvest and on the mechanism of change: for example, when fruit are harvested early, a similar high level of firmness is found in all fruit in a batch, but during softening a large range of firmness levels can be found in the same batch. If other sources of variation exist beside maturity, the stochastic kinetic model can be extended to multiple stochastic variables (Hertog et al., 2007). There is a need for integration of pre- and postharvest research, because quality is built in the field (Tijskens and van Kooten, 2006). The use of stochastic kinetic models, combined with non-destructive measurements, allows description of physiological processes while dealing with biological variation which is the main challenge in the supply chain management to provide fruit and vegetables of known maturity and quality. It is envisaged that this approach will be further extended and thoroughly studied, in order to develop applications for the supply chain management of fruits and vegetables, to improve their quality and uniformity and to provide end consumers with reliable, good quality produce.

10.8 Sources of further information and advice

The classic text of Seymour et al. (1993) is a source for general information on the biochemistry of fruit ripening. It provides an overview of the biochemical pathways involved in the ripening syndrome and an insight in the role of ethylene. In the last 10–15 years, most developments have been made in the molecular biology of ripening, and especially in knowledge of ethylene-related molecular mechanisms. Alexander and Grierson (2002) give an accurate account of what is known about tomato fruit, as a model for climacteric fruit. Golding et al. (2005) review the
most recent findings in fruit ripening, focusing on the genetic regulation of ethylene production, ethylene perception and aroma development. Research on the molecular regulation of aroma production draws particular attention to the need to integrate physiology, biochemistry and consumer expectations of fruit ripening and quality.

Inaba (2007) recently reviewed research on tomato and other fruit, such as banana, persimmon, pear and melon. Ethylene biosynthesis and regulation of fruit softening was studied in different climacteric fruits and in climacteric and non-climacteric varieties of the same species. Even in climacteric fruits, different feedback regulation systems of ethylene biosynthesis and different ethylene-dependent manners of ripening related gene expression operate in different kinds of fruits. Ripening phenomena in non-climacteric fruits are not different from those in climacteric fruits with respect to all events such as sugar accumulation, acid decrease, colour development and aroma production.

Studies on ripening and maturity of different species are scattered in several papers. Stone fruit maturity indices are described by Crisosto (1994). Handbook n. 66 of USDA (Gross et al., 2004) is a general reference for storage of fresh fruits, vegetables, cut flowers and other horticultural crops, but includes information on quality characteristics and maturity indices of a large number of species of fruits and vegetables. It is available on the Internet (http://www.ba.ars.usda.gov/hb66/).

10.9 References


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Role of maturity for improved flavour


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LESCOURRET F AND GÉNARD M (2005), ‘A virtual peach fruit model simulating changes in fruit quality during the final stage of fruit growth’, Tree Physiology 25, 1301–1315.


ROLE OF MATURITY FOR IMPROVED FLAVOUR


11

Process flavors of Allium vegetables

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11.1 Flavor compounds in Allium

There are more than 600 different species in the genus Allium of the family of Liliaceae found throughout North America, Europe, North Africa, and Asia. However, only a few of them are considered to be important vegetables; the great majority of Allium species is considered to be wild plants and most of them have little or no economic importance. Among the Allium family, garlic (Allium sativum L.), onion (Allium cepa L.), leek (Allium ampeloprasum L. var. porrum), scallion (Allium fistulosum L.), shallot (Allium ascalonicum auct.), great-headed (‘elephant’) garlic (Allium ampeloprasum L. var. holmense), wild garlic (Allium ursinum), chive (Allium schoenoprasum L.), and Chinese chive (Allium tuberosum L.) are vegetables which have been widely used to flavor foods. The characteristic aromas of the Allium species are attributed to the sulfur-containing volatiles in these plants. The compositions and formation of volatiles in garlic and onions have been extensively studied and reviewed (Whitaker, 1976; Freeman and Whenum, 1975; Fenwick and Hanley, 1985a,b; Carson, 1987, Yu et al., 1989 a,b,c; Yu et al., 1991; Whitfield and Last, 1991). Unlike the preformed volatiles, such as esters and terpene compounds in fruits and spices, which are biosynthesized while plants develop, the volatile components of the genus Allium are released from their nonvolatile precursors, \( S \)-alk(en)ylcycteine sulfoxides, by an enzyme-mediated degradation process which takes place when the plant tissues are disrupted. The alk(en)yl groups of these flavor precursors are mainly a combination of propyl, allyl, 1-propenyl and methyl groups, depending on the species (Block et al., 1992a,b; Block et al., 1993).
The chemical structures, properties, origin, and formation of the compounds responsible for the characteristic flavor of Allium vegetables have been well studied. Intact samples of leaves, stems and bulbs of Allium vegetables have no distinctive flavor or odor, nor do they exhibit any lachrymatory properties. In the intact cell, the flavor precursors, alk(en)ylcysteine sulfoxides, are located in the cytoplasm while the enzyme alliinase, that is responsible for the conversion of these flavor precursors to the characteristic odor compounds associated with these species, is located in the vacuole (Lancaster and Collin, 1981). Only when the cells are disrupted can the enzyme be released to act on the precursors.

11.1.1 Nonvolatile sulfur-containing precursors
Plants of the genus Allium are good natural sources of sulfur. The major sulfur-containing nonvolatile compounds found in the genus Allium are listed in Table 11.1. Many nonvolatile sulfur-containing compounds have been found in plants such as garlic and onion. Of the components listed in this table, some are not reported to occur in leek, chive, scallion and shallot. The most significant of these constituents in terms of their quality, flavor generating potential, and biological activity are the S-alk(en)yl-L-cysteine sulfoxides and their precursors, \( \gamma \)-glutamyl-S-alk(en)yl-L-cysteine sulfoxides. A general structure of the S-alk(en)yl-L-cysteine sulfoxides is shown in Fig. 11.1. The \( R \) group can represent a methyl, propyl, (\( E \))-1-propenyl or 2-propenyl (allyl) group depending on the species. In a few instances, the \( R \) group can also represent an ethyl, butyl or vinyl group. These precursor compounds are contained in the intact cells of Allium and are converted enzymatically into the flavor compounds and/or lachrymatory principles associated with that particular species. The general structure of \( \gamma \)-glutamyl-S-alk(en)yl-L-cysteine sulfoxide found in the genus Allium is shown in Fig. 11.2. Here, the \( R \) group can represent methyl, (\( E \))-1-propenyl or allyl. \( \gamma \)-Glutamyl peptidase or \( \gamma \)-glutamyl transpeptidase can convert the \( \gamma \)-glutamyl derivatives of S-alk(en)yl-L-cysteine sulfoxides to the S-alk(en)ylcysteine sulfoxides (Whitaker, 1976).

Although nonvolatile, sulfur-containing Allium flavor precursors were thought to be odorless, the flavor and mouth-feel effects of some of these compounds have been observed (Ueda et al., 1990). The key compounds that give rise to this effect were found to be the sulfur-containing components, primarily S-allyl-L-cysteine sulfoxide (alliin), (+)-S-methyl-L-cysteine sulfoxide, and \( \gamma \)-glutamyl-S-allyl-L-cysteine sulfoxide.

S-Alk(en)yl-L-cysteine sulfoxides
Stoll and Seebeck (1948) were the first investigators to identify the flavor precursors of Allium vegetables. They isolated the crystalline amino acid, S-allyl-L-cysteine sulfoxide (alliin), from garlic. Only S-methyl-, S-propyl-, S-1-propenyl-, and S-allyl- moieties of L-cysteine sulfoxide, have contributed significantly to the flavor, pungency and lachrymatory characteristics of these plants. The presence of S-vinyl-, S-ethyl-, and S-butyl- derivatives of L-cysteine in Allium has been
### Table 11.1  Some sulfur-containing nonvolatile compounds found in the genus Allium

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Onion</th>
<th>Garlic</th>
<th>Leek</th>
<th>Chive</th>
<th>Scallion</th>
<th>Shallot</th>
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<td><strong>Amino acids</strong></td>
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<td>L-Cysteine</td>
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<td>L-Methionine</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>gamma-Glutamyl-S-(trans-1-propenyl)-L-cysteine</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>gamma-Glutamyl-S-(2-propenyl)-L-cysteine</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>gamma-Glutamyl-S-(1-propenyl)-cysteine</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Significant amounts observed.

(References: Carson, 1987; Fenwick and Hanley, 1985b; Granroth and Virtanen, 1967; Kameoka and Hasimoto, 1983; Kameoka et al., 1984; Lancaster and Shaw, 1989; Lawson et al., 1991b; Lawson, 1992; Matikkala and Virtanen, 1967; Stoll and Seebeck, 1948; Ueda et al., 1990; Whitaker, 1976.)
suggested but not confirmed (Fenwick and Hanley, 1985b). (+)-S-Methyl-L-cysteine sulfoxide (MeCySO) was first isolated from onion in 1959 (Virtanen and Matikkala, 1959). This compound, however, is expected to be present in all plants of the genus *Allium*. Like MeCySO, (+)-S-propyl-L-cysteine sulfoxide (PrCySO) was also first isolated from onion in the same period (Virtanen and Matikkala, 1959). Unlike MeCySO, this compound was considered to occur in a limited number of *Allium* plants. (+)-S-Allyl-L-cysteine sulfoxide (AllCySO), more commonly referred to as alliin, was first discovered by Stoll and Seebeck (1948). AllCySO is also present in low amounts in other *Allium* species. However, intact garlic bulbs are reported to contain up to 12–14 mg/g fresh weight of this compound (Lawson, 1992). (+)-S-(E)-1-propenyl-L-cysteine sulfoxide (Pren-CySO) is the major S-substituted-L-cysteine sulfoxide found in onion and is also found in garlic (Lawson, 1992). This compound is considered to be the precursor of the onion lachrymatory factor. Evidence also shows that it is a precursor of cycloalliin, which is not affected by alliinase in onion tissue nor does it contribute to onion flavor.

**Gamma-glutamyl peptides**

*Gamma*-glutamyl peptides (GGPs) make up most of the reserve function, and are present in dormant seed and resting bulbs of *Allium* vegetables. A noticeable amount of these flavor precursors were reported to be present in intact *Allium* tissue (Whitaker, 1976; Lawson, 1992). GGPs are rapidly hydrolyzed by either *gamma*-glutamyl peptidase or *gamma*-glutamyl transpeptidase upon seed germination or sprouting. Both enzymes are widely distributed in nature. However, they are absent, or occur in very low amounts in dormant onions (Whitaker, 1976).

The *gamma*-glutamyl derivatives of S-alk(en)ylcysteine sulfoxide represent only ‘potentially available’ flavor precursors because this class of compounds cannot be cleaved by alliinase (*S*-alk(en)yl-L-cysteine sulfoxide lyase).
Nevertheless, \( S \)-alk(en)yl-L-cysteine sulfoxides are important enhancers of the aroma of \textit{Allium} plants and their products, and, can be released from their \textit{gamma}-glutamyl derivatives by the action of peptidases or transpeptidases. A mechanism for the biosynthesis of various \( S \)-alk(en)ylcysteine sulfoxides from their corresponding \textit{gamma}-glutamyl peptides was proposed by Lancaster and Shaw (1989).

### 11.1.2 Flavor intermediates

Enzymatically formed sulfenic acids are key intermediates in \textit{Allium} vegetables. Figure 11.3 shows the general structure of the major sulfenic acids found in the genus \textit{Allium}. Allyl sulfenic acid, \( (E) \)-1-propenyl sulfenic acid, methyl sulfenic
acid and propyl sulfenic acid were proposed to be the flavor intermediates that are enzymatically generated from allyl-, \((E)-1\)-propenyl-, methyl- and propyl thiosulfinitates, respectively (Block, 1992; Block et al., 1992a,b; Block et al., 1993). The lachrymatory factor (thiopropanal S-oxide, LF) of onion and shallot develops during crushing of the plant tissues. This highly volatile, water soluble compound that is responsible for eye irritation and tears when onions are freshly cut, arises from the enzymic hydrolysis of \((E)-(\pm)-S-(1\text{-propenyl})\text{-L-cysteine sulfoxide, as shown in Fig. 11.4 (Virtanen, 1965; Virtanen and Spare, 1961, 1962).}

### 11.1.3 Flavor compounds

It is known that sulfur-containing components are responsible for the flavor and pungency of *Allium* vegetables. Among the volatile constituents in the *Allium* species, alk(en)yl di- and trisulfides are almost ubiquitous. The alk(en)yl groups of these sulfides are mainly a combination of propyl, \((E,Z)-1\)-propenyl, allyl and methyl groups, depending on the species of the plant from which it originates.

#### Primary flavor compounds

The major thiosulfinitates found in *Allium* plants are shown in Table 11.2. At least 19 thiosulfinitates have been identified in these plants. Most of these compounds have been reported to be very unstable. Allicin (diallyl thiosulfinate), the main thiosulfinate observed in fresh garlic extract and which is responsible for the intense characteristic aroma of freshly cut garlic bulbs, undergoes nonenzymic rearrangement to form the sulfides, sulfur dioxide and thiosulfonates. This rapid chemical transformation results in a corresponding shift in the flavor profile and intensity of cut garlic bulbs.

#### Secondary flavor compounds

The secondary flavor compounds are those derived from primary flavor compounds. In *Allium* plants, secondary flavor compounds are those compounds derived from alk(en)yl thiosulfinitates. The flavor compounds obtained from *Allium* plants are different depending on the isolation methods. By using mild cold isolation methods (e.g. solvent extraction, vacuum distillation), the primary flavor compounds can be obtained; by using thermal isolation methods (e.g. steam distillation), secondary flavor compounds are the dominant compounds. It is well recognized that primary flavor compounds of *Allium* vegetables are very unstable and will undergo decomposition or condensation to form secondary flavor compounds, even at room temperature. It is also recognized that secondary flavor compounds can be generated from primary flavor compounds of *Allium* vegetables during instrumental analysis (e.g. GC or GC-MS analysis).

Different *Allium* vegetables contain different types of flavor compounds. Diallyl disulfide is known as the characteristic aroma compound of cooked or processed garlic. A typical essential oil of garlic consists of diallyl-, dimethyl-, and allyl methyl sulfide, disulfide, and trisulfide. All are the nonenzymatic rearrangement products of diallyl thiosulfinate (allicin) and its homologues. The breakdown
Table 11.2 Some thiosulfimates found in the genus *Allium*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Onion</th>
<th>Garlic</th>
<th>Leek</th>
<th>Chive</th>
<th>Scallion</th>
<th>Shallot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dipropyl thiosulfinate</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diallyl thiosulfinate</td>
<td>?</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di(<em>trans</em>-1-propenyl) thiosulfinate</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl propyl thiosulfinate</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl allyl thiosulfinate</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl <em>trans</em>-1-propenyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propyl methyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Propyl allyl thiosulfinate</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propyl <em>trans</em>-1-propenyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allyl methyl thiosulfinate</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Allyl propyl thiosulfinate</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allyl <em>trans</em>-1-propenyl thiosulfinate</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>trans</em>-1-Propenyl methyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>trans</em>-1-Propenyl propyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>trans</em>-1-Propenyl allyl thiosulfinate</td>
<td>?</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>cis</em>-1-Propenyl methyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>cis</em>-1-Propenyl propyl thiosulfinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

† Reported to be found at significant levels.
* Tentatively identified.

(References: Auger and Thiobut, 1979; Bayer *et al*., 1989a; Block *et al*., 1992a,b; Block *et al*., 1993; Lancaster and Kelly, 1983; Lawson and Hughes, 1989; Lawson, 1992; Shankaranarayana *et al*., 1982; Whitaker, 1976.)

of allicin, the major compound in garlic solvent extracts, together with other thiosulfimates, plays a major role in the formation of flavor compounds of garlic (Brodnitz and Pascale, 1971; Yu *et al*., 1989a). Volatile compounds of crushed and uncrunched garlic cloves, stems (white part and green part) and leaves obtained by Likens–Nickerson (L–N) steam distillation/solvent extraction were compared by Yu *et al*. (1991). Among the 54 volatile components identified, diallyl trisulfide, diallyl disulfide, and methyl allyl trisulfide were the major volatile compounds in all garlic samples. However, crushed leaves and crushed green stems were found to contain more *trans*-2-hexenal than crushed cloves and crushed white stems.

Methyl- and propyl *1-propenyl* disulfides have been recognized as important constituents of onion oil by Brodnitz *et al*. (1969) and Boelens *et al*. (1971). The latter researchers classified the flavor components of onion oil into seven categories: oxygenated compounds, thiols, monosulfides, thiophenes, disulfides, trisulfides, and tetrasulfides. The oxygen-containing compounds were predominately the carbonyls, with propanal being one of the most important aroma components in raw onion.

Various compounds contribute to the characteristic flavor of raw and processed onions. Propanethiol was reported to contribute to the sweetness of cooked onion (Yamanishi and Orioka, 1955). Alk(en)yl thiosulfimates and thiosulfonates display a distinct odor characteristic of freshly cut onion (Boelens *et al*., 1971). The propyl and *propenyl*-containing di- and trisulfides impart the flavor of cooked onion.
(Galetto and Bedarczyk, 1975). Boelens et al. (1971) reported that 2,4- and 3,4-dimethylthiophenes had the characteristic flavor of fried onion. However, later research by Galetto and Hoffman (1976a,b) reported that synthesized isomeric mono- and dimethylthiophenes did not possess the odor characteristics previously described. Methyl propyl disulfide, methyl propyl trisulfide, and dipropyl trisulfide were considered to make the greatest contribution to the flavor of onion oil (Galetto and Bedarczyk, 1975). The most prominent compounds were dipropyl disulfide, methyl propyl disulfide, propyl 1-propenyl disulfide (E and Z), methyl 1-propenyl disulfides (E and Z), 1-propanethiol, dipropyl trisulfide, methyl propyl trisulfide, 2-methyl-2-butenal, S-propyl thioacetate, as well as two novel compounds tentatively identified as 3-ethyl-1,2-dithio-5-ene and 3-ethyl-1,2-dithi-4-ene. Methyl 3,4-dimethyl-2-thienyl disulfide and its isomers were found to be the major flavor component (up to 55.5% of the volatiles) in the supercritical CO₂ extract of onions (Sinha et al., 1992).

Ledl (1975) observed many furans in the volatile composition of onions fried in butter. These furans were considered to have arisen from the sugars present in onion by Maillard-type reactions during heating. Triethyl dihydrodithiazine, also found by Ledl (1975) in fried onion, was considered to be a significant flavor component of this product.

The major volatile compounds contributing to the odor of leek include propanethiol, methyl 2-propenyl disulfide, methyl propyl disulfide, dipropyl disulfide, and methyl propyl trisulfide (Schreyen et al., 1976). In a later study on leek flavor, Stephani and Baltes (1992) discovered 85 compounds in leek oil which were not previously reported. These researchers determined that, while one isomer of 3-ethyl-5-methyl-1,2,4-trithiolane possessed a typical, mild, leek-like odor, all the other isomers had an onion-like flavor. The varied odor characteristics observed for these isomers of the 3-ethyl-5-methyl-1,2,4-trithiolane is of special interest and an important occurrence in the flavoring system of leeks. Besides the mono- and disulfides, the occurrence of tri-, tetra-, and pentasulfides was also observed. Six kinds of acetal derivatives were also identified from leek oil. 3,4-Dimethyl-2,5-dioxo-2,5-dihydrothiophene was found in the vacuum distillate of leek and onions by Albrand et al. (1980).

The composition of the volatile flavor compounds of chive is similar to that of leek, shallot, and onion. An exception to this is that chive contains methyl pentyl disulfide and pentyl hydrosulfide (Kameoka and Hashimoto, 1983). Dipropyl- and methyl propyl disulfides were reported to be the major flavor components of chive (Iida, 1983). Dipropyl disulfide, methyl pentyl disulfide, pentyl hydrosulfide, and (E and Z)-3,5-diethyl-1,2,4-trithiolane were reported to be the most important volatile compounds in steam-distilled oil of chive (Hashimoto et al., 1983). The major volatiles of Chinese chive were reported to be dimethyl trisulfide, methyl allyl disulfide, and methyl allyl trisulfide (Kameoka and Hashimoto, 1983).

Volatile compounds of green onion were analyzed by Kameoka et al. (1984). They reported the constituents of steam distilled volatile oils from Welsh onion (Allium fistulosum) and scallion (Allium fistulosum L. var. caespitosum). The compounds identified from the neutral fractions of each volatile oil were also
reported. Among the sulfur compounds identified in green onion, dipropyl disulfide was predominant. Along with the sulfurous compounds, Welsh onion oil also contains a large quantity of trideca-2-one and 2,3-dihydro-2-octyl-5-methylfuran-3-one. Volatile compounds isolated from Welsh onion and scallion by steam distillation or solvent extraction were identified and quantified by Kuo and his coworkers (Kuo, 1991; Kuo et al., 1990; Kuo and Ho, 1992). The novel flavor compounds observed in the distilled oils can be grouped as the 1-[alk(en)ylthio]alkyl alk(en)yl disulfides, the alkyl tetra- or pentathiaalkanes or alkene(s), and the thiaheterocyclics. Methyl propyl trisulfide, 3,5-diethyl-1,2,4-trithiolane, dipropyl disulfide, methyl (E)-1-propenyl trisulfide, dimethyl trisulfide, 3,4-dimethyl thiophene, dipropyl trisulfide, 1-(methylthio) propyl methyl disulfide, propyl 1-propenyl trisulfide, methyl trans-1-propenyl disulfide and methyl propyl disulfide were the major volatile compounds identified from these two distilled oils. The major volatile compounds in the solvent extract of Welsh onion were 1-propanethiol, 2-methyl-2-pentenal, thiopropanal sulfoxide, and propyl 1-propenyl trisulfide, while the major volatile compounds in the solvent extract of scallion were methyl sulfhydryl sulfide, dipropyl disulfide, propyl 1-propenyl disulfide and methyl 1-propenethiosulfonate. The major flavor compounds found in shallot are methyl 1-propenyl disulfide, dipropyl disulfide, propyl 1-propenyl disulfide, and methyl propyl trisulfide (Wu et al., 1982). Table 11.2 compared the major thiosulfimates found in Allium vegetables.

11.1.4 Formation of flavor compounds
Sulfur and carbonyl compounds are two major categories of the volatiles observed in Allium plants. Sulfur compounds are directly produced from alk(en)yl-L-cysteine sulfoxides by the action of alliinase, while the carbonyl compounds originate from secondary reactions of the primary products of enzymatic reaction.

Alliinase (S-alk(en)yl-L-cysteine sulfoxide lyase) is a pyridoxal 5'-phosphate dependent α,β-eliminating lyase. This enzyme is considered to be present in most of the genus Allium (Tsuno, 1958). When intact tissues of Allium species are disrupted, alliinase is released and will act on S-substituted-L-cysteine sulfoxides, the major precursor to Allium flavor compounds. After enzyme reaction, sulfenic acids (the flavor intermediates) are generated. These sulfenic acids are very unstable and can further undergo condensation to form the thiosulfimates. An illustration of the formation pathways of thiosulfimates of Allium plants generated from S-alk(en)yl-L-cysteine sulfoxides via sulfenic acid intermediates, is shown in Fig. 11.5. These thiosulfimates, along with the polysulfides they form, were assumed to be the principal sources of flavor in the genus Allium. Among the sulfenic acids, 1-propenyl sulfenic acid can also undergo the formation of propanethial S-oxide (the lachrymatory factor, LF), primarily observed as its cis-isomer. The structures of the LFs are shown in Fig. 11.6.

Figure 11.7 illustrates the enzymic formation of sulfur-containing flavor compounds from (+)-S-propyl- and (+)-S-(1-propenyl)-L-cysteine sulfoxides in onion and other Allium vegetables (Fenwick and Hanley, 1985b). The enzymic
Fig. 11.5 Formation of some of the flavor intermediates and primary flavor compounds in *Allium* from precursors (Block *et al.*, 1992, 1993).
Fig. 11.6  Structures of propanal S-oxides.

Fig. 11.7  Enzymic production of flavor compounds from flavor precursors in *Allium* (Fenwick and Hanley, 1985b).

decomposition of (+)-S-allyl-L-cysteine sulfoxide in garlic and the formation of the important secondary volatiles are shown in Fig. 11.8. Enzymic reactions of (+)-S-methyl-cysteine sulfoxides proceed in a similar way. In general, through the action of the enzyme alliinase, alk(en)ylcysteine sulfoxides are cleaved to yield alk(en)yl alkanethiosulfinates, pyruvic acid and ammonia, with alk(en)yl sulfenic
acids as the proposed intermediates in this pathway (Stoll and Seebeck, 1951). Pyruvic acid, an enzymic decomposition product of cysteine sulfoxides, has been used as a measure of the strength of garlic flavor (Alfonso and Lopez, 1960). It is also used widely to measure onion pungency and flavor (Schwimmer and Guadagni, 1962).

Symmetrical thiosulfonates are considered to result from bimolecular condensation of sulfenic acids with the same alk(ene)yl radicals. Based on a microwave spectroscopic study conducted by Penn et al. (1978), sulfenic acids exist as $[RS–O–H]$ rather than the usually described $[RS(O)H]$. Thiosulfonates with unsymmetrical alk(en)yl groups also occur. They have been reported to form by the condensation reaction of two different sulfenic acids, by the interaction of two different thiosulfonates, or by the catalytic effect of thiols as shown in Fig. 11.9 (Lukes, 1971; Isenberg and Grnidic, 1973; Yagami et al., 1980).

Thiosulfonates are pungent volatile compounds. Being rather unstable, thiosulfonates tend to transform spontaneously at room temperature to thiosulfonates.

**Fig. 11.8** Action of alliinase on S-allylcysteine sulfoxide in crushed garlic (Block, 1985).
and disulfides. Thiosulfonates have been found in small amounts in the extract of freshly cut onion by Boelens et al. (1971). They reported that thiosulfonates with four or more carbon atoms display a powerful and distinct odor of freshly cut onion. Thiosulfonates are, however, considered to be less significant as intermediate flavor compounds than the corresponding thiosulfimates (Fenwick and Hanley, 1985b). The absence of thiosulfonates in distilled onion oil (Boelens et al., 1971) is possibly due to the expulsion of sulfur dioxide to yield the corresponding monosulfides (Fenwick and Hanley, 1985b). Derived from thiosulfonates, disulfides can also undergo transformation to form trisulfides and monosulfides as illustrated in Fig. 11.10 (2).

The formation of carbonyl compounds in onion and other Allium vegetables containing 1-propenylcysteine sulfoxide is shown in Fig. 11.11 (Boelens et al., 1971). Among the oxygen-containing volatiles of onion, acetaldehyde and propanal are the most important. The former is derived from pyruvic acid while the latter is formed from thiopropanal S-oxide. Propanal was reported to be the most important flavor compound in raw onion (Beolens et al., 1971). 2-Methylpent-2-enal, can be formed by aldol condensation and subsequent dehydration of two molecules of propanal. 2-Methylbutanal can be formed by reduction of 2-methylbut-2-enal which is an aldol condensation product of propanal and acetaldehyde. Because of
the lack of the lachrymatory factor thiopropanal S-oxide, garlic contains a much smaller amount of carbonyl compounds than onion.

11.2 Effect of thermal processing on Allium flavor generation

Allium plants have been extensively used worldwide as vegetables, spices, and seasonings. Many kinds of Allium products such as the oils, powders, salts, pastes, and flakes have been used in homes and industries as food flavorings and seasonings. According to their preparation methods, these products can be categorized into the following groups: raw, dried, boiled, baked, and fried. Differences in the method of preparation can result in different flavor attributes of these Allium products.

It has been reported that the flavor precursor, alliin (S-allylcysteine S-oxide), is converted to allicin, a pungent aroma, by the enzyme alliinase when the cellular tissue of this species is disrupted (Stoll and Seebeck, 1951). When alliinase is deactivated by boiling or homogenization prior to tissue rupture, no pungent odor was detected and alliin was not converted to allicin (Stoll and Seebeck, 1951; Ueda et al., 1990). Along with alliin, S-methylcysteine S-oxide, S-(E)-1-propenylcysteine S-oxide, and their precursors, \( \gamma \)-glutamyl alk-(en)ylcysteines, can also be converted...
Table 11.3  Comparison of relative content of sulfur-containing volatiles in raw and processed shallot

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Freeze-dried</th>
<th>Baked</th>
<th>Deep-fried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanethiol</td>
<td>Trace</td>
<td>Trace</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Propanethiol</td>
<td>0.1</td>
<td>0.4</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>(1-Propenyl) propyl sulfide</td>
<td>0.3</td>
<td>0.1</td>
<td>0.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>2.0</td>
<td>0.1</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Methyl 1-propyl disulfide</td>
<td>3.6</td>
<td>0.5</td>
<td>3.0</td>
<td>10.9</td>
</tr>
<tr>
<td>Dipropropyl disulfide</td>
<td>4.2</td>
<td>5.1</td>
<td>3.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Methyl-cis-(1-propenyl) disulfide</td>
<td>2.9</td>
<td>1.9</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Methyl-trans-(1-propenyl) disulfide</td>
<td>5.1</td>
<td>2.1</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>cis-(1-Propenyl) propyl disulfide</td>
<td>2.8</td>
<td>9.2</td>
<td>1.4</td>
<td>0.7</td>
</tr>
<tr>
<td>trans-(1-Propenyl) propyl disulfide</td>
<td>4.4</td>
<td>9.9</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Dimethyl trisulfide</td>
<td>18.8</td>
<td>1.4</td>
<td>2.8</td>
<td>5.6</td>
</tr>
<tr>
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<td>19.9</td>
<td>3.7</td>
<td>15.3</td>
<td>12.9</td>
</tr>
<tr>
<td>cis-(1-Propenyl) propyl trisulfide</td>
<td>4.5</td>
<td>1.0</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>trans-(1-Propenyl) propyl trisulfide</td>
<td>5.5</td>
<td>2.3</td>
<td>10.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Dipropropyl trisulfide</td>
<td>5.6</td>
<td>4.6</td>
<td>2.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Ethyl-1-(methylthiopropyl) disulfide</td>
<td>6.4</td>
<td>3.1</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Methyl (1-propenyl) trisulfide</td>
<td>4.9</td>
<td>3.3</td>
<td>14.4</td>
<td>Trace</td>
</tr>
<tr>
<td>3-Methylthiophene</td>
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<td>Trace</td>
<td>Trace</td>
<td>0.2</td>
</tr>
<tr>
<td>2,4-Dimethylthiophene</td>
<td>Trace</td>
<td>0.4</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>2,5-Dimethylthiophene</td>
<td>0.2</td>
<td>Trace</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>3,4-Dimethylthiophene</td>
<td>1.4</td>
<td>3.3</td>
<td>3.9</td>
<td>11.7</td>
</tr>
</tbody>
</table>

(Reference: Wu and Wu, 1981)

to their alk(en)ylcysteine S-oxides during storage (Lawson et al., 1991b; Lawson, 1992). Upon tissue disruption, these alk(en)ylcysteine S-oxides could be converted enzymatically to related alk(en)yl thiosulfonates, the primary flavor compounds in *Allium* plants. Most of these thiosulfonates, especially alllicin, are very unstable and can decompose or rearrange to form sulfide compounds, vinyldithiins, or ajoenes, which give different flavor sensations (Block et al., 1986; Yu and Wu, 1989; Iberl et al., 1990; Lawson et al., 1991c; Lawson, 1992; Block, 1992).

Thermal processing, such as frying, baking, and microwave heating, is an important technique for the generation of flavors. Formation of flavor specific compounds is related to the different types of thermal treatments employed. The volatiles generated from different thermal processing conditions on shallot, including baking and deep-frying, was reported by Wu and Wu (1981). The major flavor compounds found in shallot are methyl 1-propenyl disulfide, dipropyl disulfide, propyl 1-propenyl disulfide, and methyl propyl trisulfide (Wu et al., 1982). The oil from baked or fried shallots contained lesser amounts of alkyl propenyl disulfides and greater amounts of dimethyl thiophenes than oil from raw shallot. A comparison of the relative amounts of sulfur-containing volatiles in shallot is shown in Table 11.3.

Yu et al. (1993) compared volatile compounds from deep-oil fried, microwave-heated, and oven-baked garlic slices. A total of 41 volatile compounds were
Process flavors of *Allium* vegetables

Table 11.4 Composition of some important volatile compounds in garlic samples

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diallyl sulfide</td>
<td>3.87</td>
<td>5.96</td>
<td>11.45</td>
<td>5.28</td>
<td>3.71</td>
</tr>
<tr>
<td>Diallyl disulfide</td>
<td>49.08</td>
<td>35.13</td>
<td>28.76</td>
<td>41.63</td>
<td>45.81</td>
</tr>
<tr>
<td>Diallyl trisulfide</td>
<td>5.81</td>
<td>7.27</td>
<td>3.63</td>
<td>40.75</td>
<td>36.41</td>
</tr>
<tr>
<td>Methyl allyl sulfide</td>
<td>0.81</td>
<td>3.28</td>
<td>3.76</td>
<td>0.51</td>
<td>0.59</td>
</tr>
<tr>
<td>Methyl allyl disulfide</td>
<td>10.12</td>
<td>12.07</td>
<td>9.34</td>
<td>2.69</td>
<td>3.01</td>
</tr>
<tr>
<td>Methyl allyl trisulfide</td>
<td>6.27</td>
<td>3.44</td>
<td>3.31</td>
<td>3.85</td>
<td>3.83</td>
</tr>
<tr>
<td>Allyl alcohol</td>
<td>1.42</td>
<td>11.99</td>
<td>5.21</td>
<td>0.51</td>
<td>1.17</td>
</tr>
<tr>
<td>Vinylthiins</td>
<td>11.88</td>
<td>11.07</td>
<td>14.66</td>
<td>1.09</td>
<td>1.05</td>
</tr>
<tr>
<td>Nitrogen-containing</td>
<td>1.43</td>
<td>1.39</td>
<td>3.71</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>Oxygen-containing</td>
<td>0.35</td>
<td>0.28</td>
<td>0.44</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>Other volatile compounds</td>
<td>8.96</td>
<td>8.12</td>
<td>15.73</td>
<td>3.53</td>
<td>4.19</td>
</tr>
</tbody>
</table>

A = Fried garlic; B = Oil-cooked garlic; C = Microwave-fried garlic; D = Baked garlic; E = Microwave-baked garlic.

*Allyl alcohol was not included.*

identified in this study. These volatile compounds can be categorized into four groups: (1) acyclic sulfur-containing compounds, which can be further divided into thiol, monosulfide, disulfide and trisulfide compounds with methyl, allyl, 1-propenyl, and propyl groups attached to the sulfur atoms; (2) cyclic sulfur-containing compounds, such as 2,5-dimethylthiophene, 2 vinylthiophene, 1,2-dithiacyclopentane, 3-vinyl-4H-1,2-dithiin and 2-vinyl-4H-1,3-dithiin; (3) nitrogen-containing compounds, such as pyridine, 2-methylpyridine and 2-methyl-5-ethylpyridine, which contain one nitrogen atom on the aromatic ring and 2,3-dimethyl-, 2,5-dimethyl-, 2,6-dimethyl-, ethyl- and trimethylpyrazine, which contain two nitrogen atoms on the aromatic ring; (4) oxygen-containing compounds, such as acetaldehyde, allyl alcohol, 2-methyl-2-butenal and 4-heptenal. More recent studies on garlic flavor precursors showed that only methyl, allyl and 1-propenyl groups were observed (Lawson *et al*., 1991a–c; Lawson and Hughes, 1992; Lawson, 1992; Block, 1992; Block *et al*., 1992a,b). Propyl groups were not reported to be found in these studies.

However, propyl sulfide, allyl propyl sulfide, methyl propyl disulfide, and allyl propyl disulfide have been identified in fried and baked garlic. Different yields of volatile compounds were observed in these different thermally treated garlic samples. This difference is greatest with oil-treated and baked garlic samples. Besides their contribution to the flavor generation process, frying and baking treatments were also thought to impact flavor loss. The composition of the major volatile compounds identified in garlic is shown in Table 11.4. Diallyl disulfide and diallyl trisulfide were found to be the dominant compounds in baked and microwave baked garlic. Diallyl sulfide, methyl allyl trisulfide, and methyl allyl disulfide were also major compounds in baked garlic. The flavor compositions of baked and microwave-baked garlic were found to be very similar to that found in garlic oil prepared from garlic slices. However, oxygen-containing and nitrogen-containing compounds were not found in extracted garlic oil samples. Diallyl
Table 11.5  Comparison of the volatile compounds generated in baked blanched garlic (BBG), fried blanched garlic (FBG), and blanched garlic (BG)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BBG  FBG BG</td>
</tr>
<tr>
<td>Compounds generated from thermal degradation of nonvolatile flavor</td>
<td>34.68 138.66 8.07</td>
</tr>
<tr>
<td>precursors of garlic</td>
<td></td>
</tr>
<tr>
<td>Compounds generated from thermal interactions of sugars and nonvolatile</td>
<td>1.79 2.88 0.00</td>
</tr>
<tr>
<td>flavor precursors of garlic</td>
<td></td>
</tr>
<tr>
<td>Compounds generated from thermal interactions of lipids and nonvolatile</td>
<td>3.43 5.57 0.00</td>
</tr>
<tr>
<td>flavor precursors of garlic</td>
<td></td>
</tr>
<tr>
<td>Compounds generated from thermal interactions of sugars, lipids, and</td>
<td>0.00 0.61 0.00</td>
</tr>
<tr>
<td>nonvolatile flavor precursors of garlic</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39.90 147.72 8.07</td>
</tr>
</tbody>
</table>

Disulfide was again the dominant volatile compound in fried, oil-cooked and microwave fried garlic samples. In addition, diallyl sulfide, methyl allyl disulfide, methyl allyl trisulfide, vinylthiins, and allyl alcohol were also found to be important volatile compounds in these oil-treated garlic samples. Unlike the extracted oils, significant amounts of oxygen-containing and nitrogen-containing compounds were found in these oil-heated garlic samples.

Once garlic is sliced, a portion of its flavor-generating precursors, including alliin and deoxyalliin, is transformed to the thiosulfinates (Stoll and Seebeck, 1951; Block et al., 1992 a,b; Block et al., 1993). However, blanched garlic cloves retain most of their flavor precursors. Yu et al. (1994d) compared the volatile compounds from blanched, fried blanched, and baked blanched garlic slices. Blanched garlic prepared in this study had a distinct cooked popcorn flavor with a sweet and slightly pungent garlic character. Fried blanched garlic slices possessed a typical fried garlic flavor but lacked the pungency of raw garlic or the sulfury note of air-dried garlic that was observed in fried unblanched garlic slices. Baked blanched garlic slices possessed a typical baked garlic flavor but also lacked the sulfury air-dried garlic flavor of baked unblanched garlic. Comparison of the yields of volatile compounds generated in the garlic samples in this study is shown in Table 11.5. The volatile compounds identified in these garlic samples can be grouped as follows: (1) those generated from thermal degradation of nonvolatile flavor precursors of garlic; (2) those generated from thermal interactions of sugars and nonvolatile flavor precursors of garlic; (3) those generated from thermal interactions of lipids and nonvolatile flavor precursors of garlic; and (4) those generated from thermal interactions of sugars, lipids, and nonvolatile flavor precursors of garlic. Disulfide compounds are the major compounds of group (1), while different pyrazine compounds mainly occur in group (2). Some benzene compounds, 2,4-decadienal and a few pyridines occur from group (3) thermal interactions.
11.3 Thermal flavor generation in model systems containing *Allium* components

Alk(en)yl cysteine sulfoxides and γ-glutamyl alk(en)yl cysteine dipeptides are two important groups of nonvolatile flavor precursors in intact *Allium* vegetables. Both groups of compounds are not stable under storage and thermal processing conditions. Lawson (1992) found that the content of γ-glutamyl-S-2-propenyl cysteine sulfoxide decreased from 7.5 mg to 3.5 mg/g of fresh garlic within 23 weeks of storage at 4 °C. S-allylcysteine sulfoxide was the predominant compound generated when γ-glutamyl-S-allylcysteine sulfoxide was boiled. The stability of alliin heated at 100 °C was studied by Mochizuki *et al.* (1988). These researchers reported that 94.7% of alliin was retained after 20 minutes of heat exposure, 88.3% after 40 minutes and 79.4% after 60 minutes. In his study on the thermal stability of alliin, Lawson (1992) reported that alliin was completely lost after eight hours of boiling. Yu *et al.* (1993) demonstrated the occurrence of certain volatile compounds, converted from these flavor precursors, in deep-oil fried microwave-heated and oven-baked garlic slices.

11.3.1 Thermal degradation of flavor precursors

The pH and thermal effects on the formation of volatile compounds from the degradation of alliin, garlic homogenate, garlic oil, and diallyl disulfide have been well studied (Yu and Wu, 1988, Yu *et al.*, 1989a,b,c; Block *et al.*, 1988). However, less is known of the stability of the flavor precursors of *Allium* plants. Of the *Allium* vegetables studied, most of the attention has been focused on alliin and deoxyalliin. In the thermal processing, flavor compounds can form from one of two major pathways: the thermal decomposition of flavor precursors, and the thermal interaction of flavor precursors with reducing sugar or carbonyl compounds.

Stoll and Seebeck (1951) reported that alliin remained stable in an aqueous solution even at a fairly high temperature. However, when heated in diluted methanol or ethanol to 100 °C, the solution immediately formed a dark red coloration and ammonia and carbon dioxide were emitted. Sreenivasamurthy *et al.* (1961) also reported that alliin remained stable during storage over a long period of time, either in an aqueous extract or as the dehydrated garlic powder. Studies relating to the decomposition of cysteine sulfoxide have shown that volatile compounds can also be generated upon irradiation treatment (Nishimura and Mizutani, 1972, 1973a,b; Nishimura *et al.*, 1970, 1971). Yu *et al.* (1994a–c) and Kubec *et al.* (1997) showed that volatile compounds generated from alliin and deoxyalliin in an aqueous solution at different pH as well as the formation of flavor volatiles in general was influenced by temperature, time of heating and water content. Allyl alcohol and acetaldehyde were the predominant volatile compounds from alliin at pH 3, 7 and 9, while the major volatile compounds generated from the degradation of deoxyalliin at these same pH values were diallyl sulfide, 2-methyl-1,4-dithiepane, (allylthio)acetic acid, diallyl disulfide, 2-ethyl-1,3-dithiane, 4,6-dimethyl-1,2,5-trithiepane, 3,6-dimethyl-1,4-dithiane and allyl mercaptan (Yu...
et al., 1994d). This study also found that, at pH 5, the major volatile compounds generated from the degradation of alliin were acetaldehyde, 2-acetylthiazole, sulfur dioxide, ethyl acetate, and 1-propene. At pH 3, the major volatile compounds generated from the degradation of deoxyalliin were (allylthio)acetaldehyde, 3-(allythio)propanal, 3,6-dimethyl-1,2,5-trithiepane and (allythio)acetic acid. These researchers also proposed mechanisms for the formation of some important volatile compounds from the degradation of alliin and deoxyalliin (shown in Figs 11.12 and 11.13). Kubec et al. (1999) studied volatile compounds generated from thermal degradation of S-propylcysteine and S-propylcysteine sulfoxide. Thermal degradation of S-propylcysteine sulfoxide generated dipropyl disulfide, dipropyl trisulfide, propylthiol and dipropyl thiosulfonate as the predominant volatile compounds, while dipropyl disulfide and 2-(propylthio)ethylamine were the major breakdown products of S-propylcysteine.
11.3.2 Thermal interaction of flavor precursors

Most of the attention on *Allium* flavors has been focused on their enzymatic generation from nonvolatile flavor precursors. However, in thermal processing, the nonenzymatic degradation of nonvolatile flavor precursors also contributes significantly to the characteristic aroma of processed *Allium*. A further important process for flavor generation in *Allium* plants is thermal interaction of flavor precursors with reducing sugar, carbonyl compounds, or products of lipid oxidation. Flavor precursors, which in *Allium* vegetables are predominantly the derivatives of the amino acid cysteine, can partake in Maillard-type reactions for flavor formation. In addition, 2,4-decadienal, a major aldehyde formed from the lipid oxidation of oils, also plays an important role in flavor generation of fried foods.
The identification of volatile compounds generated from the reaction between sulfur containing amino acids and reducing sugars or carbonyl compounds has been previously investigated. Kato et al. (1973) demonstrated aroma compounds produced by the thermal reaction of L-cysteine and L-cystine with D-glucose or pyruvaldehyde. Kimura et al. (1990) reported the occurrence of unique and interesting sensory profiles arising from the thermal treatment of glucose (Glc) with \( S \)-methyl-L-cysteine (MCS), \( S \)-allyl-L-cysteine (ACS), \( S \)-propyl-L-cysteine (PCS) and \( cis \)-1-propenyl-cysteine (PeCS). The MCS-Glc, ACS-Glc, PCS-Glc and PeCS-Glc reactions generated intense pickled radish, garlic, roasted onion, and rice cracker notes, respectively. Strong rice cracker-like odor was also observed for the ACS-Glc reaction. The major volatile compounds responsible for these perceived odors were thought to form from the decomposition of these alkyl-L-cysteines. In the MCS-Glc and ACS-Glc systems, sulfides such as dimethyl disulfide, dimethyl trisulfide, diallyl sulfide, and diallyl disulfide were the major flavor compounds generated. In the PeCS-Glc and PCS-Glc systems, pyridines, pyrazines and the disulfides were the major compounds observed. Flavorful alkylpyrazines were also observed in the PCS-Glc reaction system. Yu et al. (1994a) indicated that meat-like flavors could be produced from thermal interaction of glucose and alliin or deoxyalliin. Volatile compounds identified from the thermal interactions of glucose and alliin or deoxyalliin can be classified into three groups: those generated from the decomposition of alliin or deoxyalliin, those generated from thermal degradation of glucose, and those generated from interactions between glucose and alliin or deoxyalliin. Allyl alcohol was the predominant volatile compound found in a thermally treated solution of alliin, while diallyl sulfide, mercaptomethylcyclopentane, diallyl disulfide, and (allylthio)acetic acid were the predominant volatile compounds from heated deoxyalliin solution. Nine volatile compounds were thought to be the degradation products of glucose. Among these compounds, only 2-furfural and phenylacetaldehyde were found in a system in which only glucose was present. Since none of these nine compounds were found in the thermal degradation of alliin and deoxyalliin solutions (Yu et al., 1994a,b), alliin and deoxyalliin were clearly participants in the degradation reaction of glucose. It is likely that these two compounds may simply take the role of amino acids in a Maillard reaction. A comparison of the amounts of some important volatile compounds generated from these Alliin-Glc and Deoxyalliin-Glc model systems is shown in Table 11.6. The major interaction products of glucose and alliin reaction were the pyrazines and thiazoles. Among them, methylpyrazine, ethylpyrazine and 2-acetylthiazole were the predominant compounds observed. Pyrazines, predominantly 2,5-dimethylpyrazine, methylpyrazine, trimethylpyrazine, and 2-ethyl-3-methylpyrazine were the main interaction products from the reaction of glucose and deoxyalliin. The major differences between these two model reaction systems were that the formation of thiazoles was favored in the glucose–alliin system, while the glucose–deoxyalliin system favored the formation of allylthio-containing compounds. Yu et al. (1994b) also reacted alliin and deoxyalliin, with and without glucose, in propylene glycol. In their study, a considerable amount of allylthio-containing and cyclic sulfur-containing...
Table 11.6  Comparison of the important volatile compounds generated from glucose model reactions with alliin and deoxyalliin

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (mg/mol of alliin or deoxyalliin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose+alliin</td>
</tr>
<tr>
<td>Thiazoles</td>
<td>186.3</td>
</tr>
<tr>
<td>Pyrazines</td>
<td>85.2</td>
</tr>
<tr>
<td>Allylthio-containing compounds</td>
<td>0</td>
</tr>
<tr>
<td>Thiophenes</td>
<td>76.0</td>
</tr>
</tbody>
</table>

compounds were found in the deoxyalliin systems, while no allylthio-containing compounds and only a small amount of cyclic sulfur-containing compounds were found in the alliin systems. Some pyrazines were identified in both alliin and deoxyalliin model systems even in the absence of glucose. Propylene glycol was proposed as a participant in the formation of these pyrazines.

Isomers of 2,4-decadienal have been found to be the major lipid oxidation products of linoleic acid-containing vegetables oils such as soybean oil, corn oil, and sunflower oil (Snyder et al., 1988). The interaction of 2,4-decadienal with sulfur-containing amino compounds such as cysteine and glutathione has also been reported (Zhang and Ho, 1989). Yu et al. (1994e) studied the volatile compounds generated from thermal interaction of 2,4-decadienal with alliin and deoxyalliin. The volatile compounds generated in these model systems can be classified into three groups: those generated from the thermal degradation of alliin or deoxyalliin, those generated from the thermal degradation of 2,4-decadienal, and those generated from the interactions between 2,4-decadienal and alliin or deoxyalliin. The major products observed from the degradation of alliin and deoxyalliin were the same as those identified in the alliin or deoxyalliin and glucose model systems. The volatile compounds generated from degradation of 2,4-decadienal are shown in Table 11.9. Hexylthiophenes, 2-pentylpyridine, 2-pentylbenzaldehyde, and 5-formyl-2-pentylthiophene were found to be the major interaction products of 2,4-decadienal and alliin or deoxyalliin (Tables 11.7 and 11.8). Chyau and Mau (1999) reported volatile compounds from microwave

Table 11.7  Volatile compounds identified from thermal interaction of alliin and (E,E)-2,4-decadienal

<table>
<thead>
<tr>
<th>Compound</th>
<th>mg/mole of alliin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allyl caproate</td>
<td>14.9</td>
</tr>
<tr>
<td>2-Butylthiophene</td>
<td>31.5</td>
</tr>
<tr>
<td>4-(3-Hydroxy-1-propenyl) phenol</td>
<td>1.9</td>
</tr>
<tr>
<td>2-Pentylthiophene</td>
<td>6.1</td>
</tr>
<tr>
<td>2-Pentylpyridine</td>
<td>78.4</td>
</tr>
<tr>
<td>Methylpentylthiophene</td>
<td>2.7</td>
</tr>
<tr>
<td>2-Hexylthiophene</td>
<td>61.1</td>
</tr>
<tr>
<td>2-Hexanoylthiophene</td>
<td>31.5</td>
</tr>
<tr>
<td>2-Pentylbenzaldehyde</td>
<td>394.1</td>
</tr>
<tr>
<td>5-Formyl-2-pentylthiophene</td>
<td>110.2</td>
</tr>
</tbody>
</table>
Table 11.8  Volatile compounds identified from thermal interaction of deoxyalliin and (E,E)-2,4-decadienal

<table>
<thead>
<tr>
<th>Compound</th>
<th>mg/mole of deoxyalliin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexanethiol</td>
<td>0.4</td>
</tr>
<tr>
<td>2-Butylthiophene</td>
<td>17.2</td>
</tr>
<tr>
<td>2-Pentylpyridine</td>
<td>202.6</td>
</tr>
<tr>
<td>2-Hexylthiophene</td>
<td>17.6</td>
</tr>
<tr>
<td>3-Hexylthiophene</td>
<td>308.0</td>
</tr>
<tr>
<td>2-Pentylbenzaldehyde</td>
<td>150.9</td>
</tr>
<tr>
<td>5-Formyl-2-pentylthiophene</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Table 11.9  Volatile compounds identified from thermal degradation of (E,E)-2,4-decadienal

<table>
<thead>
<tr>
<th>Compound</th>
<th>mg/mole of 2,4-Decadienal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td>45.2</td>
</tr>
<tr>
<td>Pentanal</td>
<td>133.6</td>
</tr>
<tr>
<td>1-Pentanol</td>
<td>10.7</td>
</tr>
<tr>
<td>Hexanal</td>
<td>2050.0</td>
</tr>
<tr>
<td>2-Pentanone</td>
<td>15.4</td>
</tr>
<tr>
<td>Heptanal</td>
<td>128.2</td>
</tr>
<tr>
<td>3-Nonen-2-ol</td>
<td>29.3</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>37.1</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>35.7</td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>47.7</td>
</tr>
<tr>
<td>3-Ethyl-2-methyl-1,3-hexadiene</td>
<td>17.6</td>
</tr>
<tr>
<td>2-Octenol</td>
<td>669.9</td>
</tr>
<tr>
<td>n-Butylbenzene</td>
<td>170.9</td>
</tr>
<tr>
<td>Acetal</td>
<td>12.8</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>99.1</td>
</tr>
<tr>
<td>2,4-Decadienal</td>
<td>520.6</td>
</tr>
<tr>
<td>(E,E)-2,4-Decadienal</td>
<td>49770.3</td>
</tr>
<tr>
<td>2,4-Decadienal</td>
<td>3182.1</td>
</tr>
</tbody>
</table>

heating of garlic juice with 2,4-decadienals. In their study, 23 compounds were identified, among which dithio(1-propenyl)propionate, dihydro-2(3H)-thiophenthione and n-hexanethiol were not previously reported.

11.4 References


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Part IV

Genetic background and future prospects
12

Genetic background of flavour: the case of the tomato

M. Causse, INRA, France

12.1 Introduction

The tomato is one of the most important vegetables in the world. Adapted to a range of conditions (from the open field to soilless cultivation in greenhouses), fresh tomatoes are produced all year round. Consumption has regularly increased over recent years, but consumers have become more and more concerned by tomato fruit quality (Bruhn et al., 1991; Hobson, 1988). Tomato fruit quality for fresh consumption is determined by external (size, colour, firmness) and internal fruit properties (taste, aroma, texture). These internal properties can be assessed by sensory analysis and are related to fruit composition. In this chapter, flavour is understood as all the perceptions one feels in the mouth, and thus encompasses taste, aroma and texture.

Tomato taste is usually described by sweetness and sourness. It is mostly related to the fruit content in reducing sugars and organic acids (Stevens et al., 1977; Malundo et al., 1995; Janse and Schols, 1995), and to their ratio (Stevens et al., 1979; Bucheli et al., 1999). Tomato fruit is primarily composed of sugars and acids, which represent about 60% of the dry matter weight (Davies and Hobson, 1981). In mature tomato, glucose and fructose constitute the major sugars, and citric and malic acids are the major organic acids. Depending on the study, acidity is either related to the fruit pH or to the titratable acidity (Baldwin et al., 1998; Auerswald et al., 1999). Both sugars and acids contribute to the sweetness and to the overall aroma intensity (Baldwin et al., 1998), but sweetness seems more
influenced by the content in fructose than in glucose, while acidity is mostly due to the citric acid, present in higher amounts than malic acid in mature fruits (Stevens et al., 1977).

In addition to the overall tomato aroma, several attributes have been proposed to characterize the aroma, such as fruity, green, grassy, earthy, musty, floral, candy, citrus, grapefruit or pharmaceutical aromas (Bucheli et al., 1999; Causse et al., 2001; Baldwin et al., 2004). These aromas are related to the volatiles contained in fruits. More than 400 aroma volatiles have been identified in tomato fruit (reviewed in Petro-Turza 1987), among which about 30 seem to be important for tomato aroma (Baldwin et al., 2000; 2004).

Firmness, mealininess, juiciness, meltability or skin toughness are the major texture attributes. Texture attributes are more or less difficult to correlate to instrumental measurements. Firmness in the mouth is partly related to the instrumental measurement of fruit firmness (Causse et al., 2002), and mealininess was found to be related to the texture parameters of the pericarp (Verkeke et al., 1998). Actually texture is related to several processes, such as fruit morphology (size of the pericarp relative to locules, locule number), cell size and shape, cell adhesion and cell wall properties (Lee et al., 1999; Devaux et al., 2005).

High tomato-like aroma intensity and sweetness, but intermediate acidity are the most important characteristics for consumer preferences (Jones, 1986; Baldwin et al., 1998). Malundo et al. (1995) showed that given levels of sweetness correspond to optimal acid concentrations, beyond which acceptability decreases. Baldwin et al. (1998) related the overall acceptability to the ratio of sugars to titratable acidity and to the concentration of several aroma compounds. Verkeke et al. (1998) underlined the major role of texture traits in the preference of consumers.

For 50 years tomato breeders have improved fruit yield and stability, plant adaptation and disease resistances. Fruit size and appearance (lack of defects, attractive colour), firmness and shelf life have also been tremendously modified by breeding, but flavour has not been a target for long. Due to the complaints of consumers about tomato taste, genetic improvement of tomato fruit quality is now required (Bruhn et al., 1991; Causse et al., 2003). The complexity of tomato fruit quality (due to the number of parameters to take into account, their polygenic inheritance and their multiple interactions) has limited genetic progress. Today, molecular markers enable the dissection of the genetic basis of complex traits and the increasing knowledge about the tomato genome offers new efficient tools to breeders.

This chapter first presents the knowledge on genetic diversity and inheritance of tomato flavour related traits. The genetic basis of fruit quality traits in tomato has been studied in several progenies and we will try to summarize the information provided by QTL studies. Results of a marker-assisted selection scheme designed to improve fruit quality will then be presented. Finally the major genes identified as involved in fruit quality will be presented before presentation of the future prospects offered by the new high throughput genomic approaches available today.
12.2 Genetic variability and relationship among quality traits

For a successful breeding programme, breeders need efficient selection criteria and must know the potential for improvement, i.e. the range of genetic variability available, the mode of inheritance and the respective influence of cultivar and environmental conditions on the traits to improve. Genetic variability for quality traits has been reviewed by Davies and Hobson (1981), Stevens (1986) and Dorais et al. (2001). Most of the studies on genetic variation of fruit quality describe a few cultivars or compare groups of cultivars. Preferences of consumers faced with genetic variability have been rarely studied (Lengard and Kermit, 2006). Cherry tomatoes have been identified as having the best flavour (Hobson and Bedford, 1989), with fruits richer in acids and sugars than large fruited lines. In contrast, long shelf life cultivars have been described as generally less tasty than traditional ones (Jones, 1986), with lower volatile content (Baldwin et al., 1991). Several studies concerned the qualitative and quantitative composition of aroma volatiles in tomato varieties (Buttery et al., 1987; Baldwin et al., 1991; Krumein and Auerswald 1998; Krumein et al., 2004). Aroma profiles have been shown to be influenced by the variety (Langlois et al., 1996), the ripening stage (Baldwin et al., 1991) and the storage conditions (Stern et al., 1994). Tikunov et al. (2005) using a novel approach for exhaustive volatile analysis characterized 94 genotypes for their content in 322 different compounds. The content in phenolic-derived volatiles (particularly phenylethanol and benzyl alcohol) discriminated cherry tomato from round and beef cultivars. Volatiles from the phenylpropanoid pathway, including methylsalicylate, guaiacol and eugenol separated the large-fruited genotypes into two groups. Cherry tomatoes were also characterized by high levels of lipid derivatives and low levels of compounds derived from terpenoids or Leu/Ile amino acid products.

In order to analyse the inheritance of various components of tomato fruit quality, including physical and chemical traits, but also sensory attributes assessed by a trained panel and hedonic tests by randomly chosen consumers, Causse et al. (2003) have analysed the genetic variation of quality attributes in 35 hybrids and their 13 parental lines, grown in two contrasted environments. The 13 parental lines had various origins (old traditional inbred cultivars, experimental lines bred in the 1980s and lines used as parents of modern hybrid varieties). Each experiment was grown in spring under soilless glasshouse conditions and in summer in the open field or under unheated plastic tunnels, in order to estimate the overall influence of environmental conditions on quality traits. As fruit size influences the judgement of taste panels, two experiments were set up, one involving large fruits, the other small fruits from hybrids between cherry tomato lines and large fruited lines.

Several results on the genetic control of quality traits were obtained:

- Among parental lines, the influence of recent selection could be detected as fruits from modern lines were on average rated less sweet, more acid, but firmer than the older lines. However, modern lines also differed from one another. The firmness of hybrids significantly differed when they were grouped according to
their parental origins in old × old, modern × modern or old × modern hybrids. The hybrids between old lines also rated on average sweeter, even though their sugar content was not significantly higher. This could be attributed to the different perception of fruit texture in hybrids involving old lines that appeared less firm, juicier and mealier than hybrids between modern lines. Such interaction between taste and texture traits has also been mentioned by Wolters and van Gemert (1990) and Causse et al. (2001). Hybrids between modern and old lines seemed thus to benefit from the qualities of the two groups, being sweeter, with a stronger aroma intensity, and having juicier and less mealy fruits. Nevertheless a large variability was detected among hybrids. Hybrids with cherry tomato had high sugar and acid contents, in agreement with Hobson and Bedford (1989), but a wide range of variability was shown depending on the parental line.

- Consumers perceived significant differences among hybrids and seemed to particularly appreciate the hybrids between old and modern lines with intermediate firmness. The highest scoring hybrid was sweet and juicy, not very firm, nor mealy. The pleasantness note was positively correlated with sugars, titratable acidity, overall aroma intensity, sweetness and negatively correlated with mealiness. The preference for the hybrids between large and cherry tomatoes confirmed the major role of sweetness and acidity in preference, which appeared more important than texture traits, as already mentioned by Stevens et al. (1977). The important contribution of sugars and acids to the overall tomato flavour and to consumer preference, already mentioned by Jones and Scott (1984), was confirmed. The results also showed the importance of texture and flavour in consumer preference, as also mentioned by Wolters and van Gemert (1990). Although texture came second, if a good flavour is obtained, a good texture is the second criteria needed (Harker et al., 1997), at least in large-fruit hybrids. The aroma, particularly fruitiness, which was mentioned by Bucheli et al. (1999), did not appear as the most important attribute in this experiment.

- Most of the physico-chemical traits, flavour attributes and firm texture showed a simple additive inheritance, in contrast to the aroma and other texture traits. Growing conditions are known to influence quality traits at the composition level (as reviewed by Dorais et al., 2001 for glasshouse conditions), and at the sensory level (Hobson and Bedford, 1989). We observed differences between the two environments for most of the traits. The genotype by environment (G × E) interaction was strong for fruit weight and aroma intensity but not very significant for firmness and fruit composition. The G × E interaction was not usually strongly significant in comparison with the main effects, as also observed by Auerswald et al. (1999) and Johansson et al. (1999), who found that variety differences affected fruit quality more than growing conditions.

- Specific networks of relationships among traits were shown in hybrids. Among sensory attributes, acidity and tomato aroma were positively correlated, and juiciness and mealiness were negatively related. Between sensory attributes and instrumental assays, sweetness was correlated with sugar content and acidity with titratable acidity (as already shown by Baldwin et al., 1998), a firm texture
was correlated to the instrumental firmness (as also mentioned by Lee et al., 1999), and mealiness was positively correlated to the soluble solid content.

- Several mutations affecting fruit ripening and shelf life are known. The most widely used in tomato breeding is \textit{rin} (ripening inhibitor), which, in the heterozygous state, enables fruits to be kept for a few weeks (Davies and Hobson, 1981). Long shelf life cultivars have invaded the tomato market, but in the 1990s their quality, particularly their colour and flavour has been criticized by consumers (Jones, 1986; McGlasson et al., 1987). We have produced and compared seven pairs of nearly isogenic hybrids, with or without the \textit{rin} mutation at the heterozygous level. The presence of the \textit{rin} mutation reduced the consumer preference (Fig. 12.1). Differences were detected by sensory profiles, \textit{rin} hybrids having fruits on average 17% less sweet, with a 12% lower tomato aroma, a 17% higher ‘strange’ aroma and 10% high mealiness. Instrumental firmness and sugar content were not different. These differences were largely dependent on the genetic background. McGlasson et al. (1987) attributed the bland flavour of \textit{rin} hybrids to the lack of specific aroma compounds. These results confirmed the negative influence of the \textit{rin} mutation on consumer preference, but also indicated that, when transferred into a hybrid with high flavour, the negative influence of the mutation is reduced. Selection could thus be carried out to obtain much sweeter and perfumed lines combined with shelf life in \textit{rin} hybrids.

12.3 The genetic control of fruit quality traits in tomato

Molecular markers allow the dissection of quantitative traits into discrete Quantitative Trait Loci (QTL) that can be located on a genetic map. The existence of a QTL in a chromosome region means that at least one polymorphic gene segregating in the region is responsible for part of the variation of the trait (Tanksley, 1993).

12.3.1 Quantitative trait loci (QTL) analysis for tomato flavour in the progeny of a cherry × large fruit progeny

In order to study the genetic control of organoleptic quality, a population composed of 144 recombinant inbred lines (RIL) was developed from the cross between a cherry tomato line with a remarkable overall aroma intensity and an inbred line with a common taste but large fruits. The fruits of each line were characterized at physical (fruit weight, colour and firmness) and chemical levels (titratable acidity, pH, content in soluble solids, sugars and 12 aroma volatiles). A large sensory panel (56 judges) were trained and quantified sensory attributes: taste (sweetness and sourness), aroma (overall aroma intensity, together with candy, lemon, citrus and pharmaceutical aromas) and texture (firmness, meltiness, mealiness, juiciness and embarrassing skin). RILs showed a large range of variation for each trait. Molecular markers were used to construct a genetic map of the progeny and to map quantitative trait loci (QTL) for each trait. One to five QTL
Fig. 12.1  Principal Component Analysis of 20 hybrids with large fruits from the crosses between old lines (noted A, K, M, S), modern lines (B, L, G, R) and a line isogenic to M but carrying the rin mutation (I). Hybrids are indicated by the combination of letters of their parental lines. The lines were characterized by sensory analysis by a trained panel, physical measurements (colour: L, A, B, fruit weight and firmness), chemical measurements (pH, titratable acidity-TA, sugars, vitamin C, lycopene and soluble solids content). Consumer preference, scored by 300 people, is plotted as supplementary factor.
were detected per attribute. The percentage of phenotypic variation explained per QTL ranged from 9% to 45% per QTL and major QTL were detected for six aroma volatiles (Saliba-Colombani et al., 2001). Most of the favourable alleles came from the cherry tomato line, showing the potential usefulness of this line for tomato flavour improvement (Causse et al., 2001, 2002). Fruit weight was not correlated to sweetness, although it was correlated to sugar and acid content. A positive but loose correlation was detected between instrumental firmness and firm texture. Some correlations were expected, for example between sugar content and sweetness, overall aroma intensity, or candy aroma. A positive correlation was also detected between sourness and titratable acidity. The correlation between sourness and pH was lower, as previously observed by Kader et al. (1977) and Stevens et al. (1979). This low correlation was also due to the low variability of the pH in the population. Both sugar content and titratable acidity contributed to the overall aroma intensity, in an equal manner. This result is consistent with the observations of Stevens et al. (1979) and Kader et al. (1977). The lemon aroma was positively correlated to the titratable acidity, on the contrary to the citrus fruit aroma, which was only correlated to the sugar content.

Correlations between the volatile contents and the aroma descriptors were low when significant, with the exception of the pharmaceutical aroma which was strongly correlated with the content in two phenolic compounds, orthomethoxyphenol and eugenol. The analysis by trained sniffers of these two compounds associated their presence with odours of clove and camphor, which can be related to the pharmaceutical aroma. Another correlation can be mentioned, between the pentanal content and the descriptors related to sweetness and aroma intensity. In the same way, the 3-methylpentan-1-ol content was related to sourness and lemon aroma. Baldwin et al. (1998) noted a positive correlation between sourness and the hexanal content, which was not observed in Saliba-Colombani et al. (2001). Baldwin et al. (1998) also observed a positive correlation between aroma intensity and the content in hexenal, hexenol and beta-ionone. None of these correlations were significant in the cherry × large fruit population. The overall aroma intensity was only positively correlated to two volatile compounds, the 2-methylbut-2-enal and the pentanal. The only common correlation between this study and that of Baldwin et al. (1998) concerned the negative correlation between methyl-heptenone and sweetness.

Several clusters of QTL were identified, mainly on chromosomes 1, 2, 3, 4, 8, 9, 11 and 12 (Fig. 12.2). These clusters may reveal results from a fortuitous linkage, or the segregation of a unique QTL controlling two traits because of causal relationships among traits or because of related metabolisms. These colocations were compared with the correlations, as two related traits are expected to share common QTL. QTL colocations were observed for related sensory and instrumental traits. For instance, QTL of titratable acidity, sourness and lemon aroma were in the same regions on chromosomes 1, 2, 3 and 9. QTL for sugar content and sweetness mapped in the same regions on chromosomes 2 (two regions) and 11. A QTL for fruit weight with an opposite effect was also detected in each of these three regions. Only one common QTL location, on chromosome 3, could be responsible
Fig. 12.2  QTL detected for quality traits in a population of recombinant inbred lines derived from the cross between a cherry tomato and a large fruit line. Taste attributes were sweetness (SWE) and sourness (SOU). Aroma attributes were overall aroma intensity (ARO), candy aroma (CAN), lemon aroma (LEM), citrus fruit (other than lemon) aroma (CIT) and pharmaceutical aroma (PHA). Texture attributes were flesh firmness (FIT), mealiness (MEA), meltability (MEL), juiciness (JUI) and difficulty of swallowing skin (SKI). Physical and chemical measures: firmness (fir), sugar content (suc), titratable acidity (ta), contents in 12 aroma volatiles: pentanal (pna), 2-methylbut-2-enal (bea), hexanal (hxa), 3-methylpentan-1-ol (mno), hex-3-en-1-ol (×3o), 2-(methylthio)ethanol (meo), 3-(methylthio)propanal (mta), 6-methylhept-5-en-2-one (mhn), 2-isobutylthiazole (ibt), 2-phenylethanal (pea), orthomethoxyphenol (myp), eugenol (eug); adapted from Causse et al., (2002).
for the negative correlation detected between sweetness and sourness. The contribution of sugars and acids not only to sweetness and sourness but also to the overall aroma intensity (Hobson and Bedford 1989) was confirmed. The QTL for overall aroma intensity, which mapped on chromosomes 2 (top), 9 and 12 were close to QTL for sourness, while the QTL at the bottom of chromosome 2 was close to a QTL for sweetness.

Only a few colocations between aroma descriptors and volatile content were expected, in agreement with the low correlations observed. The strong correlation between pharmaceutical aroma and the eugenol and orthomethoxyphenol content was corroborated by two colocations on chromosomes 2 and 9. The content in eugenol was dependent on loci on the two chromosomes, with an epistatic interaction, as both alleles from the large fruited line were necessary to detect a significant quantity of eugenol. On the contrary, the orthomethoxyphenol content only depended on a major gene on chromosome 9. On chromosome 4, several QTL for volatiles, identified as having a tomato or grass odour, were mapped to the same region as a grapefruit aroma QTL. Colocations of QTL of texture attributes and taste or aroma attributes were not frequent, with the exception of sweetness and mealiness, which showed common QTL locations on chromosomes 3 and 9.

12.3.2 Information provided by combining several quantitative trait loci studies

In tomato several studies have been performed by the groups of Steve Tanksley (Cornell University, USA) and Dani Zamir (Jerusalem University, Israel) to map QTL controlling yield and fruit quality related traits (Paterson et al., 1988, 1990, 1991; Azanza et al., 1994; Goldman et al., 1995; Grandillo and Tanksley 1996; Tanksley et al., 1996; Fulton et al., 1997, 2000, 2002a; Bernacchi et al., 1998a; Chen et al., 1999; Doganlar et al., 2002; Frary et al., 2004; Eshed and Zamir 1995). These studies were all performed on interspecific crosses between wild relative species and processing tomato inbreds. Some of the interesting quality traits for processing tomato are common to fresh market tomato (sugar content, soluble solid content, pH, acidity, firmness …) and QTL locations can thus be compared across the progenies.

Figure 12.3 summarizes the chromosome regions carrying QTL of sugar content or related traits (Brix, fructose, glucose or sucrose content), based on 14 populations involving eight different species. From three to 19 QTL were detected per progeny, with a total of 95 QTL gathered in 56 chromosome regions. In 28 regions, QTL were detected in more than one population, and could correspond to the same QTL. The same result could be obtained for acid content (Fulton et al., 2002a; Causse et al., 2002; 2004), with only a few regions common to acid and sugar content. In contrast, frequent colocations between QTL for sugar content and fruit weight (Grandillo et al., 1999) with opposite allelic effect could be detected, suggesting a pleiotropic effect of some common QTL.

Figure 12.4 summarizes the locations of QTL for fruit firmness detected in seven populations involving six species. Thirty-eight QTL were detected, located
Fig. 12.3  Summary of QTL for sugar content or related traits (brix or hexose content) in one of the following progeny: *S. lycopersicum* × *S. cheesmaniae* F2 population (Paterson et al., 1991); *S. lycopersicum* × *S. cheesmaniae* recombinant inbred population (Goldman et al., 1995); *S. lycopersicum* × *S. chmielewskii* F2 and advanced backcross lines (Paterson et al., 1988; 1990; Azanza et al., 1994); *S. lycopersicum* × *S. habrochaites* advanced backcross population (Bernacchi et al., 1998a); *S. lycopersicum* × *S. neorickii* advanced backcross population (Fulton et al., 2000); *S. lycopersicum* × *S. pimpinellifolium* advanced backcross population (Tanksley et al., 1996; Doganlar et al., 2002); *S. lycopersicum* × *S. pimpinellifolium* backcross populations (Grandillo and Tanksley, 1996; Chen et al., 1999); *S. lycopersicum* × *S. pennellii* introgression lines (Eshed and Zamir, 1995 and Causse et al., 2004); *S. lycopersicum* × *S. pennellii* advanced backcross population (Frary et al., 2004); *S. lycopersicum* × *S. peruvianum* advanced backcross population (Fulton et al., 1997); *S. lycopersicum* cv. *S. cerasiforme* × *S. lycopersicum* recombinant inbred line population (Saliba-Colombani et al., 2001). The data concerning the advanced backcross involving *S. pimpinellifolium*, *S. peruvianum*, *S. neorickii* and *S. habrochaites* were summarized by Fulton et al., 2002a. The QTL were positioned on the tomato reference map (Tanksley et al., 1992), based on their closest marker. An arbitrary 10-cM interval around the most likely position was attributed.

in 22 regions. QTL common to two or more populations were detected in nine regions. Figure 12.2 shows the location of QTL for 12 volatile aromas detected in the progeny of the cross involving a cherry tomato (Saliba-Colombani et al., 2001). Tieman et al. (2006) also identified QTL for 23 volatiles in a population of introgression lines derived from *S. pennellii*. Twenty-five loci altered in one or more volatiles were identified. Although ten volatiles were analysed in both studies, only three QTL were detected in the same regions, for phenylacetaldehyde
Fig. 12.4  Summary of QTL for firmness in one of the following progeny: *S. lycopersicum* × *S. pennellii* introgression lines (Causse et al., 2004, unpubl. data); *S. lycopersicum* × *S. pennellii* advanced backcross population (Frary et al., 2004); *S. lycopersicum* × *S. habrochaites* advanced backcross population (Bernacchi et al., 1998a); *S. lycopersicum* × *S. pimpinellifolium* advanced backcross population (Tanksley et al., 1996; Doganlar et al., 2002); *S. lycopersicum* × *S. peruvianum* advanced backcross population (Fulton et al., 1997); *S. lycopersicum* × *S. neorickii* advanced backcross population (Fulton et al., 2000); *S. lycopersicum cv cerasiforme* × *S. lycopersicum* (Saliba-Colombani et al., 2001). The QTL were positioned on the tomato reference map (Tanksley et al., 1992), based on their closest marker. An arbitrary 10-cM interval around the most likely position was attributed.

on chromosome 8 (confirming the effect of the QTL named *Malodorous* by Tadmor et al., 2002), on chromosome 9 for 2-methylbutanal and on chromosome 12 for pentanal. In both studies, QTL for several volatiles were frequently in clusters. These clusters correspond in a few cases to volatiles deriving from the same metabolism (fatty acids, carotenoids or amino acid degradation), suggesting the action of a gene related to specific pathways but also to volatiles deriving from various metabolisms, suggesting a gene acting on the regulation of several pathways.

### 12.3.3 Information from quantitative trait loci studies

Several conclusions on the genetic control of flavour traits can be drawn:

- QTL are detected in most cases, sometimes with strong effects. A few QTL explaining a large part (20 to 50%) of the phenotypic variation, acting together with minor QTL, are frequently detected. Most of the QTL act in an additive
manner, but sometimes dominant and even over-dominant QTL were detected (Paterson et al., 1988, 1991; de Vicente and Tanksley, 1993). Epistasis (interaction among QTL) is rarely detected unless a specific experimental design is used (Eshed and Zamir, 1996).

- QTL can be separated into two types: QTL stable over the environments or years, and QTL more specific for one condition (Paterson et al., 1991). Firmness and the content in some volatiles appeared strongly variable across years or environment (Chaib et al., 2006; Tieman et al., 2006).

- Some regions involved in the variation of a trait are found in progenies derived from different accessions of a species, or from different wild species related to S. lycopersicum and could correspond to a polymorphism in the same gene acquired during domestication (Fulton et al., 1997, 2002a; Bernacchi et al., 1998b).

- Fine mapping experiments allow the precise mapping of QTL in a chromosome region and confirmation of the existence of several linked QTL in the same region (Paterson et al., 1990; Frary et al., 2003, Lecomte et al., 2004a). For example, Lecomte et al., (2004a) identified in a 20-cM region two QTL for fruit weight flanking a QTL for sugar content. Fine mapping is also an important step for cloning a QTL, as shown by the success in cloning a QTL controlling soluble solid content (Fridman et al., 2000).

- Wild species, in spite of their unfavourable characteristics in comparison to cultivars, can carry alleles which may contribute to the improvement of most of the agronomic traits (de Vicente and Tanksley, 1993; Bernacchi et al., 1998b). Gur and Zamir (2004) made progress by pyramiding independent yield-promoting regions introduced from the wild species S. pennellii. Wild species may provide original aromas, either favourable to tomato quality, as found in an S. peruvianum accession (Kamal et al., 2001) or unfavourable as the Malodorous locus found in an S. pennellii accession (Tadmor et al., 2002).

12.4 Molecular markers for improving tomato fruit flavour

Breeding for fruit organoleptic quality is difficult due to the complexity of this characteristic. In fresh-market tomato, selection for sensory traits requires complex and expensive evaluation. Sensory profiling relies on the judgement of several trained panellists. Some traits such as sweetness and sourness may be replaced by the measurement of sugar and acid content, but aroma and texture characteristics are not precisely predicted by instrumental measurements. Furthermore, most of the quality traits exhibit a polygenic inheritance and are strongly influenced by environmental conditions. A negative relationship between sugar content and fruit size also limits genetic progress. Marker-assisted selection has thus been proposed as an alternative to phenotype-based breeding for improving tomato flavour.

The previously described programme of QTL detection for fruit quality traits involving a population derived from the cross between a cherry tomato line and a
large-fruited line was followed by marker-assisted selection. As several clusters of QTL had been identified and most of the favourable alleles for tomato quality improvement came from the cherry tomato line, a marker-assisted backcross scheme has been set up in order to transfer the five regions of the cherry tomato genome with the largest effect on fruit quality into three recurrent lines with large fruits and different levels of fruit firmness. The QTL regions were chosen according to the QTL effects and their involvement in complementary quality traits. Marker-assisted selection was performed during three successive backcrosses, followed by two selfing generations necessary to fix the five QTL and recover the genome of the recurrent parents for non-carrier chromosomes (Lecomte et al., 2004b). Plants carrying one to five QTL were selected in order to study their individual or combined effects. Most of the QTL were recovered in lines carrying one introgression region and new QTL were detected (Chaïb et al., 2006). The lines carrying the five segments were crossed to several other lines and the fruit quality of the hybrids was assessed by fruit composition and sensory evaluation. It appeared that, although fruit size was reduced, prototype hybrids had improved

**Fig. 12.5** Sensory profiles of the prototypes obtained by marker-assisted selection. The line L5 having five regions introgressed from a cherry tomato line and the hybrids between L5 and two large fruited lines (L5D and L5B) were compared to the original recurrent line L for several sensory attributes and for fruit size (M. Causse, unpubl. results).
fruit quality, in comparison to parental lines, promising potential improvement for the satisfaction of consumers. Figure 12.5 illustrates the improvement obtained for several quality traits in the line carrying the favourable alleles for five QTL at the homozygous or heterozygous states. The same trends were observed in the other genetic backgrounds. Nevertheless fruit weight in these genotypes was always lower than expected due to the effect of unexpected QTL, whose effect was masked in the RIL population. The breeding efficiency strongly varied according to the recurrent parent and significant interactions between QTL and genetic backgrounds were shown for all the studied traits (Lecomte et al., 2004b; Causse et al., 2007).

The advanced backcross QTL analysis is another strategy proposed for the rapid discovery and transfer of valuable QTL alleles from unadapted donor lines into established elite inbred lines (Tanksley and Nelson, 1996). Steve Tanksley and his colleagues have applied this strategy to screening for positive alleles in five wild species, *S. pimpinellifolium* (Tanksley et al., 1996; Doganlar et al., 2002), *S. chmielewskii* (Azanza et al., 1994), *S. habrochaites* (formerly *L. hirsutum*; Bernacchi et al., 1998a), *S. peruvianum* (Fulton et al., 1997), *S. pennellii* (Frary et al., 2004) and *S. neorickii* (formerly *L. parviflorum*; Fulton et al., 2000). They showed a number of important transgressions potentially useful for processing tomato and demonstrated that beneficial alleles could be identified in unadapted germplasm and simultaneously transferred into elite cultivars, thus exploiting the hidden value of exotic germplasm (Bernacchi et al., 1998b).

### 12.5 Genes involved in tomato quality traits

Physiologists have identified many genes whose function is important in the processes leading to fruit quality. Fruit development is divided into four distinct phases: (1) ovule fertilization and fruit set; (2) cell division; (3) cell expansion; (4) ripening (Gillaspy et al., 1993). Dramatic changes occur during fruit development and ripening. The third phase, corresponding to cell expansion, is related to the accumulation in fruit cell vacuoles of water, organic acids and minerals (Coombe, 1976). A peak of transient starch accumulation is also shown, which is later converted to reducing sugars (Wang et al., 1993). The ripening phase is characterized by fruit softening, colouring and sweetening (Giovannoni, 2001). Thus early stages of fruit development are particularly important in the latter characteristics of mature fruits, their fruit weight, as well as their composition in primary metabolites, while the accumulation of volatiles and changes in texture is dependent on events occurring in the latter stages.

The expression of several genes may thus be responsible for the variation of fruit composition. They could either be genes involved in carbon metabolism or partitioning, or any gene specifically expressed during synthesis and accumulation of reserves. The enzymes involved in sink/source relations (sucrose synthase, sucrose phosphate synthase, invertase, ADPG pyrophosphorylase) have been proposed as responsible for fruit composition in sugars (reviewed by Herbers and
Sonnemwald, 1998). The importance of early starch accumulation underlined the role of ADPG-pyrophosphorylase (Schaffer and Petrikov, 1997). The impact of a polymorphism in the gene coding for an apoplastic invertase involved in the uptake of sucrose from the phloem was demonstrated by Fridman et al. (2004) and Baxter et al. (2005a). The role of sucrose synthase during early fruit development was proposed by D’Aoust et al. (1999) based on antisense plants, but has not been confirmed at the level of natural variation. Some fruit specific genes coding for other enzymes such as phosphoenolpyruvate carboxylase, fructokinase or hexokinase were identified as potentially involved in fruit composition (Guillet et al., 2002; German et al., 2003; Roessner-Tunali et al., 2003). Screening the variation among wild relative species has shown the tremendous variation for sugar content in leaf and fruit, but TCA cycle intermediates seem much less variable across species (Schauer et al., 2005; Carrari and Fernie, 2006). The regulation of the TCA cycle is still poorly characterized, with the exception of the role of a mitochondrial malate dehydrogenase and of aconitase that were shown to be involved in fruit size and dry matter content (Nunes-Nesi et al., 2005, Carrari et al., 2003). Nevertheless, showing that a polymorphism in a gene is responsible for the genetic variation in a fruit quality trait requires the colocalization between the candidate gene and a mutation or a QTL (positional proof), and/or the identification in the sequence of the polymorphism responsible for the variation. Confrontation of QTL maps and candidate gene maps allowed the identification of a few colocations (Causse et al., 2004), but the validation of the putative candidate genes requires fine mapping experiments and the identification of a causal polymorphism that is not obvious. Mutations of enzymes involved in the carbon metabolism were found in S. chmielewskii and in S. habrochaites, leading to particular sugar compositions: The sucr mutation in an invertase gene, in S. chmielewskii, provides fruits with sucrose instead of glucose and fructose (Chetelat et al., 1995). In S. habrochaites, an allele of the ADP glucose pyrophosphorylase enzyme was identified as much more efficient than the allele of the cultivated species, leading to an increase in the final sugar content of the fruit (Schaffer et al., 2000). Another locus Fgr modulates the fructose-glucose ratio in mature fruit, the S. habrochaites allele yielding a higher ratio (Levin et al., 2000). More recently, Levin et al. (2004) showed that the alleles of S. habrochaites at two loci interact in increasing this ratio. These loci remain to be characterized. A gene encoding an apoplastic invertase Lin5 has been shown to be a QTL modulating sugar partitioning, the allele of S. pennellii leading to higher sugar concentrations than the S. lycopersicum one, because of a difference in the activity of the enzyme (Fridman et al., 2004).

Fruit ripening and softening is strongly influenced by ethylene production and initial molecular studies were devoted to the isolation of genes involved in the regulation of ethylene (Grierson et al., 1992). The impact of the enzymes involved in cell wall disassembling during ripening on fruit firmness and shelf life has been extensively studied and modifications of endo-polygalacturonase or pectin methyl esterase activity were proposed to increase fruit shelf life and modify texture properties (Hobson and Grierson, 1993; Brummell et al., 2002; Powell et al., 2003). Expansin genes were also identified as involved in cell wall properties
Genetic background of flavour: the case of the tomato

(Rose et al., 2004). Oke et al. (2003) showed that a reduction in phospholipase activity through antisense transformation resulted in increased membrane stability, and higher firmness and level of certain volatiles such as hexenal. Several mutations responsible for a longer fruit shelf life have been recently cloned by positional cloning. The ripening inhibitor (rin) mutation corresponds to a deletion in a MADS-box transcription factor (Vrebalov, 2002) while the Colourless non-ripening (Cnr) mutation, modified in cell-to-cell adhesion, is due to an epigenetic mutation in a SBP-box transcription factor (Manning et al., 2006).

Volatiles are derived from the degradation of amino acids, fatty acids, carotenoids or phenolic compounds. Due to the diversity of compounds, little is known about the genes controlling their accumulation, but a few genes have been identified as responsible for their accumulation. The ADH gene coding for an alcohol dehydrogenase is involved in the ratio of hexanal to hexanol in the fruit (Speirs et al., 1998). TomloxC, a gene coding for a fruit specific lypoxygenase has been shown to be related to the generation of volatile C6 aldehyde and alcohol compounds including hexanal, hexenal and hexenol (Chen et al., 2004). Two genes LeAADC1 and LEAADC2 are responsible for the decarboxylation of phenylalanine and subsequent synthesis of phenylethanol and related compounds (Tieman et al., 2006).

Tomato flavour has also been modified by introducing in transgenic plants genes from unrelated species, such as a thaumatin gene from *Thaumatococcus daniellii* that enhanced tomato flavour (Bartoszewski et al., 2003). Aroma composition was modified by introducing new enzymes such as yeast delta-9 desaturase (Wang et al., 1996), borage delta-6 desaturase (Cook et al., 2002) or S-linalool synthase from a flower *Clarkia breweri* (Lewinsohn et al., 2001). Several examples of transgenic tomatoes can be mentioned but the only case developed for commercial use is the use of the antisense polygalacturonase gene for the modification of fruit texture (Kramer and Redenbaugh, 1994).

### 12.6 High throughput genomics for quality trait analysis

In tomato, more than 120 000 expressed sequence tags (ESTs), derived from more than 23 cDNA libraries were sequenced (Moore et al., 2002). Contigs were created, allowing the definition of more than 30 000 unigenes (Van der Hooven et al., 2002; http://www.sgn.cornell.edu). They represent a large set of candidate sequences for many physiological processes. Today, high throughput technology enables the simultaneous quantification of the transcript abundance of these genes during fruit development (Alba et al., 2004; Fei et al., 2004) or in different parts of the fruit (Lemaire-Chamley et al., 2005).

Comparison of lines with contrasted quality traits allows the identification of differences in gene expression, and gives clues on the major changes in metabolism linked to the phenotypic variations (Baxter et al., 2005b). High throughput metabolic profiling is another new approach used to get insight into the overall metabolic changes in tomato fruits during fruit development (Fernie et al., 2004;
Schauer et al., 2005) or in various genotypes (Overy et al., 2005). Proteome analysis may also give access to the variations in the quantity of hundreds of proteins and provide complementary information (Rose et al., 2004; Mihr et al., 2005; Faurobert et al., 2007).

Tomato is a model crop for the study of fleshy fruit development and a number of genes expressed during fruit development and ripening have been cloned. The identification of a set of conserved ortholog genes between S. lycopersicum and A. thaliana also facilitates synteny studies and speeds up gene and QTL characterization (Fulton et al., 2002b). An international initiative has recently started to sequence the euchromatin parts of the tomato genome, comprising the majority of the genes (Mueller et al., 2005). This will provide other tools to rapidly elucidate the key regulators of fruit development and metabolism. All the genomic tools available will allow a better knowledge of gene function and regulation, as well as the development of precise and more efficient gene-assisted selection, avoiding introgression of large segments. Once an important gene has been characterized, it will be important to find new allelic variants. Populations of mutants in a unique genetic background have been produced (Menda et al., 2004) and new techniques proposed for screening them for mutations in a specific gene (Comai and Henikoff, 2006), which in the future may help in the discovery of such new alleles.

12.7 Conclusion and perspectives

Tomato quality is a complex characteristic because of the number of components and because it depends on events occurring throughout whole plant and fruit development. Tools for exhaustive measurement of fruit composition in primary metabolites as well as in volatiles are now available, but texture traits are still difficult to predict by instrumental measurements. Modelling the processes related to fruit quality and the relative influence of genetics, environment and management will allow integration of novel insight from metabolic profiling and pathways of taste-related compounds, ultimately enabling the analysis of gene networks responsible for fruit maturation processes (Struik et al., 2005; Yin et al., 2004).

The genetic variation for fruit quality is large, particularly if one considers the possibilities offered by related species. A few mutations have been shown to be involved in fruit quality, particularly in ripening, but many QTL studies revealed a number of genomic regions involved in the variation of quality traits. A few genomic hot spots where many QTL mapped were identified. These clusters of QTL for several quality traits permit marker-assisted selection to be planned, even for several traits. Very few QTL have been identified, but one can expect a rapid increase in the number of genes identified in the near future, thanks to systems biology approaches combining transcriptomic, proteomic and metabolomic studies and the information from the genome sequence. These discoveries will facilitate breeder’s work to improve fruit quality.
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Genes involved in the biosynthesis of aroma volatiles and biotechnological applications

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13.1 Introduction

Aroma volatiles contribute to a large extent to the overall sensory quality of fruit and vegetables. Research during recent decades has been dedicated to the identification of volatile compounds and to the elucidation of some of the biosynthetic routes by either bioconversion or by tracing of precursors (Sanz et al., 1997; D’Auria et al., 2002; Dudareva et al., 2004). Recently, research efforts have been directed to the isolation of the corresponding genes in fruit and vegetables (Aharoni et al., 2000; Yahyaoui et al., 2002; Beekwilder et al., 2004) or in flowers (Shalit et al., 2003; Dudareva and Pichersky, 2006; Pichersky and Dudareva, 2007). Aroma is generally a complex mixture of a wide range of compounds. Each product has a distinctive aroma which is function of the proportion of the key volatiles and the presence or absence of unique components. The most important classes of aromas are: monoterpenes, sesquiterpenes, lipids-, sugars- and amino acid-derived compounds. Therefore, the strategies developed to improve aroma volatiles emitted by fruits and vegetables include a wide range of targets comprising the different metabolic pathways, but also regulatory elements such as hormones and transcription factors, and finally mechanisms involved in the storage or sequestration of volatile precursors, such as glycosylation and storage into the vacuoles (Fig. 13.1). It is noteworthy to mention that the production of aroma volatiles can be unexpectedly affected by engineering other fruit attributes. For
instance, down-regulation of polygalacturonase (PG), pectin methyl esterase (PME) and PG+PME in transgenic fruit (Baldwin et al., 2000) that results in lower degradation of pectins, can reduce flavor volatiles by a so far unexplained mechanism.

The aims of the chapter are the following: (1) review the information available on genes and gene families identified as participating in the synthesis of different class of aroma volatiles; and (2) describe the potential biotechnological applications for improved aromas in fruit and vegetables and other perspectives.

13.2 Genes involved in the biosynthesis of aroma volatiles

Genes involved in the generation of fatty acids derived volatiles
Fatty acids, essentially from membrane lipids, are major precursors of volatile compounds participating in the aroma of many fruit and vegetables. Volatile fatty acids derivatives include saturated and unsaturated short-chain alcohols, aldehydes and esters. They are generated by the lipoxygenase (LOX) pathway.

Modifications of fatty acids composition
Fatty acids in plants are first produced in saturated forms which are thereafter desaturated to mono- and polyunsaturated fatty acids successively by Δ9, Δ12 and Δ15 desaturases that add double bonds at specific positions in the chain. In most plants the level of mono-unsaturated fatty acids is low as compared to saturated and
poly-unsaturated fatty acids, indicating that the first desaturation by ∆9 desaturase could be a limiting factor of polyunsaturation. Indeed, by expressing a gene encoding a yeast ∆9 desaturase, the levels of 16:1 fatty acids were highly increased in tomato leaves (Wang et al., 1996). The levels of 18:1 and 16:3 fatty acids were slightly increased and the level of 18:3 was significantly decreased. As a consequence, certain volatile compounds derived from 16:1, 16:3 and 18:1 fatty acids were increased to more than 3-fold, such as 1-hydroxy-2-butanone, 1-penten-3-ol, heptanal, 3-hexen-1-ol, 2-octanol, cis-3-hexenal, hexanal and 2-nonenal (Wang et al., 2001). Surprisingly, several C-6 compounds normally derived from 18:3 such as trans-2-hexenal also increased to a similar extent and compounds not known to be derived from fatty acids such as 2-ethyl-furan, 5-ethyl-2-[5H]-furanone, eugenol and 2-ethylthiophene also sharply increased in transgenic leaves. No information is available on the emission of volatile compounds by the fruit.

A tomato mutant spr2 was identified as being deficient in jasmonic acid biosynthesis due to considerably lower levels of 18:3 fatty acids than wild type. The mutation corresponded to a gene encoding a chloroplastic ω-3 desaturase now called LeFAD7 (Li et al., 2003). In parallel with the reduction of 18:3 fatty acids, there was a 1.5 to three-fold increase in 18:2 linoleic acid (Cañoles et al., 2006). The production of unsaturated C-6 aldehydes: Z-3-hexenal, Z-3-hexenol, and E-2-hexenal and the alcohol Z-3-hexenol derived from 18:3 fatty acids was markedly reduced in leaves and fruit of the mutant line. Conversely, the production of the saturated C-6 hexanal and hexanol were significantly higher in the mutant. Sensory tests indicated that the mutant had lower flavor. It is not known whether the overexpression of the LeFAD7 ω-3 desaturase would increase the flavor of the tomato.

Phospholipases
Phospholipases are involved in the formation of polyunsaturated free fatty acids that are generally considered as substrates for lipoxygenase (Feussner and Westermann, 2002). The pathway for the catabolism of phospholipids has been studied in several senescing systems and involves the sequential action of a number of enzymes including phospholipase D (PLD), phosphatidate phosphatase, lipolytic acyl hydrolase and lipoxygenase (Paliyath and Droillard, 1992). Among these, phospholipase D-α (PLD-α) is involved in the mediation of various senescence processes promoted by ethylene or abscissic acid (Bargmann and Munnik, 2006). During tomato fruit development, a PLD-α is induced and its activity peaks at the mature green and turning stages (Pinheiro et al., 2003). Tomato antisense lines targeted against PLD-α (Oke et al., 2003) produced fruits that released, after blending, higher quantities of LOX-derived aldehydes, E-pentenal and 2-hexenal. A possible explanation is that low expression of PLD-α delayed the senescence process thus resulting in the preservation of membrane integrity and in providing higher level of precursors for the LOX pathway. However, it cannot be ruled out that other unknown mechanisms associated with the slow-down of senescence be involved.
**Lipoxygenases**

Lipoxygenases (LOX) catalyze the hydroperoxidation of polyunsaturated fatty acids containing a cis,cis-pentadiene structure. The principal substrates of LOX in plants are linoleic and linolenic acid. There are two classes of LOX: 9-LOX, which generate specifically 9-hydroxyperoxides and 13-LOX which generate specifically 13-hydroxyperoxides. LOX are supposed to be involved in stress responses, wounding, and pathogen attack (Feussner and Wasternack, 2002). But LOX are also involved in the biosynthesis of volatile compounds, such as hexanal, hexenal and hexenol that participate in the aroma of fruit and vegetables. In tomato, five LOX genes (TomloxA, B, C, D, and E) are expressed during fruit ripening (Ferrie et al., 1994; Heitz et al., 1997; Griffths et al., 1999a). TomloxA, B and E are quite similar with 72% to 77% identity at the amino acid level. TomloxC and D, which have a chloroplast targeting signal (Heitz et al., 1997), show 42% and 47% identity to TomloxA, respectively, and 46% identity to each other. The specific role of the five LOX in stress responses, defense or biosynthesis of flavor compounds during fruit ripening has been one of the major questions addressed in recent years through gene silencing. Antisense suppression of TomloxA and TomloxB in tomato fruit has resulted in no significant changes of the fruit flavor volatiles (Griffiths et al., 1999b), while co-suppression of TomloxC strongly affected the production of flavor volatiles (Chen et al., 2004). TomloxC is therefore a good target gene for increasing aroma production through biotechnology in tomato. A homolog of TomloxC, LOX H1, has been down-regulated in potato resulting in the depletion of volatile aliphatic C6 aldehydes formation (Leon et al., 2002).

**Hydroxyperoxide lyases**

Hydroxyperoxide lyase (HPL) form very unstable hemiacetals from hydroperoxides (HPO) generated by LOX from polyunsaturated fatty acids leading to the generation of aldehydes and aldehydes enols by spontaneous dissociation. HPL belong to a family of cytochrome P450 proteins (CYP74) that include also allene oxide synthases (AOS) and divinyl ether synthases (DES). HPL can be divided into three subfamilies according to their substrate specificity.

- 13-HPLs show strong preference for 13-HPO (see Fukushige and Hildebrand, 2005a) and cleave the 13-HPO into 12-oxo-(9Z)-dodecenoic acid and C6 aldehydes such as hexanal or (3Z)-hexenal.
- 9/13-HPLs can act both on 9- and 13-hydroperoxides but have often preference for 9-HPO (Matsui et al., 2000; Tijet et al., 2001). Beside cleaving 13-HPLs as indicated above, they can also cleave 9-HPO into 9-oxononanoic acid and C9 aldehydes such as (3Z)-nonenal or (3Z,6E)-nonadienal. The (3Z)-aldehydes easily isomerize to their (2E)-enals.
- A 9-HPL gene has been recently isolated in almonds encoding an HPL with almost exclusive preference for 9-HPO (Mita et al., 2005).

13-HPLs or 9/13 HPLs are considered to be targeted to the chloroplast outer membrane. The 9-HPL protein of almond is associated with lipid bodies (Mita et al.,
2005). HPLs have differential substrate specificity towards HPO derived from linolenic acid (HPOT) or linoleic acid (HPOD). For instance, watermelon HPL is three times more active for 13-HPOT than HPOD, guava ten times; green pepper, 12 times; sunflower leaf 16 times; alfalfa 1.5 times; sunflower hypocotyl two times (Fukushige and Hildebrand, 2005a).

In order to modify the flavor properties of tomato fruits, a cucumber HPL gene which encodes an enzyme having preference for 9-PHOS to form C9-aldehydes hydroxyperoxides has been introduced in tomato plants (Matsui et al., 2001). Despite a high activity of the introduced HPL in leaves and fruit of transgenic tomatoes, little changes have been observed in the composition of volatile short-chain aldehydes and alcohols emitted by the fruit. Such a result was unexpected because tomato fruit have high lipoxygenase activity to form 9-HPO. Possible explanations are that the access of HPL to 9-HPO may not be possible due to compartmentation and/or the affinity of the introduced HPL for 9-HPO is low.

The expression of a 9/13 HPL of watermelon having much higher affinity for 13-HPOs (specially those derived from linolenic acid) than for 9-HPOs in tobacco and Arabidopsis plants resulted in enzyme activities that were up to 50 times higher than in wild type plants (Fukushige and Hildebrand, 2005b). However, the effect of the transgene on the emission of volatiles by transgenic leaves has not been quantified in details. The biotechnological relevance of this strategy therefore remains to be proved.

The silencing of LOX and HPL has been performed separately in potato plants (Salas et al., 2005). The down-regulation of HPL induced an increase in LOX activity and of the content of most of the C5 volatiles and a decrease of most of the C6 compounds in the leaves. This resulted in an increase in the sweet note and decrease in the green note odor. Suppression of lipoxygenase caused a severe decrease in the amount of volatiles produced by the leaves and in the intensity of their aroma estimated by sensory evaluation. These data open some perspectives, if not the improvement of the existing flavor, at least for the modification of aromas in leafy vegetables by genetic engineering.

Alcohol dehydrogenases

Alcohol dehydrogenases (ADH) catalyze the reversible conversion of aldehydes to the corresponding alcohols. They have been involved in the response to a wide range of stresses (Chase, 1999). However, ADH genes that are suspected of participation in the production of aromas are expressed in a developmentally regulated manner, particularly during fruit ripening (van der Straten et al., 1991; Speirs et al., 1998; 2002; Echeverria et al., 2004; Manriquez et al., 2006). In grapes, three ADH genes are expressed during fruit development. VvADH1 and VvADH3 transcripts accumulate transiently in young developing berries, while VvADH2 transcripts strongly increase at the onset of ripening named véraison (Tesnière and Verriès, 2000). Fruit-specific dehydrogenases so far characterized belong to the medium-size zinc-containing class (Chase, 1999). Partial cDNA clones putatively encoding short-chain ADHs have been reported in tomato (Picton et al., 1993) and in pear (Fonseca et al., 2004). In melon, two fruit-specific
CmADH genes belonging to both the medium- and short-chain types have been isolated. After expression in yeast and purification, it was demonstrated that the two encoded enzymes preferentially work as aldehyde reductases and have specific substrates preferences (Manriquez et al., 2006).

In tomato fruit, one of the two ADH genes, LeADH2, participates in the formation of flavor volatiles during fruit ripening. Overexpression of LeADH2 has led to improved flavor of the fruit by increasing the level of alcohols, particularly Z-3-hexenol (Speirs et al., 1998). Fruits overexpressing LeADH2 were identified by a sensory panel as having a more intense ‘ripe fruit’ flavor.

Alcohol acyl-transferases

Alcohol acyl-transferases (AAT) catalyze the transfer of an acyl-CoA to an alcohol. These enzymes are capable of combining different alcohols and acyl-CoAs resulting in the synthesis of a wide range of esters accounting for the diversity of esters emitted by the fruit. A number of genes encoding AAT have been isolated and characterized in fruit and vegetables (Aharoni et al., 2000; Beekwilder et al., 2004; Souleyre et al., 2005; Wang and De Luca, 2005; El-Sharkawy et al., 2005). In melon, three AAT genes (Cm-AAT1, Cm-AAT3 and Cm-AAT4) have been isolated that are specifically expressed in fruit under the control of the plant hormone ethylene. They have different substrate specificities that contribute to the production of a wide range of esters in the melon (El-Sharkawy et al., 2005). Among the three AATs, Cm-AAT1 has the biggest capacity to produce thioesters (Lucchetta et al., 2007) that have considerable sensory importance in cantaloupe melon (Wyllie and Leach, 1990). The importance of AAT substrate specificity has also been outlined in apple (Souleyre et al., 2005) or in grapes where an AAT recently described in Concord grapes is responsible for the distinctive ‘foxy’ aroma (Wang and de Luca, 2005). A large number of acyl-transferase genes are present in plants with around 70 members encountered in Arabidopsis (Pichersky and Gang, 2000). Although performing the same reaction, AAT proteins from different fruit species may be highly divergent. For instance the strawberry AAT and the CmAAT1 of melon have only 22% identity while they have similar preference for substrates (Aharoni et al., 2000; Yahyaoui et al., 2002). Given their importance in aroma accumulation, AAT have also been the target of genetic engineering. In petunia, overexpression of strawberry AAT did not change the volatile emission profiles from flowers and green parts, yet, ester production could be enhanced when coupling the AAT overexpression with precursor feeding such as isoamyl alcohol (Beekwilder et al., 2004). More recently, the RNA interference technique has been used for down-regulating AAT in Arabidopsis thaliana (D’Auria et al., 2007) and petunia (Dexter et al., 2007). In Arabidopsis, the target AAT exhibited a substrate preference similar to melon CmAAT1 and strawberry AAT. Its down-regulation resulted in a dramatic reduction in the emission of the green leaf volatile (Z)-3-hexen-1-yl-acetate (D’Auria et al., 2007). In petunia, the target AAT was found to control the synthesis of isoeugenol, likely through the esterification of coniferyl alcohol in coniferyl acetate (Dexter et al., 2007).
13.3 Genes of amino acid metabolism

Aldehydes and alcohols derived from the degradation of amino acids constitute a class of highly abundant fruit volatiles. The two compounds, 2-phenylacetaldehyde and 2-phenylethanol and their glycosides are synthesized from phenylalanine. They are abundant in various fruits such as tomato (Baldwin et al., 2000) and some grape varieties (Garcia et al., 2003). Compounds derived from leucine catabolism such as 3-methyl-butanal and 3-methyl-butanol also contribute to the tomato flavour. In addition, alcohols deriving from amino acids can be esterified into compounds having a large impact on fruit odor such as 3-methyl-butyl acetate in banana or ethyl-butanoate and ethyl-hexanoate in strawberries (Perez et al., 1992).

Recently, genes encoding enzymes responsible for the decarboxylation of phenylalanine have been identified in tomato (Tieman et al., 2006), petunia and rose (Kaminaga et al., 2006). In both studies, the enzymes described belong to the pyridoxal 5-phosphate dependent amino acid decarboxylases and display subtle differences of sequences and enzymatic properties. The antisense down-regulation of the decarboxylase gene in tomato and petunia led to reduced emission of phenylacetaldehyde and phenylethanol. Conversely, the overexpression in tomato of the amino acid decarboxylase increased up to 10-fold the amount of phenylethanol, phenylacetaldehyde, phenylacetonitrile, and 1-nitro-2-phenylethane released from the transgenic fruits (Tieman et al., 2006). This capacity to modulate the levels of phenylethanol and phenylacetaldehyde is important since these compounds can exert a dual effect: at low concentrations phenylethanol and phenylacetaldehyde are associated with pleasant sweet flowery notes, while at high concentrations the pungent aroma of phenylacetaldehyde has a nauseating and unpleasant odor (Tadmor et al., 2002).

13.4 Genes involved in terpenoid biosynthesis

Terpenoids represent a large class of aroma volatiles originating from the condensation of the five carbon precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) generated by the methylerythritol 4-phosphate (MEP) and mevalonate pathways. The condensation is performed by prenyltransferases, such as geranyl diphosphate synthase (GPPS), geranylgeranyl diphosphate synthase (GGPPS) and farnesyl diphosphate synthase (FPPS), to produce prenyl diphosphates. The prenyl diphosphate precursors are then transformed into terpenes, sesquiterpenes and triterpenes by terpene synthases (McGarvey and Croteau, 1995; Tholl, 2006). Many of the terpene volatiles derive directly from the action of terpene synthases, while others are transformed into hydroxylated, dehydrogenated or acetylated compounds. Several reviews have been made directly related to the engineering of plants for terpenoids (McCaskill and Croteau, 1998) and for monoterpenes (Mahmoud and Croteau, 2002). Engineering of the terpene pathway has been initially attempted in plants rich in essential oils such as peppermint.
13.4.1 Up-regulation of the MEP pathway
Increasing the production of monoterpenes through the elevation of IPP and DMAPP was achieved in peppermint by expressing a reductoisomerase of the MEP pathway (Mahmoud and Croteau, 2001). There was a stimulation by 50% of monoterpenes synthesis without any change in the composition of the essential oil. Application to other species of agricultural interest is awaited.

13.4.2 Introduction or up-regulation of monoterpenes synthases
Two different monoterpane synthases have been engineered in edible products. First, a *Clarkia breweri* linalool synthase has been expressed in tomato in a fruit-specific manner (Lewinsohn et al., 2001). It has resulted not only in the production of linalool but also of 8-hydroxy-linalool, probably resulting from the presence in the tomato of a P450 enzyme capable of hydroxylating linalool. More recently, tomato flavour was enriched through heterologous expression of a basil geraniol synthase (Davidovich-Rikanati et al., 2007). Very interestingly, the introduction of geraniol synthase not only resulted in the accumulation of monoterpenes uncommon in tomato fruit (geraniol, citronellol, neric acid, limonene), but the lycopene content was reduced, suggesting that the early plastidial terpenoid pathway was diverted from lycopene accumulation to monoterpenes production.

Except for these two attempts, most of the studies related to the overexpression of monoterpenes synthases have been carried out in flowers or in model plants such as tobacco. Because monoterpenes synthesis occurs in the plastids, targeting of gene expression to this organelle has proved to be more efficient than in the cytosol. As an example, transgenic tobacco plants expressing a heterologous limonene synthase produced much more limonene when gene expression was targeted to the plastids than to the cytosol. Expression in the reticulum resulted in the absence of limonene synthesis (Ohara et al., 2003). In some instances, several terpene synthases have been introduced simultaneously, which resulted in the production of new products beside the main and minor compounds resulting from the activity of the three introduced enzymes (Lücker et al., 2004a). Thus, modifying fruit flavor through monoterpenes biosynthesis pathways has proven to be very efficient.

13.4.3 Introduction of up-regulation of P450 monoterpenes hydroxylase and sesquiterpene synthase genes
The introduction of a P450 limonene-3-hydroxylase in a tobacco transgenic line expressing three monoterpane synthases producing limonene, γ-terpinene and (–) β-pinene as the main products resulted in the hydroxylation of (+) limonene into (+) trans-isopiperitenol, an uncommon compound in the plant kingdom (Lücker et al., 2004b). The synthesis of sesquiterpenes has been stimulated by expression of a fungal sesquiterpene cyclase in tobacco (Hohn and Ohlrogge, 1991). A sesquiterpene synthase of citrus involved in the synthesis of the sesquiterpene valencene has been isolated (Sharon-Asa et al., 2003), but biotechnological applications have not been reported.
13.4.4 Reduction of undesirable monoterpenes compounds
Reduction of menthofuran, an undesirable monoterpane oil component of peppermint, was achieved by down-regulating the methofuran synthase (Mahmoud and Croteau, 2001). Similar experiments in fruit and vegetables have not been published.

13.4.5 Genes involved in the generation of volatiles from carotenoids
Carotenoids are the precursors of C14 and C13 volatile compounds that contribute to flavor and aroma of many fruit, vegetable and flowers, such as β-ionone, geranylacetone (6,10-dimethyl-5,9-undeca dien-2-one) and pseudoionone (6,10-dimethyl-3,5,9-undecatrien-2-one). Because they originate from coloured carotenoids, a link has long been observed between pigmentation and aroma production in fruit. Lewinsohn et al., (2005) have correlated the pigment content of a number of mutants or genotypes of tomato and watermelon with aroma production. For instance, tomato mutants rich in δ-carotene produce high amounts of α-ionone, while those rich in β-carotene produce more β-ionone. These observations indicate that engineering fruit for increasing the level or for modifying the balance of carotenoids in fruit has probably an effect on the production of aroma volatiles. So far, papers dealing with biotechnology of carotenoids (Rosati et al., 2000; Römer et al., 2000; Fraser et al., 2002) have not taken aroma production into account.

The C14 and C13 carotenoid-derived volatile compounds are predicted to derive from carotenoids through an oxidative cleavage mechanisms. In the recent years genes encoding carotenoid cleavage dioxygenase (CCD) have been isolated in plants (Giuliano et al., 2003) that contribute to the generation of apocarotenoids, among which, abscissic acid (Schwartz et al., 1997; Tan et al., 1997), bixin dialdehyde an important food and cosmetic plant pigment (Bouvier et al., 2003a) also known as a contributor to tomato flavor (Baldwin et al., 2000) and crocin, the main pigment of saffron (Bouvier et al., 2003b). Carotenoid dioxygenase genes have been characterized in tomato (Simkin et al., 2004a) and in petunia (Simkin et al., 2004b). In tomato, there are two closely related genes, LeCCD1A and LeCCD1B, the latter being highly expressed during fruit ripening. LeCCD1-suppressed tomatoes were generated that show strong reduction of the production of β-ionone, geranylacetone and pseudoionone (Simkin et al., 2004a). In petunia, suppression of PhCCD1 led to a 58% to 76% decrease in β-ionone synthesis (Simkin et al., 2004b). In grapes a VvCCD1 has been isolated and characterized by expression in E. coli (Mathieu et al., 2005).

A CmCCD1 gene of melon has been characterized by functional expression in E. coli (Ibdah et al., 2006). It shows up-regulation during fruit ripening. The CmCCD1 gene product cleaves carotenoids at positions 9,10 and 9’,10’ generating geranylacetone from phytoene, β-ionone from β-carotene, and α-ionone and pseudoionone from δ-carotene. Since the CmCCD1 gene is also expressed in white and pale green melons, despite the lack of (or the low) production of apocarotenoids, it can be concluded that the accumulation of β-ionone is limited by the availability
of the carotenoid substrate. From a biotechnological point of view, the over-expression of these genes in fruit may stimulate the production of aroma volatiles only when the availability of substrates is not limiting.

13.5 Genes involved in the generation of aroma volatiles from sugars

Furanone structures such as the caramel-like 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF or furaneol) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (DMMF) derive from the metabolism of sugars, primarily from D-fructose-1,6-diphosphate (Roscher et al., 1998). HDMF is present at high concentration in the aroma of strawberry (up to 55 mg/kg FW) (Larsen and Poll, 1992) and has a low odor threshold (10 ppb) (Schwab and Roscher, 1997). HDMF and DMMF are present in pineapple and a number of different fruit (Schwab and Roscher, 1997). They are not present or undetectable in root, stems, leaves or flowers. A strawberry FaOMT encoding an O-methyltransferase responsible for DMMF biosynthesis has been isolated (Wein et al., 2002). Its expression is up-regulated specifically during fruit ripening and the encoded protein was capable of methylating a number of substrates: catecol, caffeic acid, protocatechuic aldehyde, coffeoyl CoA, and DMF. It is supposed to play a role in lignification of the achenes and vascular bundles and in the synthesis of volatiles such as DMMF. The function of this FaOMT was assessed in planta using overexpression and antisense technology in strawberries (Lunkenbein et al., 2006a). The reduction of FaOMT gene expression altered the HDMF/DMMF ratio, resulting in a near depletion of the DMMF pool, thus confirming the importance of FaOMT in the DMMF formation. In addition, the dual function of this enzyme in the secondary metabolism was also proven since FaOMT down-regulation affected also the concentration of feruloyl glucose, suggesting that it is also involved in the methylation of the caffeoyl group. Recently, an enone oxidoreductase (FaQR) involved in the HDMF formation was isolated from a crude strawberry fruit extract and the corresponding gene has been cloned (Raab et al., 2006). It represents a very promising target for biotechnological engineering.

13.6 Modification of the glycosylated fraction

Many aroma precursors are also found as glycosylated compounds. Their release, by enzymatic or chemical hydrolysis, is of particular interest in fruit juice processing and in winemaking (Sarry and Gunata, 2004). The potential of this source of precursor in genetic engineering has not been fully explored. Yet, a first attempt to express an Aspergillus niger β-glucosidase in tobacco leaves had profound effects on the volatile emissions (Wei et al., 2004). Theses effects depended on the subcellular compartment targeted by the heterologous expression, suggesting that the different subcellular fractions indeed contain large pools of glycosylated
volatiles precursors which can be mobilized by metabolic engineering. In another attempt to engineer fruits flavors, Lunkenbein et al. (2006b) recently identified a UDP-glucose:cinnamate glucosyltransferase responsible for the synthesis of (hydroxyl)cinnamoyl-glucose in strawberries, which is a possible precursor of methyl and ethyl cinnamate. However, the down-regulation of the gene by antisense only decreased the pools of the cinnamoyl- and p-coumaroyl glycosides without any obvious increase or decrease of the derived volatiles. It can also be mentioned that glycosylation can be problematic in the frame of metabolic engineering since the enhanced metabolites can be stored in a glycosylated and non-volatile form. For example, metabolic engineering of terpenoid metabolism in petunia leaves (Lücker et al., 2001) or Arabidopsis flowers (Aharoni et al., 2003) by over-expression of linalool synthase resulted mainly in the accumulation of linalyl-glycosides instead of the free volatile form.

13.7 Regulators controlling aroma biosynthesis: transcription factors and hormones

Besides genes encoding enzymes involved in the aromas’ metabolic pathways, genes that regulate the pathways may also represent promising targets for genetic engineering. Two types of regulators may be considered: transcription factors and genes involved in the synthesis or perception of hormones.

Although transcription factors have not been targeted for improving aroma volatiles, they have been successfully used to increase fruit contents in antioxidant flavonoids (Bovy et al., 2002; Schijlen et al., 2004). Concerning hormones, plants altered in ethylene synthesis or perception with the aim of extending shelf-life of climacteric fruits, generally displayed a lower production of aroma volatiles (Ayub et al., 1996; Bauchot et al., 1998; Flores et al., 2002; Dandekar et al., 2004; Defilippi et al., 2004; Nuñez-Palenius et al., 2006). Since several hormones influence fruit development and sensory quality (see Klein and Goldschmidt, 2005), they represent a high number of potential targets for genetic engineering.

13.8 Conclusions and perspectives

This review shows that many genes participating in the synthesis of aroma volatiles have been isolated in recent years. In many cases functional characterization has been carried out by expression in heterologous model plants or by down-regulation in the original plant. Many studies have been carried out in flowers for which the scent is of the utmost importance and the number of studies that have been dedicated to edible fruit and vegetables are reduced. There is no doubt that the recent development in genomics and proteomics will enhance the number of target genes for genetic manipulation of aroma volatiles biosynthesis. In addition to the genes directly involved in the biosynthesis, it is probable that regulatory genes, such as transcription factors, capable of modulating the aromas’ biosynthetic
pathways will be discovered. The strategies developed for metabolic engineering should integrate other aspects. For instance, the importance of targeting the expression of the genes to specific organelles or cell compartments has been demonstrated, particularly for monoterpenes. Switching the sub-cellular localization to organelles that are not originally involved in the synthesis of aroma volatiles could result in the synthesis of new volatiles (Kappers et al., 2005). Also, the use of organ-specific promoters is desirable. Overall, the data reported in this review demonstrate the potential of genetic engineering for the improvement of aroma and taste properties of horticultural products. However, most if not all them are related so far to basic studies at the laboratory level. They provide only proof of principle that engineering one of several of these genes could be of practical interest. Field or commercial tests remain to be performed and acceptance by the consumers to be assessed.

13.9 Acknowledgements

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13.10 References


Genes involved in the biosynthesis of aroma volatiles


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14

Role of metabolome diversity in fruit and vegetable quality: multifunctional enzymes and volatiles

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14.1 Introduction

The flavour of fresh fruits and vegetables is one of the utmost external characteristics determining their quality. The human assessment of food flavour is greatly influenced by volatile compounds in the aroma mixture. Although several hundred volatiles are emitted by intact fruits and vegetables or are formed during processing, only a small contingent contributes to the overall aroma. In general, a volatile molecule produces a sensorial impression when its level exceeds the odour threshold level. Therefore, odour activity values (OAV) of volatile compounds have been determined in terms of the ratio of the concentration of the odorant in the matrix to the odour threshold, which implies that only volatiles with OAV greater than 1 contribute to the aroma (Grosch, 2001). It was found that, of the 400 volatiles released by tomatoes, only 16 reached an OAV of one or more (Yilmaz, 2001). Thus, the aroma of a particular fruit can often be characterized by the presence of a few compounds having a large impact on the odour, such as the \(\gamma\)- and \(\delta\)-lactones of peaches (Derail et al., 1999) and the 4-(4-hydroxyphenyl)-butan-2-one of raspberries (Klesk et al., 2004; Borejsza-Wysocki et al., 1992). But only in some rare cases does a single compound determine the overall flavour (flavour impact compound) (Fretz et al., 2005).

Plant volatiles are synthesized by the sequential action of metabolic enzymes in biosynthetic pathways. Great progress has been made in understanding the
enzymatic steps leading to the production of these small molecules and the molecular regulation of the pathways (Dudareva and Negre, 2005). Because most volatiles are restricted to a few lineages, it has been proposed that frequent changes in enzymatic profiles occur through evolution (Pichersky et al., 2006; Gang, 2005). Similar diversity and rapid change have also been observed for enzymes involved in the metabolism of volatiles, raising questions regarding the underlying evolutionary mechanisms (Schwab, 2003). Mechanisms that lead to the emergence of one or a few enzymes with new substrate specificities and thus new product patterns include gene duplication and divergence of enzymes used elsewhere in primary or secondary metabolism (Grotewold, 2005; Ober, 2005). In the gene copies mutations accumulate as a result of relaxed functional constraints and will be eliminated from the genome or might be fixed because of their beneficial properties, including dosage effects, subfunctionalization or the creation of a completely new function. Alternatively, alteration of spatial and temporal gene expression through mutation in the promoter broadens the chance of an encoded enzyme to find suitable substrates and produce novel products. In contrast, convergent and repeated evolution as shown for gibberellin biosynthesis (Tudzynski et al., 2002) may also cause the formation of identical volatiles in different organisms that are not closely related (Gang, 2005; Ober, 2005).

Studies have already shown how variations among biosynthetic enzymes result in the substrate and product preference that explain the diversity of volatiles present in particular fruits and vegetables. One or a few amino acid changes are sufficient to alter the substrate preference of an enzyme (Zubieta et al., 2003). On the other hand, it is also not uncommon for a single enzyme to use multiple substrates, catalyse different reactions with different kinetic properties and produce several products. Such multifunctional enzymes are considered to be the basis for the structural diversity of plant secondary metabolism and they might be the prerequisites for the evolution of new pathways and specificities (Roy, 1999; Kacser and Beeby, 1984).

14.2 Multifunctional enzymes

In literature the term ‘multifunctional enzyme’ is not well defined. Originally, only a protein which catalyses several reactions in a sequential manner starting from a single substrate was named multi-functional (Tudzynski et al., 2002). In this review the term is used in the broadest sense because enzymes may also accept a range of substrates (multi-substrate), catalyse different reactions in diverse pathways (multi-reaction) and produce a number of products from one substrate (multi-product). Moreover, multi-functionality of proteins is not restricted to catalytic activity. Besides featuring enzyme properties, proteins can simultaneously act as inhibitors, transporters, hormones, storage proteins, defence agents, transcription factors, etc. (Mosolov et al., 2001; Roymans et al., 2002).

Recently, the global properties of the metabolic map of Escherichia coli were compiled in the EcoCyc database characterizing the known network of E. coli
small-molecule metabolism (Ouzonis and Karp, 2000). Of the total of 607 \textit{E. coli} enzymes that were identified, 100 are multifunctional, either having different substrate specificities or different active sites. Some enzymes catalyse seven or even nine reactions. Because of the significantly high proportion of multi-functional enzymes, it is assumed that the genome projects are significantly underpredicting multi-functional proteins. Prominent multi-substrate enzymes of the primary metabolism are aldolase (EC4.1.2.13), hexokinase (EC2.7.1.2), transketolase (EC2.2.1.1) and uridine kinase (EC2.7.1.48) (Schuster and Zevedei-Oamcea, 2002). Isoleucine and valine are synthesized almost entirely by the same set of enzymes and four transaminases (EC2.6.1.xx) with overlapping specificities catalysing the formation of seven amino acids. Many other enzymes, which are commonly considered as monofunctional, actually catalyse side reactions, the so-called underground metabolism (D’Ari and Casadesús, 1998).

14.2.1 Broad substrate specificity
Almost all known enzymes are able to use alternative substrates, including naturally occurring metabolites (Pichersky \textit{et al}., 2006). These ‘underground reactions’ that describe reactions catalysed by enzymes acting on substrate analogues which are themselves endogenous metabolites open the door to metabolic errors but provide metabolic plasticity and lead to metabolome diversity. Such reactions offer alternative syntheses, create new metabolic capacities, bypassing the need for certain cofactors or bear alternative structures (D’Ari and Cassadesus, 1998). Underground reactions can also generate novel metabolites and pathways and provide new ways of dealing with the environment such as through the use of pesticides. These facts have not been duly taken into account in the modelling of metabolic networks. Here, I present primarily examples from the secondary plant metabolism where multifunctional enzymes have led to diversity of fruit and vegetable flavours.

\textit{Methyltransferases (EC2.1.1.xx)}
A large number of natural substances such as nucleic acids, proteins, pectins, lignins and various small metabolites such as flavonoids, phenylpropanoid, alkaloids and flavour compounds are methylated. The enzymes that catalyse the transmethylation use \textit{S}-adenosyl-L-methionine (SAM) as the methyl donor and transfer a methyl group to various acceptors carrying hydroxyl, carboxyl, amino, thiol or C–H groups thus forming methyl ethers, esters, amines, thioethers and carbon chain branches, respectively (Table 14.1). Because of their importance in the plant metabolism, the number of sequenced genes and cDNAs encoding SAM-dependent methyltransferases is rapidly growing.

Methyltransferases involved in the biosynthesis of volatiles have been characterized, among others, from rose (\textit{Rosa hybrida}) (Lavid \textit{et al}., 2002; Wu \textit{et al}., 2003, 2004), snapdragon (\textit{Antirrhinum majus}) (Murfitt \textit{et al}., 2000; Negre \textit{et al}., 2002), \textit{Clarkia breweri} (Wang and Pichersky, 1998; Ross \textit{et al}., 1999; Zubieta \textit{et al}., 2003); basil (\textit{Ocimum basilicum}) (Gang \textit{et al}., 2002), bitter fennel (\textit{Foeniculum
Table 14.1 O-, N-, S- and C-methyltransferases involved in the formation of flavour compounds. R, R₁, R₂, and R₃ refer to the references.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Products</th>
<th>Examples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-OH</td>
<td>R-OCH₃</td>
<td>Methyleugenol/methylisoeugenol</td>
<td>Wang et al., 1998</td>
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<tr>
<td></td>
<td></td>
<td>Estragole</td>
<td>Gross et al., 2002</td>
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<tr>
<td></td>
<td></td>
<td>1,3,5-Trimethoxybenzene</td>
<td>Wu et al., 2004</td>
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<tr>
<td></td>
<td></td>
<td>2,5-Dimethyl-4-methoxy-3(2H)-furanone</td>
<td>Wein et al., 2002</td>
</tr>
<tr>
<td>R₂</td>
<td>R₂</td>
<td>Methyl benzoate</td>
<td>Negre et al., 2002</td>
</tr>
<tr>
<td>R₁-NH</td>
<td>R₁-NCH₃</td>
<td>Methyl salicylate</td>
<td>Ross et al., 1999</td>
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<tr>
<td></td>
<td></td>
<td>Methyl jasmonate</td>
<td>Seo et al., 2001</td>
</tr>
<tr>
<td>R-SH</td>
<td>R-SCH₃</td>
<td>Methyl sulfide</td>
<td>Attieh et al., 2002</td>
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<tr>
<td></td>
<td></td>
<td>Methyl thicyanate</td>
<td>Attieh et al., 2000</td>
</tr>
<tr>
<td>R₁-CH₃</td>
<td>R₁-C-CH₃</td>
<td>Irone</td>
<td>Marner et al., 1988</td>
</tr>
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</table>

vulgare) (Gross et al., 2002), Stephanotis floribunda, Nicotiana suaveolens (Pott et al., 2004), Arabidopsis thaliana (Seo et al., 2001), Hoya carnosa, Petunia hybrida, Arabidopsis lyrata (Effmert et al., 2005), Vanilla planifolia (Pak et al., 2004), cabbage (Brassica oleracea) (Attieh et al., 2000, 2002) and strawberry (Fragaria × ananassa) (Wein et al., 2002). Recently, an S-methyltransferase gene has been isolated from Catharanthus roseus whose encoded protein catalysed the methylation of a number of thiols and phenols including the formation of 3-(methylthio)hexanol, an important flavour-related volatile in passion fruit (Passiflora edulis) (Coiner et al., 2006).

Methylation reactions are well suited to altering the polarity of natural metabolites and assisting volatility. Methyltransferases often accept more than a single substrate in vitro, but those that exhibit high substrate specificity also exist. The broad substrate tolerance of some methyltransferases suggests that they may be involved in more than one pathway (Schwab, 2003). But it is probably true to say that the in vivo significance of the in vitro substrate preferences still remains to be determined for a number of methyltransferases, a major problem being that the steady-state kinetic constants determined in vitro may or may not be relevant criteria for assessing in vivo routes to the products. Differences in substrate specificities between enzymes isolated from their natural sources and those expressed in E. coli have also been reported (Chiron et al., 2000). However, a dual role for a O-methyltransferase has
been demonstrated in transgenic strawberry plants where the down-regulation of the enzyme reduced the relative level of ferulic acid and of the flavour compound 2,5-dimethyl-4-methoxy-3(2H)-furanone (Lunkenbein et al., 2006).

The biochemical properties of methyltransferases and gene sequence homologies suggest that they might have evolved from a common ancestral gene which during evolution gained different expression profiles, and encoded enzymes obtained the ability to accept structurally diverse substrates. It has already been shown that substitution of a few amino acids can convert an isoeugenol O-methyltransferase (EC2.1.1.146) to a caffeic acid methyltransferase (Wang and Pichersky, 1999), a O-methyltransferase to an S-methyltransferase (Coiner et al., 2006) and even a single amino acid change can quantitatively alter substrate preference between alkaloids and phenylpropanoids (Frick and Kutchan, 1999).

Acyltransferases (EC2.3.1.xx)

Volatile esters are produced by virtually all soft fruit species during ripening and impart distinct characteristics to their flavour. They are formed by the reaction between alcohols and acyl CoAs derived from fatty acid, amino acid, phenylpropanoid and benzoate metabolism. The last step in the production of fruit esters is catalysed by alcohol acetyl-CoA transferases (EC2.3.1.xx) that have been characterized from apple (Malus sp.) (Souleyre et al., 2005; Defilippi et al., 2005; Li et al., 2006; Escheverría et al., 2004), strawberry (Fragaria × ananassa) (Pérez et al., 1996; Aharoni et al., 2000; Pérez et al., 2002), banana (Musa sapientum) (Beekwilder et al., 2004; Wyllie and Fellman, 2000), melon (Cucumis melo) (El-Sharkawy et al., 2005; Yahyaoui et al., 2002), grape (Vitis labrusca) (Wang and De Luca, 2005), and olive (Olea europaea) (Salas, 2004). ATTs capable of synthesizing benzyl benzoate, benzyl acetate and other volatile esters were also isolated from C. breweri (D’Auria et al., 2002; Dudareva et al., 1998). In all cases, both native and recombinant enzymes could use a broad range of acyl-CoAs and alcohol substrates (Fig. 14.1). This substrate tolerance enables a single enzyme to produce a range of fruit esters based on the supply of appropriate substrates.

To test the function of a rose AATs in planta, transgenic petunia plants were generated constitutively expressing the rose gene (Guterman et al., 2006). Although the preferred substrate of the enzyme in vitro was geraniol, in transgenic petunia flowers it used phenylethyl alcohol and benzyl alcohol to produce the corresponding acetate esters. Feeding the transgenic tissue with geraniol led to the production of the respective acetate. This example and others (Aharoni et al., 2000; Li et al., 2006; Wyllie and Fellman, 2000; Beekwilder et al., 2004) suggest that fruit ester production catalysed by AAT is at least partly governed by substrate availability and has to be considered when engineering volatile ester formation in plants.

In melon four members of an AAT family with amino acid identities ranging from 84% to only 22% have been reported (El-Sharkawy et al., 2005). The encoded proteins showed a differential but overlapping substrate preference and produced a wide range of volatile esters. The enzymatic activities and the expression of the AAT genes were strongly up-regulated during melon ripening. The data suggest that the multiplicity of AAT genes accounts for the great diversity of esters.
Fig. 14.1 Formation of esters catalysed by alcohol acyl-CoA transferase (AAT). R₁ and R₂ are variable substituents including saturated and unsaturated, straight and branched aliphatic carbon chains as well as aromatic and heterocyclic groups.

Formed in melon and explain why the substrate preference of a recombinant enzyme does not necessarily reflect in the representation of esters in the corresponding fruit volatile profile.

Miscellaneous

Short, to medium-length methylketones are key constituents of the aroma of dairy products but are also volatile compounds found in fruits and vegetables (Hakala et al., 2002; van Ruth et al., 1995). Although the biosynthetic pathway leading to methylketones has been well established in microorganisms, only recently a methylketone synthase gene has been isolated from a tomato expressed sequence tag (EST) database (Fridman et al., 2005). The cDNA was expressed in E. coli and the purified protein catalysed reactions in which C₁₂, C₁₄, and C₁₆ β-ketoacids bound to acyl-carrier-proteins were hydrolysed and decarboxylated to give C₁₁, C₁₃, and C₁₅ methylketones, respectively. Only one enzyme is sufficient to account for the formation of 2-undecanone, 2-tridecanone, and 2-pentadecanone in tomato and it is assumed that similar enzymes, more specific for short-length fatty acids, might form the aroma-active short-chain analogues found in different fruits (Zabetakis and Holden, 1997; Elss et al., 2005; Klesk et al., 2004).

Phenylpropenes such as chavicol, eugenol and isoeugenol are produced by plants and have been used by humans for food preservation, medicinal agents and flavoring (Gang et al., 2001). Recently it was shown that glandular trichomes of sweet basil (Ocimum basilicum), which synthesize and accumulate phenylpropenes, possess an enzyme that can use coniferyl acetate and p-coumaryl acetate together with NADPH to form eugenol and chavicol, respectively (Koeduka et al., 2006; Vassão et al., 2006). Although the eugenol synthase converted p-coumaryl acetate less efficiently into chavicol, the result demonstrated that the native enzyme shows the capability to use different substrates which occur naturally in plants.

Strawberry fruit produce an uncommon group of aroma compounds with a 2,5-dimethyl-3(2H)-furanone structure that are considered as key flavour compounds. Recently, an enzyme was characterized from ripe Fragaria × ananassa fruit which catalyses the formation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone from a newly identified natural precursor 4-hydroxy-5-methyl-2-methylene-3(2H)-furanone (Raab et al., 2006). Initially, the protein was assigned as Fragaria × ananassa quinone oxidoreductase (FaQR) due to sequence similarity with known QRs. Indeed, enzyme assays confirmed that FaQR is also able to reduce the artificial substrate o-phenanthrene quinone beside its enone oxidoreductase activity. It was assumed that the enzyme’s promiscuous activity is similar to the activity of an evolutionary related enzyme.
14.2.2 Multiproduct enzymes
Catalytically active proteins that produce more than one defined product from a substrate are called multiproduct enzymes. Typical representatives of this category are some terpene synthases. Terpene synthases (EC4.2.3.xx) catalyse among others the formation of odorous mono- and sesquiterpenes that dominate the flavour of some fruits and vegetables from geranyl diphosphate (GPP) and farnesyl diphosphate (FPP), respectively. This class of enzymes is widely distributed in nature and their products, the terpenoids, compose the largest and most diverse family of natural products. The first monoterpene synthase gene was cloned in 1993 which by similarity searches enabled the isolation and characterization of numerous terpene synthases from different plants (Colby et al., 1993; Trapp and Croteau, 2001). Terpene synthases cluster generally according to the substrate that they use but some synthases can also transform both GPP and FPP (Aharoni et al., 2004). Sequence similarity among terpene synthase genes does not necessarily translate to similarity among their products (Trapp and Croteau, 2001). Besides selective terpene synthases that form only one distinct product from the natural precursors GPP and FPP, this class is well known for their multiproduct enzymes (Sharon-Asa et al., 2003; Martin and Bohlmann, 2004; Lücker et al., 2004; Tholl et al., 2005).

Two sesquiterpene synthases have been cloned from maize that form the same complex mixture of eight sesquiterpenes from the precursor FPP but with different proportions of products (Köllner et al., 2004). These mixtures correspond to the sesquiterpene blends observed in two maize varieties, respectively. It was shown that the diversity of sesquiterpenes in these two cultivars is strongly influenced by single nucleotide changes in the alleles of the two terpene synthase genes. With regard to the diversity of terpenes, this result implies that a few synthases may account for the formation of a number of products. In light of evolution multiproduct enzymes can be seen as the result of duplicated genes that have been mutated after duplication due to relaxed functional constraints. The corresponding enzymes become less selective for their products (substrates and reactions) but under selection pressure they can gain new functions when some of the products turned out to be favourable for the survival of the plant.

14.2.3 Catalytic promiscuity
Catalytic promiscuity is defined as the ability of a single active site to catalyse more than one chemical reaction (O’Brien and Herschlag, 1999; Bornscheuer and Kazlauskas, 2004; Aharoni et al., 2005). Amidohydrolases (EC3.5.xx.xx) normally catalyse hydrolysis of amide bonds (C–N link), but also catalyse hydrolysis of phosphate triesters (P–O link) and esters (C–O link) (Roodveldt and Tawfik, 2005), while some P450 enzymes (EC1.14.21.xx) and dioxygenases (EC1.13.11.xx) can both hydroxylate substrates and epoxidize double bonds (Copley, 2003). After only five mutations to mimic the active site in a related steroid isomerase, a glutathione transferase (EC2.5.1.18) with peroxidase activity gained isomerase activity (Kazlauskas, 2005). It is assumed that catalytic
promiscuity has a natural role in evolution and occasionally in the biosynthesis of secondary metabolites. A new catalytic activity may be created by first adding an additional catalytic activity to an enzyme and second by losing the original activity (O’Brien and Herschlag, 1999). Thus alternative activities could have played an important role in the diversification of enzymes (and metabolites) by providing a duplicated gene with an advantage towards being captured by adaptive evolution. Catalytic promiscuity has not yet shown for enzymes involved in the formation of plant volatiles. One reason might be that this property of the proteins has not been analysed up to now.

14.2.4 Promiscuous function

Enzymes are expected to have evolved to serve certain catalytic functions. However, many enzymes have been found to ‘moonlight’, which means that they serve additional functions that are generally not enzymatic, but rather structural or regulatory (Jeffery, 1999; Copley, 2003). It has been demonstrated that the same protein can perform different functions in different locations within the cell. A protein in *E. coli* shows proline dehydrogenase (EC1.5.99.8) activity when it is associated with the plasma membrane, but binds DNA as a transcriptional repressor when it is in the cytoplasm. Similarly, aconitase (EC4.2.1.3) is either an enzyme or an RNA-binding protein. Glyceraldehyde-3-phosphate dehydrogenase (EC1.2.1.xx) is a glycolytic enzyme as a tetramer but the monomeric protein acts as a nuclear uracil-DNA glycosylase (Jeffery, 1999). Many lens crystallins are identical to cytoplasmic proteins (e.g. quinone oxidoreductases) and were apparently recruited during the evolution of the eye. Up to now moonlighting enzymes have not been described in plants but it is noteworthy to mention that FaQR, the enzyme involved in the biosynthesis of an important strawberry flavour molecule shows similarity to lens crystallins (Raab et al., 2006).

Moonlighting functions are difficult to predict. The existence of moonlighting may be suggested by unusual patterns of expression that are inconsistent with the enzymatic function of the protein in certain tissues or developmental stages. Moonlighting is a clever mechanism for generation of complexity using existing proteins without requiring expansion of the genome.

14.3 Multifunctional volatiles

Volatile produced by fruits, leaves and flowers serve multiple functions that are not always related to their volatility (Pichersky and Gershenzon, 2002). Plant volatiles have been implicated in defensive and attractive roles because they are involved in species-specific ecological interactions and are often restricted to specific lineages. Volatiles that derive from primary metabolism are ranked among the secondary plant metabolites (Pichersky et al., 2006). Although they provide adaptive characters under strong selective pressure, it is believed that they are not essential for plant survival. Flower and fruit aromas contain many chemicals and
the very little overlap in the profiles demonstrates the different functions of the individual molecules. Even closely related species synthesize diverse volatiles. It is generally accepted that compounds emitted by flowers serve to attract and guide pollinators but only a few studies have demonstrated the ability of individual substances to attract specific pollinators (Dudareva et al., 2004). For plants that flower at night, volatiles may be a better signal than floral colour or shape to draw insect pollinators.

Numerous investigations have shown that volatiles have anti-microbial or anti-herbivore activity and therefore it is believed that they serve to protect valuable reproductive parts of plants from enemies. For example, (S)-linalool and its derivatives produced by FaNES-expressing plants significantly repelled an agricultural pest in a dual-choice assay (Aharoni et al., 2003). A frequent vegetative volatile is isoprene, which may act to increase the tolerance of photosynthesis to high temperature by stabilizing the thylakoid membranes or by quenching reactive oxygen species (Dudareva et al., 2004). A general property of vegetative plant tissue is the release of volatiles such as fatty acid degradation products (C6- and C9-aldehydes and alcohols), terpenes or benzoids following herbivore damage (Pichersky and Gershenzon, 2002). Some of these substances also emitted by cabbage and cucumber have been demonstrated to serve as indirect plant defences through tritrophic interaction. That is, they attract arthropods that prey upon or parasitize herbivores, thus minimizing further damage to plant tissue. Volatiles may also act as direct defences through repelling or intoxicating herbivores and pathogens and some mono- and sesquiterpenes have also the potential to eliminate reactive oxygen species. It is also assumed that root-emitted volatiles function as anti-microbial or anti-herbivore substances, or exhibit allelopathic activities that increase the ecological competitiveness of the plant (Steeghs et al., 2004). Accordingly, plant volatiles can minimize the growth suppression of epiphytic bacteria by the phytopathogenic fungus Botrytis cinerea and thus affect population dynamics on leaf surfaces (Abanda-Nkpwatt et al., 2006a), while methanol serves as precursor of fruit esters and provides a carbon and energy source for epiphytic methylotrophs (Abanda-Nkpwatt et al., 2006b).

In fruits volatile emissions have evolved to facilitate seed dispersal by humans, animals and insects. The specific association of some volatiles with ripe fruits and their relative absence from vegetative tissues suggests a role in signaling ripeness and attracting seed-dispersing organisms. Unlike ripening fruits, vegetables produce most of the volatiles sensed as flavours only after their cells are disrupted. These volatile flavour compounds exhibit antimicrobial activity and have anticancer activities but can be toxic at high doses (Goff and Klee, 2006). The foundation for the flavours associated with most fruits and vegetables existed already before crop domestication. Domestication has had even a negative effect on flavour and volatile production of cultivated fruits and vegetables because breeding programmes have historically primarily focused on yield, colour, shape and disease resistance (Goff and Klee, 2006). Flavour is a complex, multigenic trait providing unique challenges to breeders and has not been a high priority up to now.
14.4 Conclusions

The complex flavour of fruit and vegetables is formed by a relatively small number of enzymes that often catalyse the production of more than one volatile from a single substrate or act on multiple substrates in the so-called underground metabolism. Thus, compartmentation of biosynthetic enzymes and accepted substrates decisively affects the composition of the volatiles emitted by fruits and vegetables.

A number of biosynthetic enzymes exhibit catalytic promiscuity, which means that a single polypeptide is able to catalyse different reactions. It is assumed that such alternative reactions play an important role in enzyme and metabolite diversification as they increase the probability that a duplicated gene gains a new function. Moonlighting functions of enzymes are the economic mechanisms of living cells to recruit already existing catalytic proteins for new, e.g. structural and regulatory functions. Finally volatiles itself serve multiple functions as they attract pollinators, seed-dispersing organisms and arthropods that prey upon herbivores but they also repel herbivores and pathogens due to their anti-microbial activity.

14.5 Future trends

Biological function is not determined by single proteins but by groups of proteins that are organized in networks of varying complexity. Thus, the in vitro function of a single gene product which has been elucidated with the recombinant protein has to be verified in planta. The in vivo functions of flavour genes can be best analysed by transgenic plants where a specific gene has been silenced or up-regulated. Fast transformation systems leading to stable and transient transformants are now available for some fruit plants (tomato, strawberry) (Fu et al., 2005; Oosumi et al., 2006). In combination with ‘-omics’ techniques these systems will accelerate the identification of the in planta function of novel flavour enzymes.

The DNA sequence of a gene, although often suggesting a plausible primary function for the gene product, is as yet far from predicting enzyme accuracy, let alone potential underground activity. High-throughput screening techniques will undoubtedly reveal additional unexpected examples of catalytic promiscuity. Increasing roles will also be given to in-silico biology, bioinformatics and modelling to understand metabolic diversity caused by multifunctional enzymes. Exact analyses of the gene expression profiles show that the differences are manifested by when, where and how individual genes or gene groups are switched on or off. This demonstrates the necessity of a systematic approach as proposed with system biology. Finally, much remains to be elucidated regarding the internal and external factors affecting volatile biosynthesis and emission. The transport, storage and emission of these compounds are neglected areas of study that must be addressed to support the efforts to improve fruit and vegetable quality.
14.6 References


Fruit and vegetable flavour


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High-throughput flavour profiling of fruit

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15.1 Introduction

Flavour perception involves the simultaneous perception of different sensations such as taste, aroma, colour, texture, but also pain and heat (Taylor and Roberts, 2004). All these so-called modalities interact in a very complex way – this is why sugar is added to lemon juice to decrease the perceived acidity. The brain interprets the signals from the different receptors and converts them into an overall flavour sensation. Flavour may actually change while the product is eaten not only because the product consistency typically changes due to mixing with saliva and mastication, but also because during this process aroma components are liberated and are even biosynthesised instantaneously (Piggott, 2000). Clearly, our lack of understanding of the multimodal character of flavour and its dynamics greatly complicates its analysis. In this chapter we will focus on the taste and aroma modalities as these flavour modalities are now understood relatively well.

Flavour is becoming one of the most important quality attributes of horticultural products to be optimised in breeding, cultivation and postharvest processes of fruit and vegetables. For example, in a typical product development cycle, consumer panels are used to measure consumer preferences, which are then mapped to flavour attributes scored by a sensory panel. Breeding programmes then aim at providing products which match these flavour attributes. Often, the capacity and cost of such sensory panels restrict large-scale screening programmes, and instrumental techniques have been developed to measure flavour attributes. The aim is usually not to replace the human sensory panel completely, but to provide tools for large-scale prescreening of new cultivars. Laboratory techniques such as GC–MS
and HPLC are available for analysing aroma volatiles and taste attributes, respectively. However, while such techniques might provide useful qualitative data, they are often too slow to analyse the large number of replicate samples which are typically required to provide statistically meaningful data – a typical analysis time is of the order of magnitude of 30 minutes. Also, the required laboratory skills to operate such equipment and the amount of sample preparation is considerable and often beyond the reach of horticultural research stations. Hence, for the analysis of the flavour of fruit and vegetables that require minimal sample preparation, there is a need for high-throughput techniques that are easy to operate at the lowest possible cost.

In this chapter we will first describe the biology of aroma and taste perception by humans as this knowledge has inspired the development of biomimetic sensors such as electronic noses and tongues. We will then introduce high-throughput spectroscopic techniques for measuring taste components, with an emphasis on near-infrared (NIR) spectroscopy. Also the principle and applications of electronic tongues will be discussed. Finally, new developments in high-throughput aroma profiling based on mass spectrometry and electronic noses will be described.

15.2 Human perception of aroma and taste

Humans can distinguish between at least five different taste attributes: sweet, acid, salt, bitter and umami. The latter attribute represents ‘savoriness’ which is related to the presence of glutamates. Taste buds for the different taste attributes are embedded in the epithelium of the papillae at the surface of the tongue which are visible to the naked eye (Fig. 15.1). Four types of papillae can be distinguished: fungiform, circumvallate, foliate and filiform; the first three cover the front, the rear sides and the back of the tongue, respectively, while the latter cover most of the dorsal surface of the tongue. All but the latter papillae contain taste buds. Taste buds are onion-like structures which consist of taste cells with microvilli at the peripheral end – thin, hair-like filaments which terminate in the taste pore. Taste components dissolved in saliva interact with a taste receptor on the microvilli and cause electrical changes in the taste cells, which then send a signal to the brain. One taste cell may contain different types of receptors. The brain transforms the signals from all triggered taste cells into a taste sensation (Rawson and Li, 2004).

Aroma perception is caused by stimulation of olfactory neurons by aroma components which must be present in a gaseous or vaporous state. The olfactory neurons are located in the olfactory epithelium on the roof of the nasal cavity. Volatiles are transported through the aqueous mucus of the olfactory epithelium by olfactory-binding proteins towards the olfactory receptors which are embedded in the membrane of the olfactory neurons. Upon binding of the volatile with the olfactory receptor, a signal transduction cascade is triggered, which involves the dissociation of a G-protein whose $\alpha$-subunit activates effectors which open ion channels. The resulting membrane depolarisation causes a signal to the brain. It is estimated that in humans there are about six million olfactory neurons and about
1000 different olfactory receptors; one olfactory neuron only has one type of receptor. The olfactory bulb in the brain processes all the signals from the olfactory epithelium simultaneously and creates a sensation of aroma (Rawson and Li, 2004; Pernollet and Briand, 2004).

15.3 High-throughput taste profiling

15.3.1 Instrumental measurement of taste components

Refractometry is generally used to measure the so-called soluble solids content (SSC) of fruit according to the AOAC 932.12 official method. The refractive index of a juice is proportional not only to the concentration of sugars but also of acids. The change of refractive index is converted into value for the SSC and is expressed in degrees Brix (°Bx). 1 °Bx corresponds to 1 g of sucrose per 100 g water. It is important to note that, while the SSC is mainly determined by sugars, it also contains information about the acid content of the juice. The total acidity of the fruit is usually determined by means of titration with 0.1 N NaOH according to the AOAC 942.15 official method and is expressed as grammes of the dominant acid per kg.

Individual sugars and acids in juice can be measured by means of HPLC (High Performance (or Pressure) Liquid Chromatography). For sugars a refractometric detector is often used, while for acids a diode array detector is most appropriate.
The sugar and acid peaks in the chromatogram are often broad and not all peaks are typically resolved well. Also, the juice cannot be injected as such but needs to be extracted. The (long) extraction procedure, typically with ethanol, is not very specific and because of the many co-extracted background components the baseline is not smooth. This may cause considerable integration errors. Also, the analysis time is typically more than 10 minutes. Because of these reasons HPLC is not suitable for high-throughput analyses of fruit.

Enzymatic kits are now available from companies such as Roche Diagnostics for measuring individual sugars and acids. The kits are based on an increase/decrease in absorbance, at a specific wavelength, caused by a change in NAD(P)H (340 nm) or formazan concentration (492 nm). The change is stoichiometrically related to the concentration of the component of interest through a cascade of several enzymatic reactions. Vermeir et al. (2007) miniaturised kits for different sugars and acids in 96 or 384 well microplates in volumes of 200 µL and 80 µL, which resulted in theoretical cost reductions of 93% and 98% compared with the traditional analysis in 3 mL cuvettes. A four-channel liquid handling system was programmed to dispense the different reagents with great accuracy. In addition, the use of the liquid handling system resulted in an increased throughput with total analysis times, including the filling and the reading of the microplate, of 45–85 s per sample per component. Such miniaturised enzymatic assays may serve as a reliable and cost-effective alternative for the HPLC-protocols.

15.3.2 Near infrared spectroscopy
Near infrared radiation covers by definition the wavelength range from 780 to 2500 nm. When radiation hits a sample, the incident radiation may be reflected, absorbed or transmitted, and the relative contribution of each phenomenon depends on the chemical constitution and physical parameters of the sample. Reflection includes both specular reflection, external diffuse reflection at rough surfaces, and scattering. The latter results from multiple refractions at cell wall interfaces, suspended particles such as starch granules, chloroplasts and mitochondria (Il’yasov and Krasnikov, 1991). Scattering may also appear due to heterogeneities such as pores, openings and capillaries that are randomly distributed through the sample.

Most absorption bands in the near infrared region are overtone or combination bands of the fundamental absorption bands in the infrared region of the electromagnetic spectrum, which are due to vibrational and rotational transitions. In complex materials such as fruit tissue, the multiple absorption bands and other peak-broadening effects result in NIR spectra that are a broad envelope with few sharp peaks. The spectra are clearly very similar and are dominated by the water spectrum with overtone bands of the OH-bonds at 760, 970 and 1450 nm and a combination band at 1940 nm (Polessello and Giangiacomo, 1981). This similarity is the reason why sophisticated multivariate statistical techniques are essential to extract useful information from an NIR spectrum.

Different measurement setups have been used to obtain near infrared spectra (Nicolaï et al., 2007). In reflectance mode (Fig. 15.2a) the light source and detector
are mounted under a specific angle, e.g. 45°, to avoid specular reflection. In transmittance mode (Fig. 15.2b) the light source is positioned opposite to the detector, while in interactance mode (Fig. 15.2c) the light source and detector are positioned parallel to each other in such a way that light from the light source cannot directly enter the detector. While Fourier transform and dispersive spectrophotometers have been used in the past, diode array systems are now the instruments of choice because of their short acquisition time (50 ms and beyond), robustness and comparatively low price.

The penetration depth of NIR radiation in fruit or vegetable tissue is limited. Lammertyn et al. (2000) found a penetration depth of up to 4 mm in the 700–900 nm range and between 2 and 3 mm in the 900–1900 nm range for apple. In a different optical configuration, Fraser et al. (2000) showed that the penetration depth in apple in the 700–900 nm range was at least 25 mm, while it became less than 1 mm in the 1400–1600 nm range. The limited penetration depth decreases the accuracy of NIR based measurements of internal quality attributes of thick-skinned fruit such as citrus. Transmission measurements, on the other hand, need very high light intensities which can easily burn the fruit surface and alter its spectral properties.

A drawback of NIR spectroscopy is that for each fruit species and cultivar a new calibration model is required, and the calibration models should be based on large datasets incorporating different orchards, seasons, cultivation systems, etc. (Peirs et al., 2002). The prediction accuracy also depends on temperature (Peirs et al., 2003). Finally, the calibration models depend on the spectrophotometer, so that model transfer even between different spectrophotometers of the same brand and type is not straightforward.

NIR spectroscopy has been used to measure the SSC of various fruit including apple (Lammertyn et al., 1998), apricot (Carlini et al., 2000), cherry (Lu, 2001), kiwifruit (McGlone and Kawano, 1997), mandarin (Kawano et al., 1993), melon (Guthrie and Walsh, 1997), and peach (Slaughter, 1995). The root mean squared error of prediction (RMSEP) is typically 0.5–1.0 °Brix. Acidity in fruit is much more difficult to measure by means of NIR spectroscopy, although some reports have been published in which reasonable accuracy was obtained (e.g. Peirs et al.,

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Fig. 15.2 Three types of reflection of radiation on a fruit or vegetable specimen: (a) specular reflection; (b) external diffuse reflection; (c) scattering.
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2002). Mehinagic et al. (2004) developed a calibration model to predict sensory attributes of apple directly from NIR reflectance spectra. A full account of NIR applications in fruit and vegetables is given by Nicolaï et al. (2007). Fruit grading lines equipped with NIR sensors are now commercially available from Aweta (IQA, www.aweta.nl), Greefa (iFA, www.greefa.nl), Mitsui-Kinzoku (www.mitsui-kinzoku.co.jp), Sacmi (F5, www.sacmi.it), TasteMark (www.taste-technologies.com) and others.

15.3.3 Fourier transform infrared spectroscopy
Infrared (IR) spectroscopy is a well-established technique in chemical analysis. Infrared radiation is absorbed by molecules, including taste components such as sugars and acids, causing molecular vibrations and rotations at specific wavelengths which depend on the chemical bonds within the molecule. The spectra have much more detail than a typical NIR spectrum, but because of the low penetration depth (typically below 0.1 mm), absorption spectra are usually measured based on thin liquid samples obtained through destructive sampling. It is, therefore, potentially useful for either batch sampling or taste screening in breeding applications. In Fourier transform infrared spectroscopy (FTIR) an interferometer is used to generate a time-domain IR absorption signal (the ‘interferogram’) which is subsequently Fourier-transformed to obtain the absorption spectrum. FTIR is relatively cheap and fast, and most IR spectrophotometers nowadays are FTIR instruments. FTIR has been used successfully to predict sugar contents in agricultural products (Paradkar et al., 2002). As in NIR, chemometric techniques are required to discriminate samples or to obtain quantitative information about taste components.

Attenuated total reflectance (ATR–FTIR) measurements offer interesting possibilities for the analysis of samples containing solids and liquids. In ATR, an infrared beam is coupled into an ATR crystal and guided by total reflection. A small part of the light, the so called evanescent field, escapes from the ATR crystal, enters the juice which has been deposited on top of it, and is wavelength-dependently absorbed (Fig. 15.3). The advantage of ATR–FTIR compared with traditional techniques such as HPLC is the speed of response and the simple sample preparation protocol. ATR–FTIR was successfully used by Beullens et al. (2006) to classify tomato cultivars based on their sugar and acid profile. In Fig. 15.4 the score plot of a canonical discriminant analysis of ATR–FTIR spectra of five tomato juices is shown. It is clear that a good discrimination could be obtained, except for two cultivars which overlap. Similar results were obtained by Rudniskaya et al. (2006) for the discrimination of apple juices.

15.3.4 Electronic tongues
An electronic tongue is essentially an array of chemical sensors with partial specificity (cross-sensitivity) to different components in the solution, in combination with an appropriate chemometric method for relating the multivariate response
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Attenuated total reflectance (ATR) cell. Radiation from the IR source enters the ATR crystal and is guided by total reflectance towards the detector. A small part of the light escapes from the ATR crystal, enters the juice which has been deposited on top of it, and is wavelength-dependently absorbed.

Score plot of the canonical discrimination analysis of juices of five different tomato cultivars (from Beullens et al., 2006, with permission from Elsevier).

Signal to a variable of interest such as a sensory descriptor generated by a taste panel. The main advantages of electronic tongues are the low cost, easy-to-handle measurement set-up and speed of the measurements.

At Kyushu University (Japan) an electronic tongue consisting of a potentiometric sensor array with lipid/polymer membranes was developed (Hayashi et al., 1990; Kikkawa et al., 1993; Toko, 1998). The array was submerged into the solution to be analysed by means of a robotic arm. Dissolved taste components
generated an electrode potential in the sensors which was then related to taste descriptors or used for discrimination. This electronic tongue has been used in a wide range of food applications, including beer, mineral water, sake, coffee and soy sauce (Toko, 1998; Iiyama et al., 2000). Kikkawa et al. (1993) were able to discriminate different tomato cultivars and to quantify taste components in tomato juices.

Electronic tongue systems based on voltammetric sensor arrays were developed by the group of Ingemar Lundström and co-workers at Linköping University for a wide variety of applications including the classification of beverages and monitoring of drinking water quality (Winquist et al., 1997), and the evaluation of milk freshness (Winquist et al., 1998). They combined the system with an electronic nose system to successfully discriminate orange, apple and pineapple juices (Winquist et al., 1999). This group also developed a hybrid electronic tongue based on a combination of potentiometry, voltammetry and conductivity to classify six different types of fermented milk (Winquist et al., 2000). The group of Andrey Legin at Saint-Petersburg University in Russia developed electronic tongue systems based on potentiometric sensors with (amongst others) chalcogenide glass membranes and plasticised polymer membranes (Legin et al., 1999, 2003). The systems were successfully used to discriminate apple and tomato juices (Rudnitskaya et al., 2006; Beullens et al., 2006). Commercial systems are available from Alpha M.O.S (www.alpha-mos.com).

15.4 High-throughput aroma profiling

15.4.1 Instrumental measurement of aroma components

Aroma analysis is traditionally done by means of gas chromatography–mass spectrometry (GC–MS). In this technique the headspace of the product is first sampled, either directly using a gas syringe, or via a concentration technique such as purge and trap or solid phase micro-extraction (SPME). The latter technique in particular has become very popular because it is simple, cheap and relatively straightforward to automate. SPME is based on extraction and concentration of the analytes either by submersion in a liquid phase or by exposure to a gaseous phase (Zhang and Pawliszyn, 1993). The SPME holder and a diagram of the SPME fibre are shown in Fig. 15.5. The fused silica fibre is coated with a stationary phase, which absorbs the volatile molecules when the fibre is exposed to the volatiles.

Different coating substrates are available and two types of fibre can be distinguished. Absorption-type fibres are coated with a liquid coating substrate such as poly(dimethylsiloxane) (PDMS). The analytes are extracted by partitioning in the liquid phase (PDMS) and these kinds of fibres are excellent for extraction of non-polar analytes. A second type of fibre is based on adsorption of the analytes. These fibres have a porous substrate (e.g. Carbowax® or divinylbenzene (DVB)) which physically traps or chemically reacts (hydrogen bonding or Van der Waals interactions) with the analytes. Polar analytes are well extracted with these fibres. The coating thus affects the eventual aroma analysis results. After this extraction
The silica fibre is coated with a stationary phase, which absorbs the volatile molecules when the fibre is exposed to the volatiles. After this extraction step, the fibre is withdrawn in the sleeve and the analytes are thermically desorbed in the injector of a gas chromatograph.

For identification purposes, every eluting component is transferred to a mass spectrometer where it is fragmented into a mass spectrum. The component can then be identified through a mass spectrum library search. It should be noted that often different volatiles have similar mass spectra. For example, terpenes typically have mass fragments at m/z 91, 93 and 136 (molecular ion), and it is often necessary also to inspect retention times relative to a standard linear alkane series. While GC–MS remains the standard aroma analysis technique to date, it requires skilled personnel and the analysis time is too long for routine fruit aroma analyses.

### 15.4.2 Headspace fingerprinting mass spectrometry (HF–MS)

Alternative systems have been developed to speed up the analysis time of GC–MS. Headspace fingerprint mass spectrometry (HF–MS) consists of introducing volatile components present in the headspace of a sample without prior chromatographic separation into the ionisation chamber of a mass spectrometer (Shiers et al., 1999). This is typically implemented by means of a short capillary column which is operated at an elevated temperature so that a broad, featureless peak is obtained (Fig. 15.6a). The spectrum resulting from simultaneous ionisation and fragmentation of the mixture of molecules introduced constitutes a ‘fingerprint’ of the actual aroma (Fig. 15.6b). Typically, vials with juice are loaded into an autosampling system equipped with an SPME injector. While the technique is much faster than traditional GC–MS – samples can be analysed every 2–5 minutes, depending on the headspace equilibration time required – headspace equilibration and extraction time and temperature must be controlled carefully to get reproducible results. A
Fig. 15.6  Typical HF–MS gas chromatogram (a) and integrated mass spectrum (b) of apple aroma. The individual volatiles are not resolved in the gas chromatogram.
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disadvantage of the technique is that it is unable to take into account variable odour thresholds. While for some products this may not be a problem, it certainly is when the headspace contains thiols or amines which have a very low odour threshold. HF–MS has been used successfully to measure ripeness of apple fruit (Saevels et al. (2004) and the aroma profile of tomato cultivars in a quality system (Berna et al. (2004). Recently, the evolution of aroma production in strawberries during superatmospheric oxygen storage was monitored using HF–MS (Berna et al., 2007).

Other techniques to speed up the gas chromatography analysis have been developed. In fast GC, a capillary column with a very small diameter and short length is used in combination with a sensitive detector. The column temperature is often established using resistive heating which allows very fast heating rates. Mondello et al. (2004) achieved an analysis time of 3.3 minutes for citrus essential oil, which represented an analysis speed gain of almost 14. Applications to rapid aroma screening of horticultural products remain to be seen.

15.5 Electronic noses

15.5.1 Principle

The concept of an artificial nose system was proposed in 1982 at the University of Warwick by Persaud and Dodd (1982). In principle, such systems rely on gas sensors, which were first developed more than 30 years ago (Gardner and Bartlett, 1994). At the beginning of the 1990s the term ‘artificial’ or ‘electronic nose’ appeared, and several commercial instruments became available. Gardner and Bartlett (1994) defined the electronic nose as ‘an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system, capable of recognising simple or complex odours’. This seems very far from the human nose, and according to Mielle (1996) this analytical system is ‘obviously electronic but not nose’.

An electronic nose system is more or less comparable to the human olfactory system as it comprises a sophisticated hardware with sensors, electronics, pumps, air conditioners, flow controller, etc. (imitation of the human nose with the olfactory epithelium), and software for hardware monitoring, data pre-processing (the analogue of the olfactory bulb), and statistical analysis tools (corresponds to the processing of the odour pattern in the brain). However, the operating principle, the number of sensors as well as the sensitivity and selectivity are very different.

15.5.2 Sensors

All types of sensors used in electronic noses exhibit interactions with the gas to be measured so that a series of physical and/or chemical interactions occurs when volatile compounds fly over the sensor. A dynamic equilibrium develops as volatile compounds are constantly being adsorbed and desorbed at the sensor
surface. Each sensor is a semi-selective chemical sensor. This feature is the key property on which the working principle of electronic noses is based. The ideal sensors to be integrated in an electronic nose should meet the following criteria: high sensitivity towards chemical compounds, that is, similar to that of the human nose (down to $10^{-12}$ g/ml); low sensitivity towards humidity and temperature; medium selectivity; they must respond to different compounds present in the headspace of the sample; high stability; high reproducibility and repeatability; short reaction and recovery time; robust and durable; easy calibration; easily processable data output; small dimensions. Hybrid systems based on different sensor technologies have also been tested (Heberle et al., 2000; Magan and Evans, 2000). These systems generally show better performances than systems based on only one sensor technique. Data fusion of electronic noses based on different sensor technologies were also more selective and sensitive (Boilot et al., 2003) than electronic noses on their own. Various kinds of gas sensors are available, but only five technologies are currently used in commercialised electronic noses.

**Metal oxide semiconductor gas sensors** (MOS) were first used commercially in the 1960s as household gas alarms in Japan under the names of Taguchi (1971) or Figaro (Figaro Inc., Japan). These sensors, also called oxide or ceramic gas sensors, rely on changes of conductivity induced by the adsorption of gases and subsequent surface reactions. Commercially available MOS sensors consist of a ceramic substrate (round or flat) heated by wire and coated with a metal oxide semiconducting material (Fig. 15.7a). The material frequently used is SnO$_2$ doped with various precious metals (e.g. platinum or palladium), but ZnO, In$_2$O$_3$, WO$_3$, Fe$_2$O$_3$ and Ga$_2$O$_3$ are also employed.

**Metal oxide semiconductor field-effect transistor** (MOSFET) sensors rely on a change of electrostatic potential. A MOSFET sensor comprises three layers: a silicon semiconductor, a silicon oxide insulator and a catalytic metal (usually palladium, platinum, iridium or rhodium), also called the gate (Fig. 15.7b). When polar compounds interact with this metal gate, the electric field and thus the current flowing through the sensor are modified. The recorded response corresponds to the change of voltage necessary to keep a constant preset drain current (Lundström et al., 1975). As in the coating of MOS sensors, the gate structure of a MOSFET sensor is either a thick, dense metal film (100–200 nm) or a thin, porous metal film (6–20 nm). MOSFET sensors are hot sensors, which means that they operate at high temperatures.

**Conducting organic polymer** (CP) sensors rely on changes of resistance caused by the adsorption of gas. However, their operating mechanism is more complex and not yet well understood. Conducting organic polymer sensors are obtained by electropolymerisation of a thin film of polymer across the gap between gold-plated electrodes (Fig. 15.7c). Conducting organic polymers such as polypyrrole (Barisci et al., 2002), polyaniline or polythiophene (Guadarrama et al., 2001) are often used as a sensing element, but also more complex polymers like poly(2,5-thienylene vinylene) (De Wit et al., 1998) can be used.

**Quartz microbalance sensors** are made of tiny quartz discs coated with materials such as chromatographic stationary phases, lipids or any non-volatile compounds.
that are chemically and thermally stable (Fig. 15.7d). Examples of coatings often used with this kind of sensors are lead zirconate titanate films (Ferrari et al., 1999), modified metallo-porphyrins (Di Natale et al., 1997), Cu-phthalocyanine dye (Fleischer et al., 2002) and zeolites (Sasaki et al., 2002). When an alternating electrical potential is applied at room temperature, the crystal vibrates at a very stable frequency, defined by its mechanical properties. Upon exposure to a vapour, the coating adsorbs certain molecules which increases the mass of the sensing layer and, hence, decreases the resonance frequency of the crystal. This change may be monitored and related to the present volatile. QMB sensors vibrate with a frequency of 10–30 MHz.

Surface acoustic wave sensors (SAW) are similar to QMB sensors in many respects. Both technologies use coated oscillators from which a frequency change is measured upon exposure to an analyte, due to adsorbed mass by the coating. In contrast with a QMB sensor, where the entire crystal oscillates, a SAW device generates Raleigh (two-dimensional) waves down the surface of a quartz or silicon substrate on to which a thin coating has been applied (Fig. 15.7e). A change in mass adsorbed on the coating generates a corresponding change in frequency. For coatings, the same material can be used as on the QMB sensors. SAW devices generally operate at much higher frequencies (100 MHz–1 GHz) than QMBs, and are thus more sensitive (D’Amico et al., 1997). However, both sensors require higher concentrations of volatiles to elicit response levels comparable to other sensors.

A major problem with all sensor designs is drift due to degradation of the sensing material and other effects. Several techniques have been suggested to compensate for drift effects (Artursson et al., 2000). Also, many sensors are notably sensitive to ethanol.

15.5.3 Applications
In horticulture, electronic noses have been successful in monitoring the aroma of melons (Benady et al., 1995), pears (Oshita et al., 2000), peaches (Moltó et al., 1999), nectarines (Di Natale et al., 2001), tomatoes (Maul et al., 1998; Berna et al., 2004) and other fruit and vegetables (Sarig, 1998, Di Natale et al., 2000). Most of the work on these fruits was limited to classification in different maturity groups based on the electronic nose measurements. The different groups were defined based on physiological properties like colour, firmness, soluble solids content and produced aroma compounds. Electronic noses are often used to measure the variety or origin of a horticultural produce. Martinez et al. (2003) applied an electronic nose for olive oil aroma discrimination of quality, variety of olive and geographic origin.

Gelperin et al. (1999) measured aroma patterns of some apple cultivars with an electronic nose and could distinguish between ‘McIntosh’, ‘Rome’, ‘Braeburn’, ‘Red Delicious’ and ‘Fuji’ apples. Measuring the headspace of apples has been an interesting challenge for electronic noses, since the aroma is an important maturity indicator that correlates well with consumer acceptance (Brezmes et al., 2001).
Fig. 15.7 Schematic diagrams of five different kinds of sensors: metal oxide semiconductor gas sensor (a), metal oxide semiconductor field-effect transistor sensor (b), conducting organic polymer sensor (c), quartz microbalance sensor (d) and surface acoustic waves sensor (e).
Hines et al. (1999) and Young et al. (1999) used an electronic nose to measure ripeness of apples. Aroma changes of apple during shelf life and the optimal picking date were successfully determined non-destructively using an electronic nose by Saevels et al. (2003) and Saevels et al. (2004). Berna et al. (2004) investigated the effect of shelf life and cultivar on the aroma of tomato using a quartz microbalance electronic nose system. These authors also correlated tomato aroma measured using an electronic nose successfully with sensory properties and consumer preference (Berna et al., 2005a,b).

15.5.4 New developments

Most of the discussed sensors are either mass transducers (QMB and SAW) or chemiresistors (MOS, MOSFET and CP). Since optical transduction is widely used in chemical sensing (Wolfbeis, 2006), an electronic nose based on the variation of the optical features of chemical interactive materials is plausible. Di Natale et al. (2000a) designed and tested an ‘electro-optical nose’. This system consists of a matrix of silicon-integrated photodiodes (the transducers), coated by different thin film of metallo-porphyrins, and of a blue LED light source with an emission peak around 450 nm, the spectral region of the main absorption band of the metallo-porphyrins which reflect a different spectrum dependent on the volatile that is adsorbed to them. This technique is promising since these optical sensors show the same behaviour as QMB sensors coated with metallo-porphyrins, but achieve a better resolution, from 3 to 22 times higher. The concept was taken one step further by Filippini et al. (2006) by using a computer display as a light source. A series of different colours is programmed in the display, and the transmission through spots of metal porphyrins on a disposable transparant support material after being exposed to volatile molecules is measured by a web camera. A comparable optical-based chemical detection system, based on the Illumina BeadArray™, was introduced by Forood et al. (2006). Rakow and Suslick (2000) reported a colorimetric sensor array for odour visualisation (Chemsensing™). This method is based on the colour change induced in array of metallo-porphyrin dyes upon ligand binding while minimising the need for extensive signal transduction hardware. The chemoselective response of a library of immobilised vapour-sensing metallo-porphyrin dyes permits the visual identification of a wide range of analytes. The authors report an extremely high sensitivity (up to ppb) and a high selective discrimination of the different tested volatiles. As the colorimetric arrays can only be used once, the reproducibility is not obvious and needs to be tested. The New Zealand company ripeSense (www.ripesense.com) developed a disposable sensor which reacts to the aromas released by the fruit as it ripens. The sensor is initially red and graduates to orange and finally yellow. The sensor can be integrated into a package and gives the consumer an idea of the ripeness of the fruit. It can be expected that similar sensors will emerge within the next couple of years.

Recently, an olfactory cell-based biosensor was described (Liu et al., 2006). Olfactory receptor neurons and olfactory bulb cells were attached to a semiconductor
chip. The extracellular potential of the neurons under stimulations of the odorants or neurotransmitters, such as acetic acid and glutamic acid, was measured successfully using a light-addressable potentiometric sensor (LAPS) as a sensing chip. Vidic et al. (2007) co-expressed olfactory receptors and an appropriate G protein \( \alpha \)-subunit in *Saccharomyces cerevisiae* cells from which membrane nanosomes were prepared and immobilised on a sensor chip. Upon exposure to volatile molecules a signal was detected by means of surface plasmon resonance. These developments pave the way for bioelectronic or bionic noses.

### 15.6 Chemometrics

The signals generated by sensor arrays such as electronic noses and tongues may be processed using a variety of techniques. Graphical analyses with bar charts, profiles, polar and offset polar plots are simple forms of data treatment that may be used. This option is suitable when visually comparing samples to a single specified reference. However, when several references are used, analysis becomes more complicated and an alternative approach may be necessary (Schaller et al., 1998).

A second way of analysing the multivariate sensor array signals is by means of multivariate analysis. Multivariate data analysis generally involves data reduction. It reduces high dimensionality in a multivariate problem where variables are partly correlated (e.g. sensors with cross-sensitivities), allowing the information to be displayed in a smaller dimension (typically two or three) (Gardner and Hines, 1997). There are many multivariate techniques to choose from: principal components analysis (PCA), principal components regression (PCR), partial least squares (PLS), canonical discriminant analysis (CDA), feature weighting (FW) and cluster analysis (CLA). These methods can be classified as supervised or unsupervised. Unsupervised learning methods are generally used in exploratory data analysis because they attempt to identify a gas mixture without prior information on the nature of the samples. These techniques, which include PCA, PCR, CLA and multi-dimensional scaling, are useful when no example of different sample groups is available, or when hidden relationships between samples or variables are suspected (Gardner and Bartlett, 1994). Conversely, supervised learning techniques classify sensor array signals by developing a mathematical model, relating training data, i.e. samples with known properties, to a set of given descriptors. Test samples are then evaluated against a knowledge base and predicted class membership is deduced.

The above multivariate techniques are all linear as a model is calculated using linear combinations of input data. Often sensors have a non-linear response versus concentration. However, these techniques work well if a low concentrations of aroma or taste components ensures an approximately linear response. In addition, the use of pre-processing algorithms, such as averaging, linearisation or normalisation, improves the performance of these analytical techniques. When high concentrations of aroma or taste components are measured, a non-linear technique would be more appropriate. Artificial neural networks (ANN) and kernel-based
techniques are the most popular non-linear techniques. The latter are of particular interest as they allow a natural non-linear extension of traditional techniques such as PCA and PLS (Nicolaï et al., 2006). Non-linear models usually need more parameters, since some of them are used to describe the shape of the non-linearity (more input data than linear models). The main advantage of such models is flexibility, i.e. the ability to adjust to more complex data variations. However, caution is necessary when choosing model flexibility; this can be achieved by selecting the number of parameters. If too many parameters are taken into account, the calculated model will also fit unwanted sensor noise.

15.7 Conclusions and outlook

The shorter commercial life cycle of fruit and vegetables and the increasing importance of flavour have necessitated the development of new high-throughput techniques for flavour analysis. While some techniques such as enzymatic biosensor arrays and fast GC(MS) are miniaturised or accelerated versions of existing techniques, others are based on the mechanism of human flavour perception. Such biomimetic sensors use sensor arrays which generate complex signals when exposed to a headspace or immersed in juices. These signals are then analysed by means of chemometric techniques and related to either sensory attributes of the fruit or vegetable, or to individual flavour components. Examples of biomimetic sensors are electronic noses and tongues, but also spectroscopic techniques such as (near) infrared spectroscopy. They typically require less sample preparation than traditional techniques and are faster. Some of them, in particular NIR spectroscopy for measuring soluble solids content, are non-destructive and have been mounted on grading lines. As a consequence, grading based on internal quality attributes rather than external appearance becomes possible and this is expected to radically change the way fresh fruit and vegetables are commercialised.

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