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What Can We Control?**

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Aroma Generation by Horticultural Products: What Can We Control? Introduction to the Workshop

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Aroma, as is true for many quality attributes of fresh and processed fruit and vegetable products, is affected by the culture of the plant material prior to consumption. Culture, in this case, refers to the cultivar selection and production and postharvest processes that affect the physiology of the plant. In this workshop, we will focus on the effects of cultural tools on the biology of aroma generation by horticultural commodities. Importantly, the aroma of some fresh horticultural products [e.g., strawberry (*Fragaria × ananassa* Duch.), tomato (*Lycopersicon esculentum* Mill.), and apple (*Malus × domestica* Borkh.)] has received an increasing amount of attention from both producers and consumers because of perceived deficiencies in the sensory quality of commercially produced cultivars, so perhaps the time is ripe for strengthening efforts to improve our understanding of this important attribute.

We have loosely grouped commodities into three distinct classes, based on the manner in which the product is typically consumed and the means by which the bulk of the compounds with aromatic character impact are generated. One class of plant products is usually consumed after some form of thermal treatment that affects the synthesis of aromatic compounds. Within this group one might place the edible portions of, for example, sweetpotato [*Ipomoea batatas* (L.) Lam.], potato (*Solanum tuberosum* L.), spinach (*Spinacea oleracea* L.), and green beans (*Phaseolus vulgaris* L.). A second class is consumed fresh, but some form of cellular disruption, and the mixing of cellular constituents normally held apart by cellular compartmentation, are

responsible for the formation of characteristic aromas. Among this group, one could include, for example, edible portions of tomato, blueberry (*Vaccinium* sp.), spinach, and radish (*Raphanus sativus* L.). In the third class, the production of characteristic aroma compounds is spontaneous, relying primarily on the developmental stage of the organ. Important in this class are fruits such as apples, bananas (*Musa* sp.), strawberries, and peaches [*Prunus persica* (L.) Batsch.].

These three classifications are somewhat artificial in that they integrate the habits of the consumer (some plant materials are consumed either fresh or cooked) and the biology of the plant. Further, the volatile profile will probably comprise compounds that are synthesized via more than one of the three routes described. Cellular disruption occurs, for instance, in all plant products as they are processed or consumed, and aromas attending this disruption will necessarily contribute to the mixture of compounds with olfactory activity. Nevertheless, there are advantages to the classifications, as they permit a focused presentation and discussion of biological systems that affect final aroma and, by extension, the manner in which plant processes can be altered by cultural techniques to affect the product.

The first of the four papers in this workshop (Kays and Wang, 2000) approaches the subject of thermally induced aromas. As part of the discussion, the authors describe the process of flavor perception by humans, segregating and then integrating the influences of taste and aroma on flavor. Flavor perception is the process linking plant biochemistry with the physiology and psychology of the consumer and is a particularly interesting and difficult area of inquiry. The authors proceed to develop the concept of thermal induction of aromas, using the sweetpotato as a primary example, and conclude with a discussion of selection through plant breeding and preharvest factors as they

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affect aroma. Among the interesting features of this work is the activity of enzymes critical to flavor development during the heating process.

The second paper in the workshop (Baldwin et al., 2000) focuses on the cell disruption-dependent synthesis of aroma in tomato. These authors also treat the subject of aroma perception, with additional perspective on the complexity and sensitivity of aroma detection by humans and other herbivores. Among the concepts discussed is the importance of the odor threshold of aromas as it pertains to perception, as well as its use as a descriptive tool. Considerable attention is given to the biochemistry of aroma generation following tissue disruption, with a discussion of possible target enzymes for improving aroma.

The third and fourth papers describe the process of spontaneous aroma generation for strawberry (Forney et al., 2000) and apple (Fellman et al., 2000) fruit. The biosynthesis of esters figures prominently in both crops. The crops differ, however, in that apple can be said to have a specific character-impact compound such as hexyl acetate or ethyl 2-methylbutyrate, whereas no such compound exists for strawberry, the aroma of which is only apparent when a mixture of esters and other volatiles are present. In both papers, the importance of cultivar and postharvest handling procedures is discussed at some length.

The task of preserving and improving the aroma of horticultural products will probably be difficult. For instance, some treatments currently used to prevent decay, or to preserve texture and color, can compromise aroma quality. Elevated CO₂, for example, is commonly used to reduce decay in strawberry and other berry crops, but can induce fermentation (Beaudry, 1993; Ke et al., 1991). Similarly, reduced O₂ levels, while extending the storage-life of apple and other pome fruit, can reduce aroma and, at stress levels, cause off-flavors (Brackmann et al., 1993). Perhaps more to the point, the task of improving our understanding through scientific inquiry appears daunting. The metabolic pathways responsible for the synthesis of aroma compounds are diverse and often highly integrated with other portions of both primary and secondary metabolism. Even so, we are poised on the brink of new breakthroughs. For instance, the protein required for the biosynthesis of esters has been characterized (Pérez et al., 1993; Ueda and Ogata, 1977) and the gene controlling its synthesis recently

identified (Aharoni et al., 2000). As we develop a better understanding of the genetic and biochemical factors that alter or control synthesis of aroma, we should be able to develop better cultural tools to maintain and/or improve flavor. We hope that the proceedings from this workshop will inspire plant scientists with these possibilities and instigate new, much-needed research.

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Thermally Induced Flavor Compounds

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Given the number of recent reviews on flavor chemistry (Acker et al., 1990; Berger, 1995; Mathlouthi et al., 1993; Schab and Crowder, 1995; Shallenberger, 1993; Spielman and Brand, 1995), especially relative to thermally generated volatiles such as those produced via the Maillard reaction (For, 1983; Ikan, 1996; Mottram, 1994; Parliment et al., 1994; Whitfield, 1992), we have confined our review to a critique of chemical components and reactions modulating flavor, touching upon how thermally derived flavors overlap into the sphere of horticulture. Why would horticulturists be even remotely interested from a professional standpoint in the flavor of cooked products? Isn't this really the realm of food scientists or food chemists, i.e., changes in food products during or after cellular death?

Thermally generated flavors are in fact a relevant horticultural topic. First, flavors of most horticultural food products are largely generated during cooking. Vegetable crops, for example, are usually cooked before they are eaten [e.g., 370 of 390 commercially cultivated vegetable crops from around the world are routinely to intermittently

cooked (Kays and Silva Dias, 1996)], and cooking significantly alters their flavor. In addition, although fruits tend to be thought of as eaten raw, a major portion of the total production is processed (Table 1). In many cases, processing involves a thermal treatment, which alters the flavor of the final product. Therefore, a major portion of horticultural food crops are cooked and much of their final flavor is the result of cooking.

Second, the eventual cooked flavor of such products varies with the chemistry of the product and how it is handled prior to cooking. There are many examples of differences in flavor among cultivars of a particular fruit or vegetable. The basic chemistry of the fruit or

Table 1. Total U.S. production of several fruits in 1995 and their use.^z

Crop	Total production (kt)	Used fresh (%)	Processed ^y (%)
Apple (<i>Malus ×domestica</i> Borkh.)	5665.5	56.2	43.8
Cherry (sour) (<i>Prunus cerasus</i> L.)	150	0.9	99.1
Peach [<i>Prunus persica</i> (L.) Batsch.]	1119	50.9	49.1
Pear (<i>Pyrus communis</i> L.)	943.5	58.6	41.4

^z1994 data (U.S. Dept. of Agriculture, 1997).

^yCanned, dried, frozen, juiced—processes generally involving thermal treatments.

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vegetable as it arrives from the field largely dictates the subsequent flavor potential of the product. Thus, alteration of the basic flavor of a product is a plant breeding problem in that food scientists can only optimize the existing flavor potential.

Flavor perception. The sensory characteristics of foods can be loosely grouped into three categories: flavor, texture, and appearance. Flavor, in particular, plays a major role in both our selection and enjoyment of foods, and is generally considered to be the combination of taste and odor. Flavor perception can also be significantly influenced by heat, pain, and tactile sensations.

The flavor of an individual food product is derived from the collective mosaic of numerous compounds that impact odor and taste. It is important to note that a taste or odor is not an inherent property of a specific compound but is the physiological and psychological assessment of the individual sensing it. Therefore, the same compound can be perceived differently by different individuals or by the same individual at different times. Interactions among stimuli may occur at the taste bud/olfactory level or at signal processing in the brain (Thomson, 1986).

The flavor quality of food, therefore, is more than just odor and taste; it is a complex pattern that has different critical characteristics depending upon the food (Thomson, 1986). In contrast to visual or auditory sensations, flavor has a complex sensory basis involving receptors in both the oral and nasal cavities. These receptors include cells sensitive not only to taste and odor but also to pressure, touch, stretch, temperature, and pain (Moulton, 1982). Although odor and taste are well integrated in their contribution to the overall flavor, odor is often considered to play a dominant role in flavor delineation. This is, in part, due to the number of odor receptors and their ability to discriminate among odors. For example, the ability to identify the flavors of molasses, whiskey, salt, and sugar are superior with odor cues than without (Mozell et al., 1969). Thus, the uniqueness of many flavor substances appears to rely upon their ability to stimulate the olfactory organ. Because of the distinct differences between taste and odor, this review is separated into sections on taste and odor, followed by an overview of how flavor chemistry can be modified through plant breeding.

Taste. Taste is a sensation assessed through the contact of water-soluble compounds with the mouth and tongue. Four primary taste sensations are widely accepted: sweet, sour, salt, and bitter, though alkaline and metallic are considered by some as important in taste responses (Moncrieff, 1967). The sensation of taste is achieved through taste buds, which are distributed over the tongue and in certain areas of the buccal cavity. The number of taste buds in humans is estimated to be ≈ 4500 (Miller et al., 1990), with individual buds consisting of ≈ 15 to 18 receptor cells. The taste buds are located within specialized structures called papillae, found mainly on the tip, sides, and rear of the upper surface of the tongue (Thomson, 1986).

Of the primary taste sensations, the taste threshold concentration on a molar basis varies considerably (Table 2). When ranked, giving sucrose a value of 1.0, perception sensitivity proceeds from bitter > sour > sweet > salty (Pfaffmann et al., 1971). For example, quinine sulfate (bitter) can be perceived at 8×10^{-6} M while potassium chloride (salty) requires 1.7×10^{-2} M. Within categories, the threshold concentration varies among compounds (Table 2). In addition, a single

compound can elicit more than one taste sensation. Sodium chloride is sweet at low (e.g., 0.020 M), but salty at higher (0.050 M) concentrations. Such interactions can greatly complicate the quantification of taste.

Taste is dominated by sugars, acids, several amino acids, and nucleotides, salts, and a number of bitter compounds (Maga, 1990). Often these are present prior to cooking. There are, however, cases where distinct taste compounds are formed during cooking. For example, some of the Maillard reaction products impact taste. Perhaps a classic example of the synthesis of taste components upon cooking is the sweetpotato [*Ipomoea batatas* (L.) Lam.], in which a major portion of the final sugar concentration develops during exposure to high temperatures (Sun et al., 1994).

a. Sweetness. Sugars are the most widespread form of sweet compounds found in plant products, and in recent history man has selected certain species that have the ability to synthesize and store large quantities; e.g., sugar cane (*Saccharum officinarum* L.) and sugar beet (*Beta vulgaris* L. Vulgaris Group). A relatively wide range of sugars is present in plants, and the individual sugars vary substantially in both concentration and relative sweetness. The common sugars (L-form) are ranked in the following order of sweetness: fructose (1.2) > sucrose (1.0) > glucose (0.64) > galactose (0.5) > maltose (0.43) > lactose (0.33) (Shallenberger, 1993). A number of the amino acids [i.e., L forms of alanine, isoleucine, leucine, valine, serine, threonine, asparagine, glutamine, arginine, lysine, cysteine, methionine, phenylalanine, glycine (D-, L-form), tryptophan, and histidine] are also sweet (Haefeli and Glaser, 1990), the latter two in particular. Most of the D-amino acids are not sweet and, in the case of tryptophan and histidine, the taste shifts from very sweet (L-form) to bitter (D-form). Generally, the concentration of free amino acids in plants is too low to significantly impact sweetness.

In addition to sugars and amino acids, a wide range of other natural and synthetic compounds are sweet (Sardesai and Waldshan, 1991). These are typically found in either small quantities or in obscure plant species and, as a consequence, do not significantly impact the sweetness of horticultural products. The range of types of compounds that exhibit sweetness is impressive: peptides, proteins, flavanones, flavonols, dihydrochalcones, isovanillyl, sesquiterpenes, urea compounds, sulfones, and others.

The methyl ester of L-aspartyl-L-phenylalanine (aspartame) is very sweet (Mazur et al., 1969). Other synthetic peptides such as alitame [L- α -aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamid] is exceptionally sweet (i.e., 2000 times sweeter than aspartame) (Glowaky et al., 1991). The discovery of aspartame led to a greatly expanded research effort on artificial sweeteners and has resulted in several commercial products (e.g., Nutrasweet®, Sucralose®) that allow a reduction in calories while maintaining sweetness in processed foods.

Sweet compounds or compounds modulating sweetness have been isolated in a number of obscure plant species. For example, miraculin, a protein found in the berries of *Synsepalum dulcificum* (Schumacher & Thonn.) Daniell, has the unique property of being able to convert the sour taste of acids into the sensation of sweetness (Inglett, 1971; Kurihara 1971). The protein reacts with the taste buds, and at very low concentrations (i.e., 7×10^{-7} M), can render 0.02 M citric acid as sweet as 0.4 M (14%) sucrose. The duration of the effect is concentration-dependent, lasting from ≈ 20 min at low concentrations of the protein to as long as 3 h at high concentrations. Another sweet protein, monellin, found in the berries of *Dioscoreophyllum cumminsii* (Stapf.) Diels, is ≈ 1000 to 2250 times as sweet as sucrose on a weight basis (Inglett and May, 1968, 1969) or $\approx 100,000$ to 130,000 times as sweet on a molar basis (Ariyoshi et al., 1991; Kim et al., 1991). Thaumatin, a protein from the fruit of *Thaumatococcus daniellii* Benth. (van der Wel and Loeve, 1972) is $\approx 100,000$ times as sweet as sucrose on a molar basis. Phyllostulcin, an isocoumarin from the leaves of *Hydrangea macrophylla* (Thunb.) Ser., is ≈ 400 times as sweet as 3% sucrose (Yamato and Hashigaki, 1979). Several flavanones and flavonols are also sweet. For example, (+)-dihydroquercetin-3-acetate from *Tessaria dodoneifolia* (Hook. & Arn.) Cabrera. (Kingham and Soejarto, 1991) is ≈ 80 times as sweet as sucrose, and (+)-dihydroquercetin-3- α -L-rhamnosyl from *Engelhardtia chrysolepis* Hance is likewise sweet (Dick, 1981). However, in virtually all instances, sweet compounds

Table 2. Molar recognition thresholds of individual compounds and relative activity ranking of taste sensations.²

Taste	Compound	Median taste threshold (mM)	Relative activity ³
Sweet	Sucrose	17	1.0
	Sodium chloride	20	
Salty	Sodium chloride	30	0.6
	Potassium chloride	17	
Sour	Hydrochloric acid	0.09	18.8
	Acetic acid	1.8	
Bitter	Quinine sulfate	0.008	24.3
	Caffeine	0.7	

²After Pfaffmann et al. (1971).

³Activity relative to sucrose.

other than sugars are either not present or are found in sufficiently low concentrations in horticultural products to be of little importance in the overall contribution to sweetness.

Some loss in sweetness can occur as a result of thermal reactions. For example, sugars represent an essential substrate for the Maillard reaction (see below); thus, losses can occur via this mechanism. This reaction, however, occurs predominately in areas that have been largely dehydrated, such as the surface of a product. As a consequence, losses are localized and typically represent only a fraction of the total sugars present. Losses of sweetness can also occur due to leaching when the product is heated in an aqueous solution. The surface-to-volume ratio of the product, solvent volume, length of cooking, and other factors can affect losses.

In a few instances, perhaps best exemplified by the sweetpotato, a pronounced increase in sugar concentration occurs with exposure to high temperature. Starch present in the storage root is rapidly hydrolyzed during cooking by the amylase system, resulting in the formation of maltose. The reaction involves two enzymes, α -amylase [(E.C. 3.2.1.1) 1,4- α -D-glucan glucohydrolase] and β -amylase [(E.C. 3.2.1.2) 1,4- α -D-glucan maltohydrolase]. Alpha-amylase cleaves the α -(1,4)-glucosidic linkages between internal glucose molecules within amylose and amylopectin (Myrback and Neumuller, 1950), yielding dextrans and small amounts of reducing sugars, chiefly maltose. β -Amylase attacks the nonreducing end of the incompletely hydrolyzed dextrans, producing maltose and low molecular weight "limit dextrans" containing α -(1,6)-glucosidic branch points that neither enzyme can attack. Hydrolysis is extremely rapid (i.e., 10^{10} to 10^{12} times faster than hydrolysis by proton catalysis with acids) (Laszlo et al., 1978), such that one β -amylase molecule can hydrolyze 250,000 glucosidic linkages per minute (Englard and Singer, 1950; Englard et al., 1951).

The final sweetness perceived is a collective function of the amounts and types of sugars present in the raw root and the concentration of maltose formed through starch hydrolysis during cooking (Morrison et al., 1993). While maltose is distinctly less sweet than the endogenous sugars present, the volume formed results in the dominant sweet taste of the cooked product. Interestingly, maltose is the sugar form in sweetpotatoes preferred by sensory panels (Koehler and Kays, 1991).

The amount of maltose formed in sweetpotatoes during cooking is temperature-dependent. The temperature optimum is 70 to 75 °C for α -amylase (Ikemiya and Deobald, 1966) and 50 to 55 °C for β -amylase, well above the deactivation temperature for most plant enzymes. During cooking, the final sugar content increases until oven temperatures above 80 °C are reached (Sun et al., 1994) (Fig. 1A). The higher temperature optimum for the intact product reflects the rate at which the final temperature is reached. During baking, the temperature is not uniform throughout the root but progressively increases, starting at the exterior and moving inward. Thus, hydrolysis and deactivation zones shift toward the center of the organ with time. The extent of hydrolysis, hence the final intensity of sweetness, is temperature- and time-dependent. The final maltose concentration is higher if the roots are placed in a cold oven and then heated rather than being placed directly into a hot oven. In the latter scenario, the time available for hydrolysis in the reaction zone is shorter, reducing the extent of hydrolysis. A similar situation occurs when microwaves are used as the heat source. Heating occurs rapidly and throughout the root rather than progressing from the exterior to the interior (Sun et al., 1994). The final result is a much lower level of maltose in the cooked product (Fig. 1B).

Sugar formation in the sweetpotato during baking is highly cultivar-dependent, and certain sweetpotato lines have very low β -amylase activity (Morrison et al., 1993). As a consequence, sweetpotato germplasm can be separated into four general classes based upon initial sugar concentration and changes during cooking: 1) low sugars/low starch hydrolysis; 2) low sugars/high starch hydrolysis; 3) high sugars/low starch hydrolysis; and 4) high sugars/high starch hydrolysis.

b. Sourness. Organic acids are primary contributors to the sour/tart taste in fruits and, to a lesser extent, some vegetables. Organic acids commonly found in fruit are *cis*-aconitic, caffeic, chlorogenic, citramalic, citric, *p*-coumarylquinic, fumaric, galacturonic, glucu-

Fig. 1. Effect of temperature on changes in levels of individual sugars in 'Jewel' sweetpotatoes (A) during baking and (B) during baking after a 2-min microwave pretreatment. (After Sun et al., 1994.)

ronic, glyceric, glycolic, glyoxylic, isocitric, lactic, malic, oxalic, oxaloacetic, α -oxoglutaric, pyruvic, quinic, shikimic, succinic, and tartaric (Ulrich, 1970). The presence or absence of a specific acid and the relative concentration when present vary widely among individual crops and cultivars within a crop. Organic acids are also significant components in vegetables and, while they generally occur in lower concentrations, may be of equal importance in flavor.

The degree of sourness of organic acids in solution is related to the hydrogen ion concentration, although sourness is not necessarily dependent upon dissociation (Beets, 1978). Sausville (1974) ranked selected organic acids relative to citric for sourness in the following order: adipic (1.10–1.15) > citric (1.0) > malic (0.89–0.94) > tartaric (0.80–0.85) > fumaric (0.67–0.72).

In addition to the organic acids, other plant constituents can contribute to the sensation of sourness. The amino acids aspartic and glutamic are sour (Haefeli and Glaser, 1990; Schiffman et al., 1981), a sensation that is transferred to peptides in which they are components. For example, leucyl-dipeptides, which are typically bitter, are sour when aspartic or glutamic acid is substituted (Ishibashi et al., 1987).

The organic acid concentration in sweetpotato remains essentially unchanged during baking, indicating that the participation of organic acids in thermally induced reactions is not quantitatively significant (Wang and Kays, personal communication). However, since organic acids are water-soluble, the method of cooking can have an effect on the final concentration because losses may occur due to leaching during boiling. Minor losses can also occur through volatilization.

c. Saltiness. Substances that taste salty, e.g., sodium and potassium chlorides, typically dissociate in solution. A salty taste is a common denominator among the halide (Cl^- , Br^- , I^-) salts of sodium. Saltiness is also conferred by the metal salts of organic acids, the most common

being acetic, citric, malic, and tartaric. Interestingly, dilute solutions of Na and K chloride taste sweet. Sodium chloride first tastes slightly sweet at a concentration of 0.01 M and progresses to strongly sweet at 0.03 M, making the transition to salty-sweet at 0.04 M, with higher concentrations being salty (Skramlik, 1926). Several synthetic peptides are also salty (Tada et al., 1984).

In general, saltiness is conferred by sodium and potassium chloride, which have recognition thresholds of 0.03 and 0.017 M, respectively (Table 2). Certain vegetable crops, especially breeding lines, can be salty. For example, several sweetpotato lines were characterized as salty (McLaurin and Kays, 1992). Sodium replacement for potassium fertilization in some crops has been advocated; however, carryover effects on saltiness have not been widely studied. For example, when 75% or 100% of the KCl used for cassava (*Manihot esculenta* Crantz) was replaced with NaCl, the cooked product had higher sweetness scores; however, alterations in saltiness were not indicated (Sudharmai Devi and Padmaja, 1996).

Thermal reactions occurring during processing or cooking have little impact on salts present within the tissue, unless cooking occurs in an aqueous medium that allows leaching of salts. Typically, however, salt is added to most vegetables during and/or after cooking, indicating that excessive saltiness is seldom a problem.

d. Bitterness. Bitter compounds are present in many horticultural crops (Rouseff, 1990a). Generally, they are considered undesirable in a food product, often indicating toxicity. Some exceptions are radicchio (*Cichorium intybus* L.) and the bitter melon (*Momordica charantia* L.), in which bitterness is considered desirable (Kays and Hayes, 1978). Bitter compounds may be present in both the raw material and in the final product. Some initially nonbitter products become bitter with processing, while some bitter products become less bitter with aging (Fenwick et al., 1990; Herrmann, 1972a, 1972b; Oberdieck, 1977; Rouseff, 1990b). In some instances, thermal reactions induce the formation or modulate the concentration of bitter compounds.

Bitterness is detected on the back of the tongue and palate and in the pharynx (Henkin and Christiansen, 1967); consequently, many foods do not taste bitter until swallowed and the intensity is frequently strongest as an aftertaste. Humans are very sensitive to low levels (i.e., a few parts per million) of certain bitter compounds, with the lower detection limits varying with the compound and the individual.

A wide range of naturally occurring bitter compounds are found in plants, and these compounds vary widely in molecular size, functional groups present, and manner of expression of bitterness. Examples are: the cucurbitacins (oxygenated tetracyclic triterpenes), of which ≈ 20 have been identified in the Cucurbitaceae (Guha and Sen, 1975; Hutt and Herrington, 1985); polyphenols, alkaloids, saponins, and furanoid norditerpenes in the Dioscoreaceae (Crockill, 1948; Ida et al., 1978; Kawasaki et al., 1968; Martin and Ruberte, 1975; Webster et al., 1984); glycoalkaloids such as α -solanine, α -chaconine (Zitnak and Filadelfi, 1985), and tomatine (Pribela and Danisova-Pikulikova, 1973; Pribela and Pikulikova, 1971) in the Solanaceae; 6-methoxymellein (Sondheimer, 1957) and 6-methoxymellein-8-O-glucoside (Carlton et al., 1961; Chalutz et al., 1969) in carrots (*Daucus carota* L.); sesquiterpene lactones, lactucin, and lactucopicrin in lettuce (*Lactuca sativa* L.) (Bachelor and Ito, 1973; Barton and Narayanan, 1958; Michl and Hogenauer, 1960) and chicory (*Cichorium intybus* L.), with the coumarins aesculetin, aesculin, and cichoriin also contributing to the bitterness in the latter (Head and Robertson, 1939; Leclercq, 1984); asparagospinin I in asparagus (*Asparagus officinalis* L.) (Kawano et al., 1977); and ipomeamarone in the sweetpotato (Uritani et al., 1980).

As mentioned above, bitterness can be induced via thermal reactions (Maga, 1990). Processing can result in the synthesis of 1,2,4-trihydroxy-*n*-heptadeca-16-ene and 1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene in avocado (*Persea americana* Miller) (Ben-Et et al., 1973) and 2-pyrrolidone-5-carboxylic acid in beetroot (*Beta vulgaris* L.) and several other vegetables (Lee et al., 1971; Mahdi et al., 1961). In citrus juice, bitterness is the result of thermally accelerated closure of lactone rings.

Proteins can be converted to bitter compounds upon heating. The taste threshold for bitterness varies from 0.005 g·L⁻¹ for heated gluten to 5 g·L⁻¹ for gelatin (Jugel et al., 1976). A number of Maillard reaction

products, e.g., 2-furfuryl, 2-furaldehyde, 5-hydroxymethyl-2-furaldehyde, are also bitter (For, 1983; Shibamoto, 1983). If proline is one of the reactants in the Maillard reaction, the chances of a bitter compound being formed is substantially increased (Shigematsu et al., 1975). Tressl et al. (1985) list a wide assortment of bitter flavors formed through reaction of proline with Maillard reaction products.

Given the wide range of bitter compounds formed via thermal reactions, preharvest and processing factors collectively must be monitored to assure a high-quality end product.

Odor. Volatile compounds are the second important component of flavor. Volatiles, which make up the aroma of foods, are extremely important in what is perceived as flavor, lending to the tremendous diversity in flavors that can be achieved. Cooking generally substantially alters the characteristic aroma from that of the raw product. The degree of alteration is a function of variables related to heating (e.g., intensity, duration, method) and the initial chemical composition of the product. Cooking causes a dramatic and extremely complex series of reactions, resulting in a myriad of new volatiles, many of which have a direct impact on the product's aroma.

In contrast to the four basic taste sensations, our level of sensitivity to odors is remarkable. Humans can discriminate over 10,000 distinct odors. In addition, human olfaction is exceptionally sensitive, capable of detecting very low concentrations of odorants. For example, a single molecule of butane-1-thiol may stimulate a single olfactory receptor in humans (De Vries and Stuijver, 1960). Human odor thresholds for anethol, citral, methyl salicylate, and safrol are markedly lower than those of a gas chromatograph (Kendall and Nielson, 1964). The differential in sensitivity between taste and odor is illustrated by ethyl alcohol, for which the taste threshold is 130 mg·L⁻¹ of water vs. an odor threshold of only 4 mg·L⁻¹ of air (Margalith, 1981).

The odor of a compound perceived can vary, not only between individuals, but with the same individual over time, the hormonal and nutritional status of the individual, and his/her degree of hunger. For example, an orange [*Citrus sinensis* (L.) Osbeck] may be perceived as having a pleasant odor; however, within an hour of ingesting 100 g of glucose in water, the same odor was considered unpleasant (Cabanac, 1971).

a. Sources of thermally induced volatiles. There are three primary sources of thermally induced aroma compounds: 1) volatilization of endogenous pools of flavor components found in the raw product; 2) volatile compounds synthesized directly in response to high temperatures; and 3) compounds requiring enzymatic and thermal components for their synthesis. In the first case, the aroma perceived upon heating typically is significantly shifted because of high temperature volatilization of compounds of low volatility at room temperature. Thus, changes in the volatile profile do not necessarily require de novo synthesis, and these compounds can significantly alter the final volatile profile. The latter two sources of thermally induced volatiles will be the focus of the remainder of this section.

During cooking and processing, the Maillard reaction between reducing sugars and amino acids (or another source of an amino group) is a primary pathway involved in the formation of flavor volatiles. The Maillard reaction does not require high temperatures. For example, Maillard reaction products have been found in seeds of radish (*Raphanus sativum* L.) and barley (*Hordeum* sp.) ≈ 1500 years old (Evershed et al., 1997). The reaction rate, however, increases markedly with the high temperatures associated with cooking, and the nature of the volatiles formed is temperature-dependent. In addition, the reactions occur most frequently in areas of the product that have been dehydrated, such as near the surface.

The first step in the Maillard reaction involves Schiff base formation between the carbonyl group of a reducing sugar and the free amino group of an amino acid, peptide, or protein (Fig. 2). The Schiff base then cyclizes to yield a N-substituted aldosylamine that is converted to the 1-amino-1-deoxy-2-ketone (Amadori product) by the acid-catalyzed Amadori rearrangement (Fig. 2A). When a ketose, rather than an aldose, sugar is involved, a ketosylamine is formed that undergoes Heyns rearrangement to form a 2-amino-2-deoxyaldose (Heyns product) (Fig. 2B). Amadori/Heyns products do not contribute to flavor directly, but are important precursor compounds. They are thermally unstable and undergo dehydration and deamination reactions to give

Fig. 2. Maillard reaction pathway for the formation of (A) Amadori and (B) Heyns intermediates through the interaction of reducing sugars and amino compounds and their subsequent decomposition. For pentoses - R' = CH₂OH and R* = H; for hexoses R' = CHO-CH₂OH and R* = CH₃. (After Hodge, 1953 and Mottram, 1994.)

Table 3. Examples of volatile aroma compounds.^{z,y}

Class	Basic Structure	Example	(Odor)
Furans			2-furaldehyde (sweet)
Furanones			4-hydroxy-2,5-dimethyl-3(2H)-furanone (sweet, cotton candy)
Pyranones			2-methyl-3-hydroxy-4H-pyran-4-one (sweet, caramel)
Pyrroles			2-acetyl-5-chloropyrrole (almond)
Pyridines			2-pentyl-pyridine (green pepper)
Pyrimidines			4,6-dimethyl-pyrimidine (roasted, nut-like)
Pyrazines			2,5-dimethyl-3-ethyl-pyrazine (earthy, baked potato)
Thiophenes			5-methyl-2-thiophene-carbaldehyde (cherry-like)
Dihydro-thiophenes			2-methyl-2,3-dihydrothiophene (cabbage-like)
Oxazoles			4,5-dimethyloxazole (green, sweet, vegetable)
Oxazolines			4,5-dipropyl-2-isopropyl-3-oxazoline (banana)
Thiazoles			4-butyl-5-ethylthiazole (bell pepper)
Thiazolines			2,4-trimethyl-3-thiazoline (nutty, onion-like)
Aldehydes		CH ₃ CHO	ethanal (green, sweet)
Ketones		CH ₃ COCOC ₂ H ₅	2,3-pentanedione (nily-buttery)
Cyclopentanones			5-imino-2-methyl-1-cyclopenten-1-ol (cooked rice)
Non-cyclic sulfur compounds		CH ₃ SH	methanethiol (cooked cabbage)

^zAfter For (1983).^yLatin binomials: green pepper (*Capsicum annuum* L. Grossum Group); cabbage (*Brassica oleracea* L. var. *capitata* L.); onion (*Allium cepa* L. Cepa Group); rice (*Oryza sativa* L.).Table 4. Variation in aroma within the pyrazines.^z

Compound	Aroma description
2-Methylpyrazine	Nutty, roasted
2-Ethylpyrazine	Buttery, rum
2-Propylpyrazine	Green, vegetable
2-Isobutylpyrazine	Green, fruity
2,5-Dimethylpyrazine	Grassy
2,6-Dimethylpyrazine	Fried potatoes
2-Ethyl-3-methylpyrazine	Raw potato, earthy, nutty
2-Ethyl-6-vinylpyrazine	Buttery, baked potato
2-Methyl-6-propylpyrazine	Burnt, butterscotch
2-Isobutyl-3-methylpyrazine	Bell pepper
2,3,4-Trimethylpyrazine	Baked potato, roasted peanut
2,3-Dimethyl-5-ethylpyrazine	Nutty, roasted
2,3-Dimethyl-5-butylpyrazine	Sweet, earthy
2,3-Dimethyl-5-pentylpyrazine	Sweet, smoked, caramel-like
2,3-Dimethyl-5-isopentylpyrazine	Caramel-like, coffee, sweet
2,3-Dimethyl-5-(1-methylbutyl)pyrazine	Honey-like, sweet
2,3-Dimethyl-5-(2,2-dimethylpropyl)pyrazine	Brown sugar-like
2,3-Dimethyl-5-(1,5-dimethyl-4-hexenyl)pyrazine	Roasted nut
2,3,5,6-Tetramethylpyrazine	Fermented soybeans ^y
2-Acetylpyrazine	Popcorn ^x , nutty
6-Acetyl-2-methylpyrazine	Popcorn ^x
2-Ethyl-3-methoxypyrazine	Raw potato, earthy
2-Propyl-3-methoxypyrazine	Bell pepper
3-Isopropyl-2-methoxypyrazine	Earthy, bellpepper, raw potato
2-Methoxy-3-isopropyl-5-methylpyrazine	Green bean ^w -like
2-Isobutyl-3-methoxy-3-methylpyrazine	Licorice ^v -woody
5-(2-Methylpentyl)-2-methoxy-3-methylpyrazine	Burdock ^u
2-Isobutyl-3-methoxy-5,6-dimethylpyrazine	Minty-camphoraceous
2-Ethoxy-3-ethylpyrazine	Raw potato
3-Butoxy-3-methylpyrazine	Floral
3-Butoxy-3-propylpyrazine	Medicinal
2-Methylthio-3-methylpyrazine	Cooked meat
5-Methylthio-2-methylpyrazine	Meaty
2-Methylthio-3-isobutylpyrazine	Roasted peanuts
2-Methyl-3-(furfurylthio)pyrazine	Coffee

^zAfter For (1983).^y*Glycine max* (L.) Merr.^x*Zea mays* L. subsp. *mays*.^w*Phaseolus vulgaris* L.^v*Glycyrrhiza glabra* L.^u*Arctium lappa* L.Table 5. Major volatile compounds formed when tristerin is heated to 192 °C in air.^z

Alcohols	Acids
Octanol	Hexanoic acid
Nonanol	Pentanoic acid
Decanol	Butanoic acid
γ-Lactones	Aldehydes
4-Butanolide	Hexanal
4-Pentanolide	Hepanal
4-Heptanolide	Octanal
Hydrocarbons	Methyl ketones
Heptadecane	2-Heptanone
Nonane	2-Nonanone
Decane	2-Decanone

^zAfter Selke et al. (1975).

numerous rearrangement and degradation products. Often these products react with other compounds in that particular product to yield a diverse assortment of volatile compounds. Thus, the steps in the Maillard reaction can be summarized as: 1) formation of a glycosylamine and its subsequent rearrangement; 2) degradation to furan derivatives, reductones, and other carbonyl compounds; and 3) conversion of furan and carbonyl intermediates to aroma compounds (Mottram, 1994)—usually via reaction with other intermediates, such as amino compounds or amino acid or lipid degradation products.

An extremely diverse array of volatile compounds is synthesized by way of the Maillard reaction, and these can be classified according to their primary precursor: 1) simple sugar degradation/fragmentation products, such as furans, pyrones (e.g., maltol), cyclopentenones, carbonyl compounds, and acids; 2) simple amino acid degradation products, such as aldehydes, sulfur compounds such as hydrogen sulfides and methanethiol, and nitrogen compounds such as ammonia and amines; and 3) volatiles produced by further reactions, such as pyrroles, pyridines, pyrazines, imidazoles, oxazoles, compounds from aldol condensations, thiazoles, thiophenes, di- and trithiolanes, di- and trithianes, and furanthiols (Nursten, 1980). Some of the classes of volatiles formed via thermal reactions are presented in Table 3. Note the diversity of classes of compounds and odors. The odors cited in the table are simply examples and are not indicative of the odor of all compounds in the class.

Diversity in odor within a class is illustrated by the pyrazines (Table 4). By simply altering side groups, the aroma goes from buttery to potato (*Solanum tuberosum* L.) to floral to meaty. Note that some individual compounds have more than one aroma descriptor listed. This is in part due to variation among individuals in odor discrimination, and descriptors such as “baked potato” generally are comprised of multiple compounds. While one individual may detect a “baked potato aroma,” another may associate it with a different odor.

The Maillard reaction has a number of effects on the product in addition to the synthesis of flavor volatiles. It also results in: color formation (i.e., browning reactions); nutritional losses because of the utilization of amino acids, ascorbic acid and other compounds that participate in the reaction; the formation of possible toxic compounds such as imidazoles and methylglyoxal, which are carcinogenic; and the synthesis of antioxidants.

Lipid degradation also results in numerous odorous aliphatic compounds having both positive and negative impacts on food quality; hence, lipids are also a significant source of aroma compounds. The compounds formed through autoxidation reactions give rise to unpleasant aromas, such as the rancid aroma of old or improperly stored oil seed crops (St. Angelo, 1996). The impact of these volatiles is illustrated by the fact that one seldom needs to place a rancid peanut (*Arachis hypogaea* L.) in the mouth before the brain emits a rejection signal. While autoxidation reactions are extremely important in flavor (both positive and negative), we will focus on the formation of volatiles from lipids by thermally mediated reactions.

Lipids comprise a significant portion of horticultural crops, ranging, for example, from 74% of the fresh weight (fwt) in seeds of some pecan [*Carya illinoensis* (Wang.) K. Koch] cultivars to 0.4% of the fwt in banana (*Musa paradisiaca* L. var. *paradisiaca*) fruits, and are primary components of the cell's membrane system. Hence, all living cells have a small but significant lipid component. Given the tremendous diversity in the chemistry of plant cells and the logarithmic increase in complexity as these compounds undergo high temperature degradative and synthetic reactions, many investigators have utilized model systems (Whitfield, 1992). Such systems have afforded the most desirable means of understanding reactions that can occur in intact systems. For example, on heating tristearin, a triacylglycerol in which the three acyl side chains are identical (octadecanoic acid), to 192 °C in the presence of air, 18 major compounds were formed (Table 5) of which aldehydes and methyl ketones comprised 36.1% and 38.4%, respectively (Selke et al., 1975). Because many fatty acids are components of triacylglycerols [for example, 22 fatty acids are found in the pecan (Senter and Horvat, 1976, 1977)], and because altering the glycerol carbon position of only two fatty acids gives six possible lipids, tristearin represents a very simple model. In addition to triacylglycerols, plant products contain a wide assortment of phospho-

Table 6. Volatile heterocyclic compounds formed during the thermal interaction of 2,4-decadienal with either cysteine or glutathione.^a

Compounds	Quantities produced ^b (mg·mol ⁻¹)	
	Cysteine	Glutathione
<i>Furans</i>		
2-Butylfuran	12.8	3.1
2-Pentylfuran	6.4	trace
2-Hexylfuran	trace	12.8
<i>Thiophenes</i>		
Thiophene	3.5	7.2
2-Methylthiophene	---	34.4
Tetrahydrothiophen-3-one	10.5	---
2-Propylthiophene	---	2.3
Methylpropylthiophene	---	3.8
2-Butylthiophene	57.2	56.6
3-Methylthiophene-2-carboxaldehyde	29.8	---
Methylbutylthiophene	---	4.6
2-Pentylthiophene	13.1	14.6
Methylpentylthiophene ¹	18.7	46.5
Methylpentylthiophene ¹	17.5	---
2-Hexylthiophene	42.0	87.8
2-Heptylthiophene	1.8	---
3-(1-Hexanoyl)thiophene	9.3	60.8
Formylpentylthiophene	15.6	21.0
<i>Thiazoles</i>		
Thiazole	25.6	16.0
2-Methylthiazole	---	15.8
5-Methylthiazole	---	14.5
3-Methylisothiazole	2.0	---
2-Acetylthiazole	2.2	3.8
<i>Other sulfur-containing compounds</i>		
Butanediol	6.2	---
2-Methyl-1,3-dithiolane	5.0	---
3,4,5,6-Tetrahydro-2,4,6-trimethyl-2H-thiadiazine	828.5	---
3,5-Dimethyl-1,2,4-trithiolane ²	122.8	162.1
3,5-Dimethyl-1,2,4-trithiolane ²	18.2	206.4
5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine	284.2	---
3-Methyl-1,2,4-trithiane	42.5	1.6
3,6-Dimethyl-1,2,4,5-tetrathiane	---	59.2
3,6-Dimethyl-1,2,4,5-tetrathiane	---	1.1
4,6-Dimethyl-1,2,3,5-tetrathiane	---	76.1
1,2,5-Trithiepane	trace	---
3,5,7-Trimethyl-1,2,4,6-tetrathiepane ³	---	6.4
3,5,7-Trimethyl-1,2,4,6-tetrathiepane ³	---	1.6
5,6-Dihydro-2,4-dimethyl-6-pentyl-4H-1,3,5-dithiazine	18.9	---
5,6-Dihydro-4,6-dimethyl-2-pentyl-4H-1,3,5-dithiazine	28.7	---
3-Methyl-5-pentyl-1,2,4-trithiolane	14.3	---
4,7-Dimethyl-1,2,3,5,6-pentathiepane ⁴	6.7	---
4,7-Dimethyl-1,2,3,5,6-pentathiepane ⁴	6.3	---
5,6-Dihydro-6-pentyl-2-propyl-4-methyl-4H-1,3,5-dithiazine	2.9	---
3-Methyl-6-pentyl-1,2,4,5-tetrathiane ⁵	---	6.4
3-Methyl-6-pentyl-1,2,4,5-tetrathiane ⁵	---	3.1
5,6-Dihydro-4-butyl-6-pentyl-2-methyl-4H-1,3,5-dithiazine	0.9	---
<i>Pyridines</i>		
1-(2-Pyridinyl)pentanone	5.1	6.8
2-Pentylpyridine	501.5	1219.0

^aAfter Zhang and Ho (1989).

^bMilligrams of volatile compound per mol of respective amino acid.

^cCompounds with the same superscript (^{1, 2, 3, 4, 5}) are isomers.

lipids and glycolipids.

Progressing to the next level of complexity in a model system by reacting a lipid with an amino acid, an even more diverse array of compounds is formed (Table 6) (Zhang and Ho, 1989). Altering the amino acid (cysteine vs. glutathione) distinctly changes the qualitative and quantitative profile of compounds. Thus, in the highly complex systems found in fruits and vegetables, with numerous lipids and hundreds of potential reactants such as those formed in the Maillard

Table 7. Volatile thermolytic products identified from sweetpotatoes, sweetpotato fractions, and sugar standards.^z

Compounds	Sweetpotato		Fractions			Sugars	
	Baked	Thermolysis	Insoluble	Nonpolar	Polar	Maltose	Standard ^y
Acetol	+ ^x	+	+	+	+	+	+
Acetic acid	—	+	+	—	—	—	—
Furyl aldehyde	+	+	+	+	+	+	+
2-Acetylfuran	+	+	+	—	—	+	+
Benzaldehyde	+	+	+	—	—	—	—
5-Methyl-2-furfural	+	+	+	+	+	+	+
Phenylacetaldehyde	+	—	—	—	—	—	—
Furfuryl alcohol	+	+	+	+	+	—	+
3,4-Dihydropyran	+	+	+	—	—	—	+
β-Ionone	—	—	—	+	—	—	—
3-Hydroxy-2-methyl-4-pyrone (maltol)	+	+	+	—	—	—	—
Unknown	+	+	+	—	—	+	+
2-Hydroxyacetyl furan ^w	+	+	+	—	—	—	+
5-Hydroxy-methyl-2-furfural	+	+	+	—	+	+	+

^zAfter Sun et al. (1995).^yA sugar standard of glucose, fructose, sucrose, and maltose (12, 12, 43, and 36 g, respectively, in 10 mL distilled water) in the same ratio as in baked sweetpotatoes.^xPresent (+), not detectable (—).^wTentatively identified using mass spectral data.

reaction, hundreds of potential volatiles can be formed. In roasted coffee (*Coffea arabica* L.) beans, >800 volatiles have been identified, a majority of which are formed by Maillard reactions (Holscher and Steinhart, 1994). Another example is the baked potato, in which over 70 compounds have been identified, with critical flavor components being a mixture of pyrazines, thiazoles, and oxazoles (Buttery et al., 1973; Coleman et al., 1981; Pareles and Chang, 1974).

Likewise, the sweetpotato also presents a complex mixture of thermally induced volatile compounds. Precursors of critical volatiles were identified by fractionating the root tissue into polar (methanol-soluble), nonpolar (methylene chloride-soluble) and insoluble fractions (Sun et al., 1995). The fractions were then heated (200 °C) and the volatiles produced identified (Table 7). Initial reactions in the formation of critical volatiles occurred in the insoluble fraction, comprised of starch, cellulose, hemicellulose, proteins and other insoluble high molecular weight components. One character-impact volatile (3-hydroxy-2-methyl-4-pyrone) forms via the Maillard reaction; however, synthesis first involves the activity of α- and β-amylase, which hydrolyze starch to maltose. Maltose is subsequently degraded to monosaccharides that undergo the Maillard reaction. Hence, the synthesis of critical volatiles in the sweetpotato involves both enzymatic and thermal reactions.

b. Factors affecting thermally generated flavors. A wide range of factors can modulate the profile of volatiles formed. In general these can be separated into two categories: cooking-related and product-related. With cooking, initial and final temperature, length of the cooking cycle, moisture conditions, method of heat introduction, and other factors are critical in determining the final flavor of the product.

The effect of temperature on the synthesis of maltose during cooking is illustrated in Fig. 1. The amylase system functions at a high temperature relative to other enzymes, most of which are denatured at lower temperatures. Heat enters the product as an inward-progressing thermal wave with the highest temperature at the outside. Maillard reactions are favored by dry conditions, hence we would anticipate that a significant portion of these aroma compounds are synthesized near the surface of the product.

The method of heat introduction is also critical. If sweetpotatoes are heated with microwaves instead of a conventional convection oven, the microwaves deactivate the amylase system. Therefore, very little maltose is formed, resulting in a pronounced shift in the aroma of the cooked product (Table 8).

Both the rate of heat introduction and the duration of thermal treatment are also critical. In the sweetpotato, rapid heating alters the formation of precursors to critical flavor compounds, in particular maltol. Likewise, the volatiles produced change with time during heating. With extended exposure, overcooked and burned flavor volatiles are eventually formed.

The cultivar used is a primary factor affecting the eventual flavor of the cooked product. Table 9 illustrates the variation in volatile

Table 8. Volatiles emanating from conventionally baked vs. microwaved 'Jewel' sweetpotatoes.

Volatile	Relative concn ^z (μg·kg ⁻¹ fresh weight)	
	Baked	Microwaved
Pyridine	1.68	0.24
1,2,4-Trimethyl benzene	0.67	0.13
3-Furaldehyde	4.81	0.01
Xylene	0.03	---
2-Furmethanol	3.84	0.48
Furfuryl alcohol	0.39	0.01
2-Acetyl furan	0.59	0.14
Benzaldehyde	0.41	0.10
5-Methyl-2-furfural	0.14	0.02
2-Pentyl furan	0.31	0.01
2,3-Pentanedione	0.18	0.04
Phenylacetaldehyde	6.27	0.04
Limonene	tr	tr
3,4-Dihydropyran	0.22	0.02
2-Acetyl pyrrole	tr	0.02
Maltol	3.70	0.04
Linalool	0.24	0.04
Isopulegone	tr	tr
Geraniol	0.20	0.01
2,4-Nonadienal	0.89	0.14
Cyclohexanol	0.49	0.04
n-Decanal	tr	---
2,2-Dimethyl-1,3-cyclohexanediol	tr	0.02
2,3-Nonadecanediol	0.73	---
2,4-Decadienal	tr	---
Octyl ketone	tr	tr
Methyl geranate	tr	tr
Germacrene D	0.28	0.06
β-Caryophyllene	tr	0.02
β-Farnesene	0.17	0.06
α-Copaene	tr	0.01
α-Bisabolene	tr	0.06
Bohlmann 176	0.16	0.06
2(4H)-Benzofuranone	0.27	0.07
β-Ionone	0.68	0.06
Nerolidol	tr	0.11
4-Decanolide	tr	---
Unknown	tr	tr
Tetradecanoic acid	0.19	0.05
Total	26.87	1.73

^zRelative concentrations were calculated from gas chromatograph (GC) peak areas relative to those of internal standard. Volatiles were collected after cooking using a purge and trap system with product held at 50 °C. Trace concentrations (tr) are <1%, based on GC peak areas; (—) = not detectable by GC or olfactory analysis. (Wang and Kays, unpublished data).

Table 9. Effect of curing on volatile constituents of two sweetpotato lines during baking.^z

Volatile compound	Relative concn ($\mu\text{g}\cdot\text{kg}^{-1}$ fresh wt)			
	Jewel		GA90-16	
	Noncured	Cured	Noncured	Cured
Pyridine	1.5	5.6	tr ^y	1.7
1,2,4-Cyclopentanetriol	0.6	1.2	tr	0.7
1,2,4-Trimethyl benzene	1.3	2.7 ^x	0.1	2.4 ^x
3-Furaldehyde	8.0	14.5	0.2	2.4
Xylene	tr	0.3	---	0.1
2-Furmethanol	13.1	14.1 ^x	1.5	1.8
Furfuryl alcohol	1.2	1.3	0.3	2.8
2-Acetyl furan	1.2	4.4	0.3	2.8
Benzaldehyde	1.0	2.1	tr	0.5
5-Methyl-2-furfural	0.2	0.9	---	0.7
2-Pentyl furan	0.2	1.2	---	0.1
2,3-Pentanedione	0.5	0.7	0.1	1.5
Phenylacetaldehyde	3.8	29.7 ^x	0.4	20.9 ^x
Limonene	---	tr	---	tr
3,4-Dihydropyran	0.4	2.1	tr	1.0
2-Acetyl pyrrole	0.6	0.3 ^x	tr	0.1 ^x
Maltol	12.3	30.8 ^x	0.4	0.7
Linalool	1.1	0.8	---	0.2
Isopulegone	tr	0.8	---	0.8
4,5-Dimethyl-4-hexen-3-one	tr	tr	---	tr
Geraniol	tr	0.4 ^x	---	0.1 ^x
2,4-Nonadienal	0.1	1.2	tr	---
2-Naphthalenone	1.5	1.2	---	---
Cyclohexanol	tr	5.4	---	tr
n-Decanal	---	tr ^x	---	tr ^x
2,2-Dimethyl-1,3-cyclohexanediol	tr	0.4	---	0.2
2,3-Nonadecanediol	0.2	0.5	---	2.5 ^x
2,4-Decadienal	tr	0.6 ^x	---	1.9 ^x
Octyl ketone	tr	tr	tr	0.3 ^x
Methyl geranate	tr	tr ^x	tr	tr ^x
Germacrene D	0.6	0.9	tr	0.6
β -Caryophyllene	tr	0.3	tr	0.2
Cyperene	---	---	0.4	1.8 ^x
β -Farnesene	tr	0.3	tr	0.3
α -Copaene	0.1	0.3 ^x	tr	0.2 ^x
α -Bisabolene	---	0.3	tr	0.2
Bohlmann 176	1.3	1.5	0.2	0.3
2(4H)-Benzofuranone	0.4	1.3	0.1	0.3
β -Ionone	1.3	1.6 ^x	0.5	0.8 ^x
Nerolidol	tr	0.2	tr	0.1
4-Decanolide	tr	tr	---	tr
Unknown	tr	1.0 ^x	---	0.2 ^x
Tetradecanoic acid	1.3	4.2	0.3	0.5
10-Heneicosene(c,t)	1.1	0.5	0.5	0.3
Palmitic acid	32.1	54.1	33.5	26.1
Octadecanol	2.1	2.6	1.5	1.6
1-Nonadecanol	16.5	24.0	16.2	6.0
9,12-Octadecadienoic acid	3.4	2.4	1.2	1.6
Total of identified compounds	109.0	227.9	58.4	84.3
Total of odor-active compounds	51.7	141.6	5.5	47.8

^zAfter Wang et al. (1998).^y(-) = Not detectable by gas chromatography (GC) and olfactory analysis. Trace concentrations (tr) are <1%, based on GC peak areas.^xCompounds are important contributors to odor.

profiles between two sweetpotato cultivars with distinctly different aromas. The first, 'Jewel', has the classical U.S. sweetpotato aroma with an intense sweet odor note provided largely by maltol. The second, in contrast, smells very much like a baked white potato. Hence, there are often extremely wide differences in the aroma profiles among cultivars, especially when genetic diversity within the gene pool is extreme (McLaurin and Kays, 1992).

A myriad of preharvest factors, even factors as simple as product size, can modulate the cooked flavor of horticultural products. When large sweetpotato roots are baked, the additional mass alters the thermal dynamics within the root, affecting the eventual flavor. The

Table 10. Outline of conventional selection schemes for potato and sweetpotato breeding programs.^z

Clonal population remaining (%)	Selection parameter
<i>Potato</i>	
100.0	Greenhouse screening for virus and nematode resistance
35.0	General agronomic traits
3.0	General agronomic traits and disease resistance
0.9	Yield quality, disease resistance, storability, agronomic assessment
0.4	Yield quality, disease resistance, storability, agronomic assessment
<i>Sweetpotato</i>	
100.0	Greenhouse screening for disease and nematode resistance
10.0	Field planting-evaluation for general agronomic characteristics, insect resistance, yield, root color.
1.0	Quality: fiber, absence of discoloration, flavor, general appearance after baking

^zData after Jones (personal communication) and MacKay (1987).

presence of disease, even extremely slight amounts, can also significantly alter aroma. In the sweetpotato, certain pathogens, insects, and stresses induce the synthesis of toxic furanoterpenoids, which have a major negative impact on the aroma. Other postharvest factors, such as handling, storage conditions and duration, and postharvest treatments can modulate eventual flavor of the cooked product. For example, the standard postharvest curing treatment significantly alters the aroma and taste of the sweetpotato after cooking (Wang et al., 1998) (Table 9).

Alteration of flavor thorough plant breeding. One of the real advantages of being a horticulturist is that we can implement ideas developed in the laboratory and actually see them through to a final product. Interfacing analytical techniques with plant breeding is one way to have a significant impact on flavor chemistry.

Most conventional breeding programs determine: 1) what traits are to be used in the selection process; 2) the priority that will be placed on each trait; and 3) what criteria will be used in assessment of individual traits. Typical selection programs for potatoes and sweetpotatoes are presented in Table 10. Selection generally occurs initially in the greenhouse, where a large number of clones can be tested for disease and nematode resistance, and other traits under controlled conditions. At each step in the selection protocol, clones not displaying the desired trait(s) are discarded. With each reduction in the population size, the chance of selecting previously unselected traits diminishes, because the rate of genetic gain is a function of the heritability selection intensity, degree of genetic variance in the population, amount of time per selection cycle, and the precision of measurement of the trait selected. In typical potato (MacKay, 1987) and sweetpotato (Jones, personal communication) breeding programs (Table 10), 98% to 99% of the lines are eliminated by the end of the first year. Thus screening for flavor usually occurs after a major portion of the clonal population has been discarded. The lower priority for flavor in the selection sequence stems in part from the difficulty of measuring flavor using conventional sensory analysis. A routine test can assess only five to eight samples at one sitting and due to the subjective nature of flavor analysis, reasonably large panels (i.e., > 15 individuals) are required to obtain an accurate estimate of preference. This greatly reduces the number of clones that can be screened for flavor, hence the lower priority in a selection process even though eating quality may be considered as a top priority (Martin and Jones, 1986). Thus the discrepancy between what is thought to be needed and what is actually practiced is often wide.

Using an analytical selection method (Sun et al., 1995), the number of clones that can be screened can be increased to ≈ 40 to 50 a day per gas chromatograph while greatly increasing precision. This allows evaluation of flavor earlier in the selection sequence. An analytical method does not, however, completely eliminate use of sensory panels, although it does allow moving large numbers of progeny through several selection cycles prior to final substantiation using conventional sensory techniques (Kays and Horvat, 1983). Use of an

analytical selection protocol requires understanding the basic chemistry of the flavor traits desired, but allows the imposition of a substantially increased selection pressure for the desired trait. Advantages of an analytical vs. a subjective approach to selection of flavor include: 1) the trait is well-defined; 2) accurate parent line selection; 3) increased sample population; 4) improved accuracy of progeny selection; 5) the ability to simultaneously select for multiple consumer groups with distinctly different flavor preferences; 6) the ability to select new, unique flavor types; 7) the potential for a centralized analytical program; and 8) a data base for future use (Kays, 1988). The approach we have taken with the sweetpotato is to: 1) identify the major positive and negative flavor components; 2) assess the range in flavor within the gene pool (McLaurin and Kays, 1992); 3) develop an analytical procedure for rapid screening of large numbers of parent lines and progeny (Sun et al., 1993); 4) characterize the chemistry of flavor preference of target consumer populations; and 5) identify desirable clones using chemical analyses interfaced with sensory analyses.

In conclusion, two critical ideas should be reiterated. First, thermally derived flavors are the norm, not the exception, for horticultural products. Secondly, we as horticulturists have the critical responsibility for creating and delivering a product to the processor or consumer that will have the desired flavor upon cooking.

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Flavor Trivia and Tomato Aroma: Biochemistry and Possible Mechanisms for Control of Important Aroma Components

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Consumers are often dissatisfied with the flavor of fresh tomatoes (*Lycopersicon esculentum* Mill.) purchased in the supermarket. There are several reasons for this, ranging from poor genetic material to harvest and handling procedures. Research is ongoing to determine the important flavor components in tomato in order to give breeders and molecular biologists access to objective flavor criteria for use in selection of high-quality material. Furthermore, harvest and postharvest handling, and shipping and storage procedures can be analyzed for their effects on important flavor components.

FLAVOR TRIVIA

To successfully conduct this research, one must first understand what comprises flavor and how it is perceived. The nose has olfactory nerve endings at the back with receptors that bind volatiles emanating from food. These reactions are somewhat analogous to enzyme/substrate stereochemical associations. Tastes such as sweet, sour, salty, or bitter are perceived because of reactions of sugars or polyalcohols, hydronium ions, sodium ions, glucosides, alkaloids, etc., with receptors located in certain regions of the tongue. The nerves in the tongue can also detect texture, temperature, metallic irritation, bite (carbonation), chemical heat [e.g., hot pepper (*Capsicum frutescens* L.)], etc., collectively known as trigeminal responses. The olfactory system is the more sensitive of the two organs, however, and the most sensitive of the five senses. It can detect odors in parts per trillion whereas receptors on the tongue can detect flavor compounds in parts per hundred. The adverse side of this extreme sensitivity is fatigue, which may be a protective mechanism against nerve damage (DeRovira, 1997). Nevertheless, the fatigue factor must be considered in sensory work with aroma compounds.

The extreme sensitivity of the olfactory organ has played an important role in the evolutionary development of mammals, allowing for the odor imprinting involved in olfactory recognition. This has helped in developing behavioral relationships, such as mother-offspring, consort interactions, and general kin recognition, not to mention detection of predator and prey (Margot and Salvadori, 1995;

Rouhi, 1996). Specific odors can evoke powerful thoughts and emotions in humans. The sense of smell, therefore, has played a key role in several areas important to species survival. In mammals at least 1000 receptor genes are devoted to encoding receptors that recognize odors; these comprise 1% to 2% of the mammalian genome (Rouhi, 1996).

Many factors can affect our perception of flavor, especially the components of flavor made up of aroma compounds. Smelling an aromatic food through the front of the nose may produce a different experience than when the aroma is perceived during chewing of food (Voirol and Daget, 1987). This difference is due to the temperature of the mouth, the disruption of food cells by chewing, and reduction of viscosity by mixing the food with saliva. Temperature, viscosity, and polarity of the food can effect relative vapor pressure and aroma release (Land, 1994; Taylor and Linforth, 1994; Voirol and Daget, 1987). This, in turn, alters the concentration in the headspace of the mouth of volatile compounds that rise through the back of the nose to bind olfactory receptors (Land, 1994; Taylor and Linforth, 1994). In addition, odor and taste can interact to give an integrated perception (Voirol and Daget, 1987). Texture can also play a role. A softer tomato may be perceived as more flavorful than a firm tomato, while a crisp, juicy apple will likely be perceived as more flavorful than a mealy one. Such observations may be related to texture and the state of the cell wall (in particular, the condition of the middle lamella). The mechanism of tissue disruption, i.e., whether fruit cells break across cell walls, releasing cellular components, or between cells (middle lamellae) as in mealy fruit, may affect juiciness and flavor impact (Vickers, 1977).

To make matters more complicated, not all odorants are alike. Primary odorants are like letters of the alphabet in that they define one odor individually and can then, in combination, define another aroma. They bind only one receptor in the olfactory bulb. Methyl salicylate, an important volatile in tomato, is an example; it alone is responsible for the aroma "wintergreen." Secondary odorants are like syllables and bind more than one olfactory receptor (Amoore, 1952). An example would be saffrole, which is thought to bind four receptors and is described as anise, with a wintergreen character, vanilla background, and camphoraceous overtone (= root beer flavor) (De Rovira, 1997). This food additive is carcinogenic and has been removed from the market. Manufacturers were able to duplicate its aroma by using a combination of primary odorants, including anise, methyl salicylate, vanillin and camphor, which bind the same four olfactory receptors as saffrole. And finally, to really confound the issue, flavors have top notes and background notes. Top notes are generally compounds of relatively low molecular weight and high volatility that are heat labile and polar (De Rovira, 1997). They are usually very noticeable in a food item. Background notes, on the other hand, are generally of low molecular weight, heat stable, and nonpolar, and have a more subtle impact on flavor than do top notes.

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IMPORTANT AROMA COMPOUNDS FOR TOMATO FLAVOR

First, how do we measure odor compounds that are present in food? Earlier studies employed the classical flavor isolation procedures of steam distillation and/or solvent extraction (Teranishi and Kint, 1993), which can modify the flavor profile of a sample qualitatively and quantitatively (Schamp and Dirinck, 1982). This method is time-consuming and not easily applied to large numbers of fruit samples. Internal standards must be incorporated to determine recovery, but the resulting concentration of material allows identification of compounds by gas chromatography-mass spectrometry (GC/MS). More recently, investigators have employed purge and trap headspace sampling methods, which involve trapping and concentrating volatile components on a solid support. The trap is later heated to release volatiles into GC or GC/MS systems (Schamp and Dirinck, 1982; Teranishi and Kint, 1993). Gas chromatographs may be equipped with a sniff port to provide some sensory information as the compounds are eluted from the column. Static headspace methods are said to more closely reflect the true flavor profile, however, although the compounds are present at lower levels and some may not be detected. Cryofocusing (cold trapping) of static headspace volatiles (Teranishi and Kint, 1993) can help overcome this problem, since samples can be concentrated without heating and the associated possibility of adulteration. The newest method available is solid phase microextraction (SPME), a rapid sampling technique where volatiles interact with a fiber-coated probe that is inserted into the headspace of a sample and then transferred to a GC injection port where the volatiles are desorbed (Song et al., 1998). Aside from GC and GC/MS methods, there are new sensors available that have a broad range of selectivity. These sensor arrays (called "electronic noses") are useful for discriminating one sample from another based on the volatile profile, rather than for identification/quantification (Maul et al., 1998a).

So now that aroma compounds can be identified and quantified, how do we know which ones are contributing to flavor? One way is to do aroma extraction dilution analysis (AEDA) or "Charm" analysis, using a sniff port on a GC while diluting the sample (Acree, 1993). A simpler method is to establish odor thresholds (Teranishi and Buttery, 1987; Teranishi et al., 1991). This is done in the food or in some similar medium since odorants' volatility can change with polarity and viscosity (Buttery et al., 1973). In tomato, for example, >400 volatiles have been reported, but only 30 are present in concentrations >1 nL·L⁻¹, as summarized in several reviews (Buttery, 1993; Buttery and Ling, 1993a, 1993b; Buttery et al., 1989). Buttery et al. (1971) determined odor thresholds (the level at which a compound can be detected by smell) for these 30 compounds. Log odor units can then be calculated from the ratio of the concentration of a component in a food to its odor threshold. Volatile compounds with positive odor units are assumed to contribute to the flavor of a food, while those with negative units may not (Buttery et al., 1989). Buttery (1993) determined concentrations, odor thresholds, and log odor units of volatiles present in tomato at levels of 1 nL·L⁻¹ or more (Table 1). Of these, 16 had log odor units >0, and therefore are likely to contribute to tomato flavor. Some fruits or vegetables have one or two odor-impact compounds that dominate the flavor of that particular commodity. Banana (*Musa acuminata* Colla) is a good example, with 3-methylbutyl acetate dominating its flavor (Berger, 1991). Not so for tomato, however, since no single compound has been found in this fruit that is reminiscent of a ripe tomato; a combination of at least 16 aroma compounds together give tomato its unique odor characteristics. Buttery (1993) has suggested that a combination of *cis*-3-hexenal, *cis*-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and β -ionone, at the appropriate concentrations, produces the aroma of a fresh, ripe tomato. However, compounds with negative odor units may still contribute to the overall flavor of tomato or other foods as background notes. For this reason synthetic flavors, vanillin, for example, do not have quite the same aroma as the natural vanilla extract, which contains many background notes not present in the synthetic product.

Tomato volatiles present at concentrations ≥ 1 nL·L⁻¹, along with their concentrations in a typical ripe tomato, odor threshold, and log

odor units (Table 1) (Buttery, 1993), demonstrate that a volatile need not be present in high levels to have an impact on flavor. For example, *cis*-3-hexenal is the most abundant (12,000 nL·L⁻¹) of the 30 volatiles present at >1 nL·L⁻¹, and has the highest log odor unit (3.7) (Table 1). However, β -ionone, a volatile present at one of the lowest concentrations (4 nL·L⁻¹), is second highest in log odor units (2.8).

Odor thresholds in the above studies were determined in water, since tomato is considered an aqueous system. Tomato homogenate (which simulates masticated tomato), however, may bind or trap volatiles, and contains high levels of methanol and other alcohols (Baldwin et al., 1991a, 1991b) that could affect volatile solubility and, therefore, odor threshold. Threshold studies of tomato aroma compounds were carried out in an aqueous alcohol system (water with the amount of methanol and ethanol found in fresh tomato homogenate) and in bland homogenate (volatiles removed by distillation). Volatiles in homogenate generally had higher thresholds (i.e., suppressed sensory perception) and different odor descriptors than did those in water (Tandon, 1998).

Some investigators have attempted to determine the relationships of different flavor compounds (sugars, acids, and volatiles) to sensory descriptors in order to understand the contribution of individual components to overall flavor. Correlations were found between concentrations of aroma compounds and the intensity of aroma as well as taste descriptors (Baldwin et al., 1998). For example, geranylacetone was related to tomato-like flavor and sweetness whereas 6-methyl-5-hepten-2-one was associated with tomato-like flavor, overall acceptability, and spoiled aroma. In three seasonal studies, sweetness intensity was related to hexanal, with contributions from *cis*-3-hexenal, *trans*-2-hexenal, or *cis*-3-hexenol. Soluble solids were more closely related to sourness than to sweetness, which, in turn, correlated more closely with sucrose equivalents (combined sweetness value of glucose and fructose).

Table 1. Tomato volatiles present in fresh tomato at levels ≥ 1 nL·L⁻¹, their odor threshold (in water) and their order of log odor units.^z

Volatile	Concn (nL·L ⁻¹)	Odor threshold (nL·L ⁻¹)	Log odor units ^y
<i>cis</i> -3-Hexenal	12,000	0.25	3.7
β -ionone	4	0.007	2.8
Hexanal	3,100	4.5	2.8
β -Damascenone	1	0.002	2.7
1-Penten-3-one	520	1	2.7
2+3-Methylbutanal	27	0.2	2.1
<i>trans</i> -2-Hexenal	270	17	1.2
2-Isobutylthiazole	36	3.5	1.0
1-nitro-2-Phenylethane	17	2	0.9
<i>trans</i> -2-Heptenal	60	13	0.7
Phenylacetaldehyde	15	4	0.6
6-Methyl-5-hepten-2-one	130	50	0.4
<i>cis</i> -3-Hexenol	150	70	0.3
2-Phenylethanol ^x	1,900	1,000	0.3
3-Methylbutanol	380	250	0.2
Methyl salicylate	48	40	0.08
Geranylacetone	57	60	-0.02
β -Cyclocitral	3	5	-0.2
1-Nitro-3-methyl-butane	59	150	-0.4
Geraniol	12	32	-0.4
Linalool	2	6	-0.5
1-Penten-3-ol	110	400	-0.6
<i>trans</i> -2-Pentenal	140	1,500	-1.0
Neral	2	30	-1.2
Pentanol	120	4,000	-1.5
Pseudoionone	10	800	-1.9
Isobutyl cyanide	13	1,000	-1.9
Hexanol	7	500	-1.9
Epoxy- β -ionone	1	100	-2.0

^zAdapted with permission from Buttery (1993), copyright 1993, American Chemical Society.

^yLogarithm of odor unit value.

^xThe exact concentration and log odor unit values are uncertain.

SYNTHESIS OF TOMATO VOLATILES

The biogenesis of aroma compounds in tomato has been the subject of several recent reviews (Buttery and Ling, 1993a, 1993b). Flavor volatiles are formed in the intact tomato fruit during ripening, or upon tissue disruption, which occurs when tomatoes are macerated, blended, or homogenized (Buttery, 1993). When cell disruption occurs, previously compartmentalized enzymes and substrates mix and new volatiles are formed. The ability to form flavor volatiles after cell disruption, however, can change during ripening, apparently because of changes in enzyme and substrate availability. To determine which volatiles are formed in intact tissue and which are formed after cell disruption, reactions were minimized by blending tomatoes in saturated calcium chloride (CaCl₂) or rapidly heated in a microwave to deactivate enzymes (Buttery, 1993; Buttery and Ling, 1993a). This assumes that enzymes were, in fact, deactivated immediately and that nonenzymatic oxidative reactions did not result in production of some volatiles. Volatiles present at $\geq 1 \text{ nL}\cdot\text{L}^{-1}$ that appeared when tissue was disrupted, those that increased upon tissue disruption, and those that showed little change are shown in Table 2 (Buttery and Ling, 1993a). Of those that appeared upon tissue disruption, only *trans*-2-pentenal was present in concentrations $>1 \text{ nL}\cdot\text{L}^{-1}$, although it has negative log odor units. Several of the volatiles that increased when tissue was disrupted, including the lipid oxidation compounds that are considered to be important to tomato flavor, had positive log odor units. All but two (1-penten-3-ol and geranylacetone) had positive odor units; these two, nevertheless, were present at $>1 \text{ nL}\cdot\text{L}^{-1}$. For volatiles that showed little change during tissue disruption, all but three (pentanol, hexanol, and geranial) had positive odor units and, thus, are likely to contribute to tomato flavor.

Volatile precursors include lipids, amino acids, carotenoids, and terpenoids (Buttery and Ling, 1993b) (Table 3). Volatiles are formed from lipids via oxidation when cells are disrupted. The enzyme lipoxygenase (LOX), along with hydroperoxide lyase (HPL) and a

hydroperoxy cleavage enzyme, convert linoleic (18:2) and linolenic (18:3) acids to hexanal and *cis*-3-hexenal, respectively, via 9- and 13-hydroperoxy- C18:2 and -C18:3 intermediates (Fig. 1). Hexanal and *cis*-3-hexenal can be reduced to hexanol and *cis*-3-hexenol, respectively, by a reductase enzyme such as alcohol dehydrogenase (ADH). Further isomerization of *cis*-3-hexenal to *trans*-2-hexenal can occur, either enzymatically or nonenzymatically (Galliard et al., 1977; Jadhav et al., 1972; Riley et al., 1996; Stone et al., 1975). This process is similar for tomato leaves and fruits (Buttery and Ling, 1993b; Galliard et al., 1977). The ability to form volatiles upon tissue disruption, however, changes during ripening (Fig. 2). Levels of hexanal, *cis*-3- and *trans*-2-hexenal, formed after tissue homogenization, increase as the fruit ripens (Baldwin et al., 1991a).

Other volatiles arise from amino acid precursors, including alanine, valine, leucine, isoleucine, and phenylalanine (Table 3). This occurs mostly in the fruit during the ripening process rather than upon cell disruption. Tomato fruit contain some highly unusual, volatile, nitro compounds (Buttery, 1993), which are not found in any other fresh fruit or vegetable, but do occur in some flowers. They can, however, be formed by degradation of amino acids and sugars in cooked foods. They appear as tomatoes ripen from breaker to mature red stages. One proposed pathway is via an amino acid, such as phenylalanine, which can be converted to 1-nitro-phenylethane and thence to phenylacetaldehyde in vitro by reducing the pH from 10.0 to 4.5 (Nef reaction) (Buttery, 1993). The proposed pathway for phenylacetaldehyde and 3-methylbutanal, therefore, is from 1-nitro-2-phenylethane and 1-nitro-3-methylbutane, perhaps by some enzymatic system in the fruit.

Alternatively, volatile aldehydes such as phenylacetaldehyde and 3-methylbutanal could be formed from enzymatic oxidation of corresponding alcohols that are released by enzymatic hydrolysis of glycosides during ripening. Glycosides can also be precursors of volatile aroma compounds (Williams, 1993). Isolation of a fresh tomato glycoside fraction yielded 3-methylbutyric acid and β -damascenone

Table 2. Volatiles present in tomato at levels $\geq 1 \text{ nL}\cdot\text{L}^{-1}$ that (A) appear after tissue disruption, (B) increase after tissue disruption, or (C) show no change after tissue disruption and are assumed to be present in the intact fruit.^a

A) Appear after tissue disruption	B) Increase after tissue disruption	C) No significant change due to tissue disruption
<i>trans</i> -2-Pentenal	<i>cis</i> -3-Hexenal <i>trans</i> -2-Hexenal Hexanal <i>trans</i> -2-Heptenal 1-Penten-3-one 1-Penten-3-ol Geranylacetone	3-Methylbutanol Pentanol <i>cis</i> -3-Hexenol Hexanol 6-Methyl-5-hepten-2-one Phenylacetaldehyde 2-Phenylethanol
Geranial	2-Isobutylthiazole 1-Nitro-2-phenylethane	

^aButtery and Ling (1993a).

Table 3. Origins of tomato volatiles present in fresh tomato leaves (L), fruit (F) stems (S), and all plant parts^a.

Lipid-L+F	Amino acid—mostly only in F		Carotenoid-F only
Hexanal	Alanine:	Acetaldehyde	Open chain:
Hexanol	Valine:	1-Nitro-2-methylpropane	Geranylacetone
<i>cis</i> -3-Hexenal	Leucine:	3-Methylbutanol	6-Methyl-5-hepten-2-one
<i>trans</i> -2-Hexenal		1-Nitro-2-methylpropane	6-Methyl-5-hepten-2-ol
<i>trans</i> -2-Heptenal		3-Methylbutanal	Pseudoionone
<i>cis</i> -3-Hexenol		3-Methylbutylnitrile	Cyclic:
Pentanal		3-Methylbutanol	β -Ionone
1-Penten-3-one		1-Nitro-3-methylbutane	β -Cyclocitral
1-Penten-3-ol		3-Methylbutyric acid	β -Damascenone
2-Isobutylthiazole			
<u>Lignin and miscellaneous</u>			
—all parts	Isoleucine:	2-Methylbutanol	Terpenoid—mostly L+S
Methyl salicylate		2-Methylbutyric acid	α -Copaene (green fruit)
Eugenol	Phenylalanine:	Phenylacetaldehyde	Linalool ^b
Benzaldehyde		2-Phenylethanol	Neral ^b
Guaiacol		1-Nitro-2-phenylethane	Geranial ^b
		Phenylacetoneitrile	

^aButtery and Ling (1993b).

^bOxygenated.

ENZYMATIC DEGRADATION OF LIPIDS

Fig. 1. Biosynthetic pathway for formation of C_6 "green" aroma volatiles from degradation of acyl lipids in blended tomato fruit (Gallaird, et al., 1977; Riley et al., 1996). LOX = lipoxygenase, HDL = hydroperoxide lyase, and ADH = alcohol dehydrogenase.

Fig. 2. Concentrations of aldehyde volatiles *cis*-3-hexenal (C3hx, HSD = 0.5), *trans*-2-hexenal (T2hx, HSD = 1.2), acetaldehyde (Acet, HSD = 1.0), *trans*-2-*trans*-4-decadienal (Deca, HSD = 0.07), hexanal (Hex, HSD = 3.1), and ethylene (ETH) production in homogenates of 'Sunny' (SUN ETH) and 'Solar Set' (SOL ETH) tomatoes sampled at the mature green (G), breaker (B), turning (T), pink (P), and red (R) ripening stages. Values are means for five replications. HSD (honestly significant difference) for ripening determined by Tukey's studentized range (after Baldwin et al., 1991a).

as major products upon hydrolysis. Other products included phenylacetaldehyde, 2-phenylethanol, linalool, linalool oxides, hotrienol, α -terpineol, 4-vinylguaiacol, 4-vinylphenol (Buttery, 1993; Buttery et al., 1990). The volatile furanone [2,5-dimethyl-4-hydroxy-3-(2H)-furanone] may also be formed from enzymatic hydrolysis of furanone-glycoside during ripening, since its concentration is little affected by blending (Buttery et al., 1995). It has a pineapple-like odor and may contribute to flavor since it has relatively high odor units (1.4). Although hexanol, *cis*-3-hexenal, and 6-methyl-5-hepten-2-one are also found after hydrolysis of glycosides, they are probably not bound as glycosides, but formed after blending (Buttery et al., 1990).

Carotenoids may also be precursors of some important flavor

volatiles (Table 3) (Buttery and Ling, 1993a). They are formed from C_8 , C_{13} , and C_{18} linear and cyclic isoprene (C_5) composites. This occurs only in the fruit and, in some cases, is dependent on cell disruption.

Finally, some volatiles are formed from terpenoids (C_{10} and C_{15}), related to lignin or of unknown origin (Table 3). Only one terpenoid volatile (α -copaene) has actually been identified in tomato fruit and occurred only in green fruit (Buttery and Ling, 1993b). However, several oxygenated terpenoid volatiles, which are important for flavor of other fruits, have been identified in ripe tomatoes, although their log odor units are below zero (linalool, nerol, and geranial). Lignin-related and miscellaneous volatiles include methyl salicylate, eugenol, benzaldehyde, and guaiacol. Of these, only methyl salicylate has log odor units >0 .

TARGETS FOR GENETIC CONTROL

Following tissue disruption, lipoxygenase is a key enzyme in the rapid formation of volatiles from lipids. The action of HPL ultimately forms hexanal and *cis*-3-hexenal. Reduction or isomerization of these compounds by ADH and perhaps an isomerase produces hexanol, *cis*-3-hexenol, and *trans*-2-hexenal (Galliard et al., 1977; Jadhav et al., 1972; Stone et al., 1975). All but hexanol in this group of volatiles have relatively high log odor units (Buttery, 1993). Conceivably, therefore, regulation of gene expression of LOX, HPL, and ADH might impact tomato fruit flavor. However, homogenation of tomato tissue may exaggerate the importance of lipoxygenase-derived volatiles because of the wounding effect, since wounding also induces formation of this enzyme (Hildebrand, 1989).

In a study of activities and subcellular location of LOX and HPL, Riley et al. (1996) reported that the majority of LOX activity was soluble, but the microsomal form of LOX changed most during ripening of tomato fruit. The activity of this isozyme increased between the green and breaker ripening stages and then decreased as the fruit turned red. Most of the HPL activity was found in the microsomal fraction and did not change during ripening. Riley et al. (1996) therefore suggested that volatile flavor formation takes place in the microsomal compartment. This was further confirmed by Riley and Thompson (1997), who also suggested that "blebbed" lipid particles (pinched off of membranes) may serve to move flavor volatiles out into the cytosol.

Two genes for LOX, *tomloxA* and *tomloxB*, have been cloned in tomato, using degenerate oligonucleotides corresponding to a purified, partially sequenced (Ferrie et al., 1994), membrane-associated LOX of ≈ 97 -kD (Bowsher et al., 1992) by probing breaker fruit cDNA library. *TomloxA* is expressed in seeds and fruit, reaches its highest level in fruit during the breaker stage and corresponds to the membrane-associated LOX. *TomloxB* is fruit-specific and reaches its highest level in ripe fruit. Another report describes two LOX activities in tomato fruit pericarp that increase in early stages of ripening and subsequently decrease, roughly paralleling ethylene production. The activities were associated with proteins of 95- and 97-kD (Ealing, 1994).

A 94-kD LOX was purified and cloned from a red-ripe fruit cDNA library (Kausch and Handa, 1997); it corresponded with a 94-kD protein that accumulated in the fruit during ripening, and reached a maximum concentration at the red-ripe stage. Expression of this gene was highest in the radial pericarp, but the highest LOX activity was in the locular gel, suggesting that LOX might have been synthesized in the pericarp and transported to the locular tissue where it accumulated during ripening. Buttery et al. (1988) reported that levels of hexanal, *cis*-3-hexenal, and *cis*-3-hexenol in the locular fluid of dissected fruit was 50% to >100% that of levels in intact fruit. The pulp contained >100% of the levels found in the intact fruit for these volatiles. This increase in volatile concentrations during or following dissection was attributed to wounding (Buttery et al., 1988). Interestingly, the 94-kD LOX gene was not expressed in the nonripening (*nor*) mutant, whereas the never-ripe (*Nr*) mutant accumulated the LOX mRNA, but not the protein. Nonexpression in *nor* fruit indicates that expression of the 94-kD LOX is linked to the ripening process. Since the *Nr* mutation blocks ethylene perception (Lanahan et al., 1994), accumulation of the LOX mRNA, but not the protein, indicates that ethylene may play a role in posttranscriptional regulation of the 94-kD LOX, perhaps through translation, or that ethylene affects the stability of the protein. Apparently ethylene perception is not required for gene expression and subsequent mRNA accumulation. The ability to synthesize hexanal, *cis*-3-hexenal, *cis*-3-hexenol, and *trans*-2-hexenal increases during ripening, peaking at the pink to red-ripe stage, and is closely associated with ethylene production (Fig. 2) (Baldwin et al., 1991a).

Another important enzyme is ADH, which reduces hexanal and *cis*-3-hexenal to hexanol and *cis*-3-hexenol, respectively. Two genes for ADH have been identified in tomato. One (*adh1*) is expressed in developing seed and pollen and responds to anaerobic stress (Longhurst et al., 1990). Hypoxic conditions are well known to increase ADH gene transcription in many plant parts (DeLisle and Ferl, 1990). The other (*adh2*) is expressed in a range of tissues, including the fruit, and

responds to anaerobic stress but also increases during ripening in the presence of normal oxygen levels. The *adh2* gene product is present in tomato pericarp 15 d prior to the initiation of ripening. The activity decreases and then increases late in ripening. Changes in cytoplasmic pH may induce ADH activity, since anaerobic stress is not a factor during tomato fruit ripening (Longhurst et al., 1990). A recent study demonstrated the effect of genetic manipulation of ADH levels in ripening tomato fruit. Tomato plants were transformed with constructs containing a tomato *adh2* cDNA. The resulting fruit, with higher or lower levels of ADH activity, exhibited corresponding higher and lower levels of hexanol and *cis*-3-hexenol. Fruit with increased levels of these alcohols were rated higher in "ripe fruit" flavor by a sensory panel (Speirs et al., 1998).

In one study, alteration of the fatty acid composition of tomato fruit led to changes in the flavor profile (Wang et al., 1996). Over-expression of the yeast Δ -9 desaturase gene increased concentrations of unsaturated and saturated fatty acids in transformed tomato fruit, some of which are precursors of important flavor volatiles. The most dramatic increases were in levels of palmitoleic acid (16:1), 9,12-hexadienoic acid (16:2), oleic acid (18:1), and linoleic acid (18:2), the latter being a precursor of hexanal. Because of the increased LOX substrates, transformed plants produced fruit with higher levels of hexanal and hexanol. This was expected since higher levels of hexanal precursor (linoleic acid) were present. Surprisingly, fruit from transformed plants also had higher levels of linolenic acid peroxidation products, such as *cis*-3-hexenal, *trans*-2-hexenal, and, subsequently, *cis*-3-hexenol via ADH.

CONSEQUENCES OF GENETIC CONTROL

Down-regulation of cell wall-digesting enzymes, such as polygalacturonase (PG) and pectinmethylesterase (PME), can alter the cell wall structure of tomato fruit. The enzyme PME demethylates pectin and PG hydrolyzes mainly demethylated pectin (Huber, 1983; Pressey and Avants, 1982). Transformation of fruit with the antisense genes for these enzymes inhibited PG to <1% of the normal level, extended shelf life, and increased disease resistance and solids content (Hobson and Grierson, 1993; Kramer et al., 1992; Schuch et al., 1991). Because cell wall structure can impact aroma binding and release, we assessed, in a preliminary study, the release of flavor volatiles into the headspace of homogenates prepared from transgenic, ripe tomato fruit with down-regulated PG, PME, and PG + PME activities (Table 4 A and B). The flavor volatile data from transgenic fruit, obtained by the method of Baldwin et al. (1992a), are shown as percentages of the values for nontransformed fruit. In the first study (Table 4A), red-ripe tomatoes with down-regulated PG (Kramer et al., 1992; Sheehy et al., 1988) produced lower levels of some volatiles, including methanol, ethanol, 1-penten-3-one, hexanal, 2+3-methylbutanol, *trans*-2-hexenal, *trans*-2-heptenal, 6-methyl-5-hepten-2-one, *cis*-3-hexenol, 2-isobutylthiazole, and geranylacetone, than did nontransformed fruit. In the second study (Table 4B), transformation of 'Ailsa Craig' fruit down-regulated PG (pTOM 6) (Grierson and Schuch, 1993; Smith et al., 1988) and reduced PME (PE1) (Hall et al., 1993) and PG+PME activities. In this case, the levels of flavor volatiles were similar to those of nontransformed controls except for a reduction in methanol in antisense PME and PG+PME fruit (Table 4B). Thus, at least some of the methanol observed in the headspace of homogenized tomato fruit (Baldwin et al., 1991a, 1991b) comes from demethylation of cell walls by PME once enzyme and substrate are mixed upon cell disruption. Nisperos-Carriedo and Shaw (1990) also suggested that this was the source of the methanol observed in the headspace of orange [*Citrus sinensis* (L.) Osbeck] juice, which also has active PME. Fruit with down-regulated PG+PME also exhibited low levels of 2+3-methylbutanol, 2-isobutylthiazole, and 6-methyl-5-hepten-2-one, the first two of which are derived from amino acids (Table 3). The third is thought to arise from carotenoid breakdown (Buttery and Ling, 1993b) and is similar in structure to geranylacetone (one isoprene unit difference between the two compounds), which was at normal levels in the transformed fruit. Despite their apparently similar origin, these two volatiles often behave differently, indicating a possible alternate mechanism for biosynthesis of 6-methyl-5-hepten-2-one. For ex-

Table 4. Tomato flavor volatiles in ripe homozygous and heterozygous transgenic fruit expressed as a percentage of concentrations in non-transformed controls (NT) including antisense PG (AS-PG), antisense ACC (AS-ACC) and its hybrid (AS-ACC x NT), antisense PME (AS-PME), antisense PG+PME (AS-PG+PME), antisense ACO (AS-ACO), pTOM99 (AS-99), and antisense phytoene synthase (AS-PSY).

Fruit type	Volatile (% of nontransformed controls) ²														
	acet	meoh	etoh	pent	hex	c3hx	mbut	t2hx	t2hp	mhep	c3hol	iso	nphn	ger	bio
<i>Study A³</i>															
AS-PG	79	59	56	67	39	123	49	68	54	46	53	50	77	61	218
AS-ACC	69	67	51	48	48	82	44	64	54	37	59	40	85	46	173
AS-ACC x NT	67	87	74	60	80	105	49	69	69	48	65	60	77	55	173
<i>Study B⁴</i>															
AS-PG	85	79	---	100	108	104	102	125	---	85	167	100	---	118	102
AS-PME	116	46	---	98	102	102	114	110	---	80	117	100	---	130	102
AS-PG+PME	69	42	---	102	84	107	57	88	---	63	175	0	---	98	102
AS-ACO	78	82	---	78	121	117	31	74	---	52	108	100	---	73	150
AS-99	113	104	---	82	108	77	83	138	---	107	175	200	---	148	271
AS-PSY	54	92	53	80	140	172	58	104	66	56	50	35	69	14	16

²Volatiles analyzed: acetone (acet), methanol (meoh), ethanol (etoh), 1-penten-3-one (pent), hexanal (hex), *cis*-3-hexenal, (c3hx), 2+3-methylbutanol (mbut), *trans*-2-hexenal (t2hx), *trans*-2-heptenal (t2hp), 6-methyl-5-hepten-2-one (mhep), *cis*-3-hexenol (c3hol), 2-isobutylthiazole (iso), 1-nitro-2-phenylethane (nphn), geranylacetone (ger), and β -ionone (bio).

³Data are means of two samples, each a composite of three ripe fruit, harvested field ripe.

⁴Data are means of three to five samples, each a composite of two to three ripe fruit, harvested 6 d past breaker stage; "—" indicates not measured.

ample, this compound did not increase following cell disruption, as did geranylacetone (Buttery and Ling, 1993a), and was a product of glycoside hydrolysis, whereas geranylacetone was not (Buttery et al., 1990).

One way to extend shelf life is to delay or reduce ethylene production. Transformed fruit with down-regulated key enzymes in the ethylene biosynthesis pathway exhibited extended shelf life (Murray et al., 1993; Oller et al., 1991). Ethylene is synthesized from *S*-adenosyl methionine via the intermediate, 1-aminocyclopropane-1-carboxylic acid (ACC), by action of ACC synthase; ACC in turn, is oxidized to ethylene via ACC oxidase (ACO, also known as ethylene-forming enzyme) (Adams and Yang, 1979; John, 1997). Fruit in which expression of ACC synthase is inhibited (Oeller et al., 1991) produce only 1% to 5% of normal ethylene levels and do not ripen completely without treatment with exogenous ethylene. Fruit in which ACO expression is inhibited (Hamilton et al., 1990) produce only 3% of normal ethylene levels, yet ripen slowly, especially if left on the plant. Ripening can be accelerated by treatment with exogenous ethylene (Murray et al., 1993). Gene pTOM99, which is identical to E8 (Slater et al., 1985), is reportedly regulated by both ethylene and other fruit-ripening signals. Meanwhile, the gene appears to negatively regulate ethylene biosynthesis (Deikman, 1997; Penarrubia et al., 1992). Since ethylene production often correlated with synthesis of pigments and flavor volatiles (Baldwin et al., 1991a; Buttery, 1993), we investigated the effect of altered ethylene production on levels of flavor volatiles.

In one study, red-ripe fruit with down-regulated ACC synthase (Oeller et al., 1991) produced lower levels of all volatiles measured except *cis*-3-hexenal, 1-nitro-2-phenylethane, and β -ionone (Table 4A). Hybrid fruit, resulting from a cross between plants with antisense ACC synthase and nontransformed plants, generally showed levels of volatiles intermediate between those of the two parents. The differences between ACC nontransformed control, hybrid, and antisense ACC fruit were significant ($P \leq 0.05$) for nine volatiles (Baldwin et al., unpublished). Transgenic fruit had less red color development, as evidenced by lower "a*" values when measured by a chromameter by the method of Baldwin et al. (1991a) than did nontransformed fruit. The hybrid fruit again showed "a*" values intermediate between those of the two parents (a* = 3.1, 4.0, and 6.8 for the transformed, hybrid, and nontransformed fruit, respectively). Levels of the major sugars, glucose and fructose, as analyzed by HPLC (Baldwin et al., 1991a, 1991b), were similar in transformed, hybrid, and nontransformed fruit (Baldwin et al., unpublished).

Fruit from the transgenic 'Ailsa Craig' line (same line as the antisense PG, PME, and PG+PME in Table 4B) exhibited less activity of ACO (pTOM13) (Hamilton et al., 1990; Murray et al., 1993), a key enzyme for the formation of ethylene, and had longer shelf life (Murray et al., 1993; Picton et al., 1993). Volatile production was little affected except for reduction in 2+3-methylbutanol and 6-methyl-5-hepten-2-one (Table 4B). Fruit with the antisense gene pTOM99

produced normal to high levels of flavor volatiles. The antisense ACC synthase fruit from the first study and the antisense ACO and pTOM99 fruit from the second study all had abnormally high levels of β -ionone.

Phytoene synthase (PSY) is a key enzyme in the synthesis of phytoene, a precursor of carotenoids (Gross, 1991). Down-regulation of this enzyme in pTOM5 tomatoes resulted in fruit with no red color (Bird et al., 1991). This resulted in negative "a*" values when measured with a chromameter (a* = -4.0 and +6.4 for antisense PSY and nontransformed control fruit, respectively). Analysis of flavor volatiles from antisense PSY fruit revealed lowered levels of all but methanol and some lipid-derived volatiles (1-penten-3-one, hexanal, *cis*-3-hexenal, and *trans*-2-hexenal). Levels of most of the amino acid and carotenoid-derived volatiles were reduced, and in the case of geranylacetone and β -ionone, to nearly trace levels. Surprisingly, however, 6-methyl-5-hepten-2-one was reduced to only 56% of the levels in nontransformed controls. This again suggests an alternate route for synthesis of this compound. The levels of eight volatiles, including the amino acid and carotenoid-derived compounds, were significantly higher in nontransformed fruit.

The effects of the various antisense transformations may have been due to repression of a specific gene product, but could also have been related to the site at which the antisense gene was inserted. In the latter case, different transgenic lines could exhibit different volatile levels.

CONVENTIONAL BREEDING AND POSTHARVEST TREATMENTS

Selecting tomatoes with higher levels of carotenoids may result in higher levels of carotenoid-derived volatiles, such as 6-methyl-5-hepten-2-one, β -ionone, β -damascenone, and geranylacetone, that have positive or borderline log odor units. Stevens (1970) reported high correlations between certain carotenoid-derived volatiles and specific carotenoids in tomato. Buttery et al. (1988) found that tomato cultivars containing higher levels of carotenoids also contained higher levels of 6-methyl-5-hepten-2-one, geranylacetone, and β -ionone than did those with lower concentrations of carotenoids. The volatile 6-methyl-5-hepten-2-one was higher in the high lycopene cultivar, geranylacetone was higher in the high lycopene and high β -carotene cultivars, and β -ionone was higher in the high β -carotene cultivar. Tomatoes cultured *in vitro* were induced to produce more lycopene pigment by addition of 2-(4-chlorophenylthio)triethylamine (CPTA), which resulted in an increase of some carotenoid-derived volatiles (Ishida et al., 1998).

Reducing ethylene production and thereby slowing down the ripening and softening process can also be accomplished using the ripening mutants, including nonripening (*nor*), ripening inhibitor (*rin*), never ripe (*Nr*), and alcobaca (*alc*). The mutants *rin* and *nor* do not ripen, do not display the climacteric rise in carbon dioxide or ethylene, and contain little PG activity. The *rin* and *nor* fruit fail to

develop normal red color. *Nr* fruit will ripen to a deeper red than either *rin* or *nor* and may contain low PG activity. *Alc* fruit have prolonged keeping qualities and can develop a light orange-red color on the plant, but do not ripen off the plant if harvested when mature green (Mutschler and Guttieri, 1987). All of these fruit have been used by breeders in crosses with normal-ripening lines to produce hybrids with extended shelf life characteristics, although *rin* hybrids have been the most successful commercially. It was generally assumed, as with the antisense PG, ACC, or EFE fruit, that quality of the ripened product would be improved because of the possibility of advanced harvest maturities (past color-break). This was based on work by Kader et al. (1977), where tomatoes ripened past breaker stage had better flavor.

Over the past several years, our laboratories have been analyzing *rin* hybrids from different sources and with different genetic backgrounds. Hybrids with the *rin* gene often have a pale red color (Baldwin et al., 1995) and both sensory and volatile analyses indicated that *rin* hybrids had lower levels of many important volatiles at the red-ripe stage (Baldwin et al., 1992a, 1992b, 1995) (Fig. 3A–D). This was true even in fruit harvested past the breaker stage when compared with normal tomatoes harvested mature green (Fig. 3A–D). Yet there was no consistent pattern of difference in soluble solids or titratable acidity (Baldwin et al., 1995) (Fig. 3 E and F). Firmness and shelf life, however, were often greater in the hybrids. Sensory work indicated

that *rin* hybrids were ranked lower than other normal fruit by consumer and experienced panels (Baldwin et al., 1995) (Fig. 3E) and were ranked lower in flavor intensity by trained panels (Baldwin et al., 1998, unpublished data). Nevertheless, incorporation of the *rin* gene into high flavor and color backgrounds may compensate for the *rin* effect on flavor and color while improving shelf life. Another study showed that both *rin* and *nor* hybrid fruit (backcross 6 generation with 'Rutgers') were deficient in volatiles found in 'Rutgers' tomatoes (McGlasson et al., 1987). The author is not aware of published information on flavor or volatile levels in *Nr* fruit.

In addition to using antisense technology and ripening-impaired mutants to indirectly improve flavor through advanced harvest maturity, breeders try to select for better-flavored tomatoes. The problem is that identifying objective measurements that signify good flavor is difficult (Baldwin et al., 1998). Which flavor components are important and what are the appropriate levels and balance for good flavor is still not understood. Analysis of flavor compounds in the aromatic component requires expensive equipment and training. Breeders can use sensory analysis, but this is often difficult to perform and requires access to a panel and considerable expertise. Even sensory descriptive data are limited in applicability as they provide information on specific flavor characteristics, but do not indicate consumer acceptability (Shewfelt, 1993). Nevertheless, significant differences exist between

Fig. 3. Headspace ketone volatiles (A) geranylacetone (Ger), (B) 6-methyl-5-hepten-2-one (Mhep), 1-penten-3-one (Pent), and β -ionone (Bio); headspace aldehyde volatiles (C) hexanal (Hex), *cis*-3-hexenal (C3hx), *trans*-2-hexenal (T2hx), and (D) *trans*-2-heptenal (T2hp) in homogenates of 'Solar Set' (S) tomato harvested mature green and in a *rin* hybrid (R) harvested at breaker stage; and (E) soluble solids (SS) and (F) titratable acidity (TA) in homogenized fruit tasted by 28 experienced panelists and rated on a hedonic scale of 1 to 9 for overall flavor (E). Data for (A–D) are means of three replicate samples $\pm$ SD, while (E) and (F) represent one composite sample for SS and TA, all from the same fruit (10 fruit/cultivar) sampled by the 28 panelists (E).

cultivars in levels of important aroma compounds (Baldwin et al., 1991b, 1995).

Climatic and cultural conditions can also affect tomato flavor. For example, heavy rains prior to harvest appear to dilute the concentrations of flavor compounds (Baldwin et al., 1995). Heavy fertilization with nitrogen and potassium reduced sensory analysis scores, and increased levels of titratable acidity, soluble solids, and several volatiles (including hexenal, phenylacetaldehyde, β -ionone, and 6-methyl-5-hepten-2-one among others) in tomato fruits (Wright and Harris, 1985). Levels of 12 out of 15 volatiles were determined to be higher in field-grown tomatoes than in greenhouse-grown fruit (Dalal et al., 1967).

Finally, harvesting and handling techniques impact the flavor of the ripened tomato fruit. The immature green fruit (determined non-destructively by number of days to break color under ethylene treatment) that can make up a significant percentage of the harvest in a gas green operation, do not ripen with acceptable flavor, as evidenced in sensory and volatile data (Maul, 1999; Maul et al., 1998ab). Bruising of fruit during harvest and handling also alter aroma volatile profiles (Maul et al., 1997; Moretti et al., 1997). Certain volatiles that increase in controlled atmosphere storage (5% O₂ and 4.4% CO₂) do not do so in air storage (Crouzet et al., 1986). Temperature abuse is also a problem that could be controlled. Temperatures below 16 °C may impair tomato flavor by lowering the volatile content and reducing "tomato-like" flavor (Kader et al., 1978). Storage at 2, 5, 10, 12.5 and 13 °C reduced levels of important volatiles (Baldwin et al., 1992b; Buttery et al., 1987; Maul, 1999). The mechanism by which volatile levels are reduced is not known, but could be related to reduced ethylene synthesis at low temperatures. Tomatoes stored at 2, 5, 10 or 12.5 °C also had less ripe aroma and tomato flavor, as well as more off-flavor, when analyzed by a trained descriptive panel (Maul, 1999).

More studies are needed to really understand the effects of climate, and of cultural and postharvest handling practices on tomato flavor. Nevertheless, poor flavor quality in tomato appears to be a result of breeding practices that do not select for flavor (because of lack of information), harvesting of green fruit (because of prevalence of immature green fruit in commercial harvests), and temperature abuse (because of storage of fruit below 16 °C, which results in impaired volatile levels). Harvest and handling practices could conceivably be altered, but information on flavor for use by breeders and molecular biologists is lacking. Considerable progress has been made in the identification of important flavor components in tomato and the determination of their concentrations in fresh fruit. Additional information is needed, however, on the optimal ranges and ratios for sugars, acids and aromatics required for good flavor. Future work needs to address what instrumental and sensory methods are most effective for evaluating important tomato flavor components. Likewise, establishing the relationship between instrumental measurements and sensory analysis is essential and will allow a more reliable assessment of the effects of breeding, genetic transformation, harvest maturity and postharvest handling on product quality.

The biosynthetic pathway for lipid-derived volatiles has been largely determined, but specific information is lacking on enzyme systems and other possible mechanisms of formation of amino acid and carotenoid groups. This information is necessary for the pragmatic manipulation of levels of flavor volatiles in the fruit. The relationship between volatile formation and ethylene-induced ripening events is not clearly understood, although lipoxygenase appears to be linked to such events. This enzyme may require posttranscription regulation by ethylene as well (Kausch and Handa, 1997). Ethylene is necessary for normal carotenoid development and, thus, may at least indirectly control synthesis of carotenoid-derived volatiles. Control mechanisms for amino acid-derived and other miscellaneous volatiles are little understood.

Thus far, flavor quality for tomato (or any other horticultural product) has been an elusive trait. We still lack quantifiable definition for tomato flavor. Increased performance of analytical instruments, availability of new sensor technology, advances in biotechnology, and development of powerful computer programs make identification and quantification of important chemical components possible. Furthermore, these components can then be traced back to sensory descrip-

tors, key enzymes, and gene products. Ultimately, identification and isolation of genes that influence eating quality would be useful. Integrating the fields of plant breeding, molecular biology, postharvest physiology and food science should provide superb opportunities for major breakthroughs in this area.

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The Composition of Strawberry Aroma Is Influenced by Cultivar, Maturity, and Storage

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Strawberry (*Fragaria × ananassa* Duch.) fruit have a unique, highly desirable flavor and are one of the most popular summer fruits. Sugars, acids, and aroma volatiles contribute to the characteristic strawberry flavor, which is dependent on the proper balance of these chemical constituents. While sugars and acids are responsible for the sweetness and tartness of the fruit, aroma volatiles provide the unique, fruity flavors that characterize a fresh strawberry.

The aroma of fresh strawberries is dependent on many factors. The large genetic variability in the nature of strawberry aroma results in differences in flavor among cultivars. In addition, the aroma changes dramatically during fruit ripening after harvest; therefore, it is important to preserve and enhance the ripe fruit aroma during postharvest handling. The loss of this desirable aroma or the development of objectionable aromas reduces the quality and marketability of fresh strawberries. In this review we will discuss the chemical nature of strawberry aroma and how it is affected by various factors, including cultivar, maturity, and postharvest environment.

CHEMICAL COMPOSITION OF AROMA

Volatile chemicals are responsible for the aroma and contribute to the flavor of fresh strawberries. These compounds comprise only 0.01% to 0.001% of the fruit fresh weight but have a major effect on its quality (Buttery, 1981). Fresh strawberries produce numerous volatile compounds; as many as 360 have been isolated (Latrasse, 1991) including esters, aldehydes, ketones, alcohols, terpenes, furanones, and sulfur compounds (McFadden et al., 1965).

Esters are quantitatively and qualitatively the most abundant class of these compounds; 131 different ones have been identified in strawberry aroma (Latrasse, 1991). Esters provide the fruity and floral notes and they comprise from 25% to 90% of the total volatiles in fresh ripe fruit (Douillard and Guichard, 1990; Ito et al., 1990; Pyysalo et al., 1979; Schreier, 1980). Other classes of compounds, which may comprise up to 50% of strawberry volatiles, include aldehydes (Schreier, 1980) and furanones (Larsen and Poll, 1992). Alcohols account for as much as 35% of the volatiles, but normally contribute little to strawberry aroma (Larsen and Watkins, 1995b). While terpenes normally comprise <10% of strawberry volatiles and sulfur compounds <2%,

they both may contribute to strawberry aroma (Dirinck et al., 1981; Schreier, 1980).

The volatile profile obtained from strawberry fruit is influenced by the analytical methods used to characterize these compounds. Volatiles from whole, intact fruit can be collected using headspace techniques; these samples can be analyzed directly or concentrated using adsorbent or cold traps. Volatiles are also collected from homogenized fruit or juice, using either headspace or solvent extraction techniques. Volatile samples are normally analyzed by gas liquid chromatography using a variety of methods of sample introduction, including liquid injection, thermal desorption, and cold on-column injection. High performance liquid chromatography (HPLC) has been used for some compounds that are thermally labile.

Each combination of techniques results in a slightly different volatile profile. Analysis of headspace compounds is dependent on their individual vapor pressure. The more volatile ones are present in higher concentrations. This reflects the compound's contribution to the fruit aroma but does not give its true concentration in the tissue. Disruption of the fruit through homogenization removes barriers to diffusion and allows for the determination of true concentrations, but may cause enzymatic changes in the volatile profile. Significant quantities of 1-hexanol, *trans*-2-hexen-1-ol, 1-hexanal, and of *trans*-2-hexenal are formed during homogenization through the actions of lipoxygenase, oxygen, and linolenic and linoleic acids (Latrasse, 1991). These C₆ aldehydes and alcohols may comprise up to 55% of the volatile profiles from homogenized fruit (Schreier, 1980), but account for <0.1% of volatiles collected from whole fruit (Ito et al., 1990).

Methods used to collect and analyze volatiles can cause the loss of certain compounds. This may explain the inconsistencies in the detection of the furanones: furaneol [2,5-dimethyl-4-hydroxy-3(2H)-furanone] and mesifurane [2,5-dimethyl-4-methoxy-3(2H)-furanone] in strawberry fruit. Pérez et al. (1992) were not able to detect furanones in 'Chandler' fruit when volatiles were collected through purge and trap of whole fruit, eluted from the trap with carbon disulfide, and analyzed using gas chromatography (GC) with cool on-column injection. However, both compounds were detected when volatiles were extracted and analyzed using HPLC (Sanz et al., 1995). Furanol breaks down at elevated temperatures and is unstable at low pH (Shu et al., 1985). Pickenhagen et al. (1981) reported that heating during volatile extraction, as well as glass capillary columns, reduced recovery of furaneol.

Among the hundreds of volatile compounds produced by fresh strawberries, only a small portion contribute to the fruit's aroma and flavor. The characteristic aroma is a blend of a number of volatile compounds; no single "character-impact" compound is responsible for strawberry aroma. The contribution of a compound to the aroma is dependent on its odor threshold and concentration in the fruit. From

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these two values an aroma value (concentration/threshold) can be calculated (Larsen and Poll, 1992). Aroma values >1 should contribute to the fruit's aroma, and the greater the value, the greater the compound's contribution. Larsen and Poll (1992) calculated aroma values for volatiles from 'Senga Sengana' strawberries using threshold values determined from the headspace over water solutions of each compound and concentrations determined from solvent extracts from fresh juice. They determined that ethyl butanoate, furaneol, and ethyl hexanoate contributed the most to the aroma; methyl butanoate, linalool, 2-heptanone, and 2-methyl butanoic acid were also important. Using similar techniques, Schieberle and Hofmann (1997) reported that mesifurane, *cis*-3-hexenal, methyl butanoate, ethyl butanoate, ethyl 2-methylpropanoate, and 2,3-butanedione were the most odor-active compounds in fresh juice from Spanish strawberries of an unknown cultivar.

The contribution of individual compounds to strawberry aroma can also be evaluated by sniffing GC effluent of individual peaks and characterizing their aroma. Using this technique, we have evaluated the headspace aroma trapped on Tenax adsorbent traps from whole fresh fruit and have found that ethyl hexanoate gave the most consistent high intensity aroma in all five cultivars sampled. High intensity peaks were produced by ethyl 3-methylbutanoate in 'Kent', 'Cavendish', and 'Micmac' fruit and by 3-methylbutyl acetate in 'Kent' and 'Micmac' fruit (Forney et al., unpublished data). Pérez et al. (1992), using similar techniques to rank the contribution of headspace volatiles from whole 'Chandler' fruit to the fruit's aroma, determined that ethyl butanoate, ethyl 2-methylbutanoate, and ethyl hexanoate were major contributors.

CULTIVAR DIFFERENCES

Strawberry cultivars vary both quantitatively and qualitatively in the volatiles they produce. We found a 35-fold difference in the quantity of volatiles evolved from different cultivars of ripe strawberries (Fig. 1A). The chemical composition of these volatiles was dominated by methyl and ethyl esters but the abundance of each ester varied with cultivar. Aromas of 'Configra' and 'Chandler' fruit were dominated by ethyl esters comprising 80% and 60%, respectively, of the total volatiles (Dirinck et al., 1981; Pérez et al., 1992). In fruit of other cultivars, including 'Hokowase', 'Kent', 'Senga Gigana', and 'Annapolis', methyl esters accounted for >70% of the total volatiles (Dirinck et al., 1981; Forney and Jordan, 1995; Miszczak et al., 1995; Ueda and Bai, 1993).

Among strawberry esters, butanoates and hexanoates predominate. Butanoates comprise 88%, 57%, 32%, 77%, and 51%, while hexanoates comprise 8%, 30%, 18%, 11%, and 40% of the total esters in 'Annapolis', 'Cavendish', 'Honeoye', 'Kent', and 'Micmac' fruit, respectively (Fig. 1B) (Forney and Jordan, 1995). Quantitatively, methyl and ethyl butanoate and methyl and ethyl hexanoate comprise the bulk of the volatile esters produced by fresh strawberries.

In addition to these esters, other volatile compounds are present in specific cultivars that gave them characteristic flavors. Ethyl 3-methylbutanoate and 3-methylbutyl acetate are predominant aroma volatiles in 'Kent' and 'Micmac' strawberries and hexyl acetate is an important contributor to aroma in 'Honeoye' fruit (Forney et al., unpublished data). Furaneol is an important contributor to strawberry flavor in many cultivars, including 'Senga Sengana', 'Parker', and 'Benton' (Larsen and Poll, 1992; Sanz et al., 1995). The monoterpene linalool is found in fruit of 'Senga Sengana' and 'Annelie' (Hirvi and Honkanen, 1982; Larsen and Poll, 1992). Other contributors to strawberry aroma in various cultivars include butanoic acid, 2-methylbutanoic acid, methyl and ethyl 2-methylbutanoate, γ -decalactone, and 2-heptanone (Fischer and Hammerschmidt, 1992; Larsen and Poll, 1992; Schieberle, 1994).

MATURITY EFFECTS

Many rapid qualitative and quantitative changes occur in strawberry fruit volatiles during ripening. Volatile content increases rapidly as fruit ripen and is closely correlated with color development (Forney et al., 1998; Miszczak et al., 1995). The fruit ripen rapidly in the field,

Fig. 1. Total concentration of volatile compounds (A) and major ester composition (B) in ripe fruit from five strawberry cultivars. Volatiles in the headspace of 4-L glass jars, each containing 100 g of whole, fresh fruit, were trapped on Tenax adsorbent traps, thermally desorbed, and analyzed by gas chromatography-mass spectrometry. The error bar represents the SE where $n = 12$ and $df = 40$. (Adapted from Forney and Jordan, 1995.)

turning from white to fully red in about 1.5 d. Volatile concentration is 5-fold as great in red-ripe fruit ($6.8 \mu\text{mol}\cdot\text{m}^{-3}$) as in fruit that is 75% red ($1.3 \mu\text{mol}\cdot\text{m}^{-3}$) at time of harvest (Forney and Jordan, 1995). Similarly, Ito et al. (1990) reported that total volatiles increase 14-fold during the 3 d in which 'Nyoho' strawberries go from white to full red. During color development many qualitative changes also occur. The concentrations of methyl esters increase ≈ 7 -fold while those of ethyl esters change very little as fruit ripen (Fig. 2) (Forney et al., 1998). In 'Chandler' strawberries, Pérez et al. (1992) found that C_6 alcohols accounted for 25% of the fruit volatiles 36 d after bloom, but only $\approx 5\%$ after 46 d. They suggest that C_6 alcohols could account for the "green" odor of immature strawberries. In addition to the increase in esters, furaneol, mesifurane, and furaneol glucoside increased with ripening in seven cultivars of strawberries (Sanz et al., 1995).

POSTHARVEST ENVIRONMENT

Volatile content of fresh strawberries increases during storage. Volatiles in 'Kent' fruit harvested fully red increased 7-fold after 4 d at 15°C (Fig. 3) (Miszczak et al., 1995). Volatile content in fruit harvested pink also peaked after 4 d, increasing ≈ 200 -fold. Volatile concentrations in pink fruit reached levels similar to those in freshly harvested, red-ripe fruit after being held for 4 d at 15°C . In this study, ethyl esters increased during storage to a greater extent than did methyl esters. After 4 d at 15°C , ethyl ester content of 'Kent' fruit increased from 7% to 44% of total volatiles. Similar increases in volatile content were observed by Forney and Jordan (1995). During 5 d at 1°C plus 2 d at 15°C , volatile content of 'Kent', 'Annapolis', 'Micmac', 'Cavendish', and 'Honeoye' fruit were 5.7, 1.9, 1.7, 1.4, and 1.3 times as high, respectively.

Storage temperature influences strawberry volatile production.

Fig. 2. Concentrations of four major esters in strawberries harvested at three stages of maturity. Fruit were harvested when 50% red (50% Red), on the day the fruit turned fully red (Red), or on the following day (Red+24 h). Volatiles in the head space of 4-L glass jars, each containing 100 g of whole, fresh fruit, were trapped on Tenax adsorbent traps, thermally desorbed, and analyzed by gas chromatography-mass spectrometry. Values are averages for five cultivars. Error bars represent the SE where $n = 15$ and $df = 75$ (Adapted from Forney et al., 1998.)

Holding pink 'Kent' strawberries at 10 and 20 °C had no consistent effect on the content of all volatile compounds (Miszczak et al., 1995). Production of methyl 3-methylbutanoate and 3-methylbutyl acetate was higher at 20 °C than at 10 °C, whereas the opposite was true for ethyl butanoate. We have found that ethyl butanoate and ethyl hexanoate increased during storage at 1 °C, while methyl butanoate and methyl hexanoate increased during storage at 15 °C (Fig. 4). These temperature-related changes in ester composition are consistent with what we have observed with strawberries ripening in the field (Fig. 2). With field temperatures ranging from 12 to 30 °C, concentrations of methyl esters increased more rapidly than did those of ethyl esters. Methyl butanoate and methyl hexanoate increased 6- and 20-fold, respectively, in fruit ripened from 50% red to red + 24 h, while ethyl butanoate and ethyl hexanoate concentrations increased <50%. The reason for this temperature effect on ester synthesis has not been explained. Understanding the mechanism underlying these changes could provide new methods to control strawberry flavor development both before and after harvest.

Exposure to light during storage also affects patterns of volatile synthesis in harvested strawberries. Miszczak et al. (1995) showed that storage in light ($200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 3 d at 10 or 20 °C increased the production of ethyl hexanoate, 3-methylbutyl acetate, ethyl 3-methylbutanoate, and methyl 3-methylbutanoate in pink 'Kent' strawberries.

Low-oxygen atmospheres and injurious levels of CO_2 can induce fermentation, causing ethanol accumulation in the fruit, which, in turn, produces off-odors. Normal mitochondrial oxidative phosphorylation can be disrupted because of a lack of O_2 or interference with normal mitochondrial function arising from membrane damage or changes in pH gradients (Ke et al., 1994). Disruption of respiratory metabolism results in the accumulation of pyruvate, which is converted to acetaldehyde and ethanol by pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) in the cytoplasm. In addition, elevated levels of CO_2 in the storage atmosphere tend to lower cytoplasmic pH (Siriphanich and Kader, 1986), which may stimulate the activity of these two enzymes (Ke et al., 1994). The resulting increased levels of ethanol may stimulate formation of ethyl esters, especially ethyl acetate (Ke et al., 1994; Larsen and Watkins, 1995b).

Accumulation of ethanol and acetaldehyde is often associated with off-odors and flavors. In strawberry fruit held in 20% CO_2 , ethanol concentrations increased 2-fold but there was no increase in acetalde-

Fig. 3. Total concentration of volatile compounds in head space over 'Kent' strawberry fruit in storage. Fruit were harvested fully red, pink, or white and held at 15 °C for 10 d. Volatiles in the headspace of 1-L glass jars, each containing 10 whole, fresh fruit, were trapped on Tenax adsorbent traps, thermally desorbed, and analyzed by gas chromatography-mass spectrometry. Error bars represent the SE where $n = 3$ and $df = 10$. (Adapted from Miszczak et al., 1995.)

Fig. 4. Concentrations of four major esters in "red-ripe" strawberry fruit at harvest (Initial), after storage at 1 °C for 5 d (5d 1 °C), or after an additional 2 d at 15 °C (5d 1 °C + 2d 15 °C). Volatiles in the head space of 4-L glass jars, each containing 100 g of whole, fresh fruit, were trapped on Tenax adsorbent traps, thermally desorbed, and analyzed by gas chromatography-mass spectrometry. Values are averages for five cultivars. Error bars represent the SE where $n = 15$ and $df = 75$. (Adapted from Forney et al., 1998.)

hyde concentration (Larsen and Watkins, 1995b). Off-odors that developed in these fruit were correlated with fruit ethanol content, but, because of its high odor threshold, ($100\text{--}800 \text{ mg}\cdot\text{kg}^{-1}$), ethanol was not responsible for the off-odor (Larsen, 1994). Increased levels of ethyl acetate induced by stressful atmospheres appear to be the true cause of many anaerobic off-odors in fresh produce. Ethyl acetate has a low odor threshold ($0.26\text{--}5 \text{ mg}\cdot\text{kg}^{-1}$) (Larsen, 1994). It has a fruity, pineapple-like odor at low concentrations, but a more chemical-like odor that often is associated with anaerobic fruit at higher concentrations. When 'Pajaro' strawberries were stored in varying concentrations of CO_2 (0% to 20%) for up to 11 d at 0 °C, the concentration of ethyl acetate was best correlated with off-flavor scores ($r = 0.85$) (Larsen and Watkins, 1995b); r values for ethanol and acetaldehyde were 0.62 and 0.3, respectively.

Esters are the most important group of volatile compounds responsible for the aroma of strawberry fruit. However, research to determine the mechanisms by which these esters are produced has been limited. The primary enzyme believed to be responsible for ester production is alcohol acyltransferase (AAT), which Pérez et al. (1993) isolated and partially purified from 'Chandler' fruit. The enzyme had a broad pH range (5.5 to 9.3) and a temperature optimum of 35 °C at pH 8.0.

This enzyme catalyzes the esterification of an acyl moiety from acyl-CoA onto an alcohol (Fig. 5). The many sources of substrates for this reaction influence the composition of the esters produced. Pérez et al. (1993) reported that AAT had greatest activity with hexanol when acetyl-CoA was used as an acyl donor although methanol and ethanol were not tested as substrates. Although it had slightly greater activity with acetyl-CoA, AAT acted on other acyl-CoAs (propionate and butanoate). The specificity of this enzyme was correlated with the ester composition in ripe 'Chandler' fruit, suggesting that ester composition is dependent on the properties of the enzyme. Pérez et al. (1996) observed that the more flavorful fruit of 'Oso Grande' had higher AAT activity than did fruit of the less flavorful cultivar I-101, supporting the importance of this enzyme for flavor development in strawberry fruit.

The ability of strawberry fruit to produce esters varies with fruit maturity. Yamashita et al. (1977) demonstrated that immature 'Hokowase' strawberry fruit, collected 5 d after flowering, converted added pentanal to 1-pentanol, but produced no esters. However, the production of 1-pentyl acetate and 1-pentyl n-butanoate increased dramatically between 30 and 40 d after flowering, as fruit ripened. Similarly, Hamilton-Kemp et al. (1996) found that C₆ alcohols added to ripe strawberries were converted to their corresponding acetate esters. To explain this ability of ripe fruit to produce esters, Pérez et al. (1996) showed that AAT activity increased as strawberry fruit ripened on the plant and was first detected in most cultivars when fruit had begun to turn pink. In addition, AAT activity did not change when fruit were stored at 1 °C for 9 d (Pérez et al., 1996).

Alcohol dehydrogenase (ADH) is another enzyme that is involved with synthesis of aroma volatiles in strawberry fruit (Fig. 5). This enzyme is involved in the interconversion of alcohols and aldehydes to supply precursors for ester synthesis and the production of other volatile compounds. When C₆ aldehydes were supplied to ripe strawberries, they were readily converted to their corresponding alcohols and acetate esters (Hamilton-Kemp et al., 1996). Mitchell and Jelenkovic (1995) observed that the specific activity of NAD- and NADP-dependent ADH to various alcohols and aldehydes corresponded to the substrates found in ripe strawberries. This enzyme may play a key role in supplying the precursors that determine what esters a strawberry fruit produces.

Substrate availability may also play a major role in the composition of esters produced by ripe strawberry fruit. Amino acids, sugars, and lipids all can act as precursors for ester substrates. Amino acid metabolism generates aliphatic and branched-chain alcohols, acids, carbonyls, and esters. Leucine gives rise to 3-methylbutyl acetate, and low levels of both leucine and 3-methylbutyl acetate were found in 'Chandler' strawberries (Pérez et al., 1992). Alanine increased 2-fold in strawberries between 30 and 36 d after bloom and could be a precursor of ethyl esters. Drawert and Berger (1981) found that feeding alanine to cultured segments of strawberry fruit enhanced formation of methyl hexanoate, ethyl hexanoate, ethyl butanoate, and ethyl decanoate.

Normal aerobic metabolism of sugars can produce precursors for ester production, and fermentation induced by anaerobiosis produces large quantities of acetaldehyde and ethanol. Associated with these increases are increases in the production of ethyl esters, including ethyl acetate, ethyl butanoate, and ethyl hexanoate (Larsen and Watkins, 1995a; Ueda and Bai, 1993).

Fatty acids from various lipids also appear to serve as ester precursors. Fatty acids are catabolized through two major pathways, β -oxidation and the lipoxygenase pathway (Sanz et al., 1997). β -Oxidation produces acyl-CoAs that can be used by AAT to produce esters. The lipoxygenase pathway is most active in disrupted plant

Ester Biosynthesis

Fig. 5. Proposed pathways involved in the biosynthesis of volatile esters in ripe strawberry fruit. ADH = alcohol dehydrogenase, AAT = alcohol acyltransferase.

cells and produces a variety of volatile C₆ and C₉ compounds, including 1-hexanol, *trans*-2-hexen-1-ol, 1-hexanal and *trans*-2-hexenal, which are found in large quantities in homogenized strawberry fruit tissue (Latrasse, 1991; Schreier, 1980). These lipoxygenase products are metabolized by the fruit into volatile esters (Hamilton-Kemp et al., 1996).

In addition to esters, furanones make an important contribution to the aroma and flavor of fresh strawberry fruit. However, our understanding of the mechanism of furanone biosynthesis is very limited. Sugars have been suggested as precursors for furanone synthesis, with fructose being the most likely candidate (Sanz et al., 1997; Zabetakis and Holden, 1997). Sanz et al. (1997) suggest that furaneol could be synthesized from an intermediate of the pentose phosphate cycle and that fructose-6-phosphate could be its precursor. The addition of 6-deoxy-D-fructose to tissue-cultured strawberry cells stimulated the production of furaneol-glucoside, suggesting its role as a precursor of furanone synthesis (Zabetakis and Holden, 1997). While furanone synthesis is believed to be enzyme-mediated, no enzyme has been identified to date. Identification of the biochemical pathway responsible for furanone synthesis and its regulation could provide new insights into strawberry flavor development.

CONCLUSIONS

The aroma of fresh strawberries is comprised of a complex mixture of volatile components, with methyl and ethyl esters predominating. Other compounds that contribute to aroma include furanones, aldehydes, terpenes, and sulfur compounds. Many factors influence the volatile composition, including cultivar, fruit maturity, and postharvest environment. We still have a limited understanding of the mechanisms controlling the synthesis of aroma volatiles. A better understanding of these mechanisms could provide us with the ability to manipulate strawberry fruit to optimize flavor at the time of consumption. Understanding properties of enzymes involved in the production of aroma volatiles may lead to genetic and environmental manipulations to improve strawberry flavor following shipping and marketing.

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Factors That Influence Biosynthesis of Volatile Flavor Compounds in Apple Fruits

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When one bites into an apple (*Malus ×domestica* Borkh.), the olfactory sensations received from volatile molecules are responsible for the perception of “apple flavor,” hence it is pertinent to examine some of the factors that can influence production of these molecules in the hope of having a better product available for a longer period of time in the marketplace. Many studies have shown the occurrence of secondary volatiles resulting from maceration (e.g., chewing) of plant tissues and these occur in apple products (Flath et al., 1967). However,

the aldehydes generated during the maceration of apple tissue are often overwhelmed by the presence of the volatile esters (Mattheis et al., 1991a, 1991b). In fact, the aroma profile changes from aldehydes (“green-notes”) to esters (“fruity-notes”) during ontogeny (Mattheis et al., 1991b). We have been studying the nature, occurrence and biosynthesis of the acetate esters, a particular class of volatile compounds noted for imparting the characteristic sweet, fruity flavor and aroma associated with apples.

Flath et al. (1967) were among the first to identify some of the compounds responsible for the taste and smell of apples, isolating 56 volatile compounds from ‘Delicious’ apples. In a more recent review, Dimick and Hoskin (1983) reported that nearly 300 volatiles have been isolated from apple. Of these, three esters (butyl acetate, 2-methylbutyl acetate, and hexyl acetate) are considered major contributors to the

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Although volatile profiles produced vary with cultivar (Kagiguchi et al., 1986), there appears to be some similarity in the major esters. In 'Rome' apples, ethyl acetate, butyl acetate, 2-methyl-1-butyl acetate, hexyl acetate, hexyl butyrate, and butyl-2-methylbutyrate were major ester components of the volatile profile (Fellman et al., 1993). Ethyl butyrate, butyl acetate, ethyl-2-methylbutyrate, and 2-methylbutyl acetate were found in the peel of nine cultivars (Guadagni et al., 1971). In 'Bisbee Delicious', major ester constituents included hexyl acetate, butyl acetate, 2-methylbutyl acetate, ethyl acetate, ethyl-2-methylbutyrate, and pentyl acetate (Mattheis et al., 1991a, 1991b), while the first three compounds were also prominent in 'Golden Delicious' (Brackmann et al., 1993). In addition to studying these three end products, our efforts have been focused on examining the terminal step in biosynthesis of acetate esters, catalyzed by alcohol acetyl-CoA transferase (AAT). The biochemical pathways involved in the interaction of various catabolic steps required for synthesis of the aroma compounds we are studying are summarized in Fig. 1. This report represents an overview of some of the information accumulated by our research efforts with regard to the influence of cultivar, growing conditions, maturity, and storage on the occurrence and biosynthesis of the principal acetate esters in apples.

Apples were obtained from research orchards at Pullman or Wenatchee Wash., or Parma, Idaho, or obtained through the generos-

ity of growers who allowed research plots to be established in commercial orchards throughout the inland northwestern United States and Okanagan Valley, B.C., Canada. Volatiles analyses were via capillary gas chromatography with a flame ionization detector (GC-FID) or by gas chromatography-mass spectrometry (GC-MS) using purge-and-trap or headspace methods developed in our laboratories (Fellman et al., 1993). Headspace volatiles were measured by placing fruits in polytetrafluoroethylene jars, and passing purified air through the jar at 100 mL·min⁻¹ for 10 min to collect 1 L of headspace atmosphere on 50 mg of Tenax, a porous polyester substrate (Alltech Associates, Deerfield, Ill.). Trapped volatiles were thermally desorbed (250 °C) into a cryofocusing loop and analyzed as described below. All associated connective tubing was made of polytetrafluoroethylene to prevent reactions with the compounds studied. To assay volatile content of flesh, mesocarp tissue was crushed, and 2.5 mL of juice diluted 1:1 with distilled deionized water was analyzed using purge-and-trap cryofocusing techniques. Samples were purged in a closed system for 5 min with helium, and water vapor was condensed from the sampling stream by passing the vapors through a cryostat held at -10 °C. Samples were injected by cryofocusing at -90 °C using a commercial purge-and-trap injector (Chrompack International B.V., Middelburg, The Netherlands) modeled after that described by Badings et al. (1985). Gas chromatographic separations were achieved using conditions reported by Mattheis et al. (1991a, 1991b), but the DB-WAX column (J&W Scientific, Rancho Cordova, Calif.) diameter was 0.32 mm with 5.0-μm film thickness. Quantitation was achieved using FID. Positive identification of volatiles was facilitated by interfacing the gas chromatograph to a Quadrupole Mass Spectrometer (Hewlett-Packard model 5971, Palo Alto, Calif.) operated in the electron ionization mode at 70eV. Identification was via Wiley/NIST library (National Institute of Standards and Technology, Gaithersburg, Md.)

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match and injection of standard compounds.

Assays of AAT were performed using the spectrophotometric method developed in our laboratory, with confirmatory analyses using purge-and-trap cryofocusing GC (Fellman and Mattheis, 1995). Esterification of butanol to butyl acetate was measured by incubating partially-purified protein extracts of apple tissue in the presence of acetyl coenzyme A and butanol. Assay components in final concentrations were: 10 mM potassium phosphate buffer, pH 7.0, 20 mM $MgCl_2$, 1 mM 5,5'-dithiobis-nitrobenzoic acid (DTNB, Ellman's reagent) in pH 7.0 phosphate buffer, 3 mM 1-butanol, and 0.49 mM acetyl CoA. Reactions were assayed by mixing ingredients in a cuvette to a total volume of 1 mL, and initiating the reaction with 0.01 mL of acetyl CoA solution. Progress of the esterification reaction was followed by monitoring solution absorbance at 412 nm, the absorbance maximum of the thiophenol product formed by reaction of DTNB with free CoA. Formation of butyl acetate, as measured in the reaction mixture by purge-and-trap capillary GC, was proportional to the amount of thiophenol generated in the reaction mixture.

Apples were sampled from orchards according to cultivar, stage of maturity, N status of trees, and different fruit-shading treatments, depending on the experiment, and placed under various controlled-atmosphere (CA) storage conditions at the Pullman or Wenatchee facilities.

Table 1. Percentage of total flavor volatiles purged from the flesh of fruit from three strains of 'Delicious' differing in skin coloration.^a

Volatile compound	Strain (Color Group ^b)		
	Hi Early (Group 1)	Topred (Group 2)	Starkrimson (Group 7)
Esters			
Butyl acetate	13.2	13.3	1.6
2-Methylbutyl acetate	25.0	54.7	39.4
Hexyl acetate	5.9	0.5	0.7

^aAverage total volatile concentration was 4021 ng·mL⁻¹ juiced tissue. Trees were grown on 'EMLA 7' rootstock.

^bColor group 1 has almost no red pigment in skin cells ("stripe"); color group 2 has low proportions of pigmented skin cells ("blush", with few or no stripes); color group 7 has the highest proportion of pigmented skin cells ("blush").

Influence of genotype

Examination of principal acetate levels indicated the influence of strain of 'Delicious' apple on ester content of the fruit. Esters, the principal compounds responsible for apple odor, were present in various amounts in the different strains, but we did not attempt to quantify the data in the initial study; rather the relative concentrations in each strain were examined. Our initial study suggested that genetic differences between 'Delicious' strains can alter the flavor pattern in apple flesh. Other researchers have classified strains according to tree type (standard-Groups 1 through 3, or spur-Groups 4 through 7) and the pigmentation levels in the skin cells (Dayton, 1964), but comparison of different 'Delicious' color classes vs. aroma profiles is enlightening. Color group 1 is a striped apple with 95% to 100% of the skin cells having no pigment; 'Starking' and 'Hi Early' belong to this category. Group 2 is blushed with few or no stripes and has low proportions of pigmented skin cells; 'Topred' is placed here. Group 3 apples would be classified as standard trees with 75% or more of the epidermal cells having red pigment, predominately blushed with few stripes. Groups 4–6 are spur-type trees with pigmentation descriptions similar to those of groups 1–3. Group 7, a blush-type, has the highest proportion of pigmented skin cells. Most modern strains are descendants of 'Starkrimson', a group 7 apple. Table 1 compares color grouping and aroma molecule profile in three strains. There are relatively lower levels of butyl acetate, and hexyl acetate in 'Starkrimson' (group 7) than in 'Hi Early' (group 1). 'Topred' (group 2) has more 2-methylbutyl acetate than the other strains. A relationship between peel coloration and ester content was apparent, with the higher-coloring strains having lower aroma content. We then performed experiments that included two 'Delicious' strains, 'Topred' and 'Starkrimson', with different capacities for accumulation of anthocyanin pigments in peel tissue.

At the same time, the capacity for immature fruits to color was manipulated by placing shade cloth around them. 'Topred', while accumulating about twice as much anthocyanin as 'Starkrimson', did not display an identical relationship between acetate ester synthesis and shading (Fig. 2). Dayton (1964) reported that 'Topred' accumu-

Fig. 2. Relationship between peel coloration and acetate ester emission of 'Topred Delicious' (top) and 'Starkrimson Delicious' (bottom) apples. Shade cloth was placed around developing fruit at midseason, and apples were harvested at 5 weekly intervals. Harvest 1 (early September; preclimacteric) fruit is compared with harvest 5 (mid-October; fully ripe) fruit. Anthocyanin values were obtained via spectrophotometry of acidified ethanol extracts of peel tissue.

lated the anthocyanin responsible for red coloration in the vacuoles of hypodermal cell tissue, while 'Starkrimson' accumulated it in the hypodermal and epidermal cell vacuoles of its peel tissue. Mutations in the *Malus* genome appear to reduce the capacity for acetate ester synthesis by limiting substrate availability through increased synthesis and sequestration of acetate moieties in the anthocyanin molecules deposited in peel cell vacuoles. The relationship between shading and ester biosynthesis is more pronounced in apples that have a larger proportion of pigmented epidermal cells in addition to the deeply colored hypodermal cells ('Starkrimson') than in those that have very little pigment in the epidermal layer of peel cells ('Topred'). Nevertheless, ester emission from 'Topred' was 50% less than that from 'Starkrimson' following intermediate shading treatments (Fig. 2), reinforcing the suggested relationship between color and synthesis of aroma. Alcohol acetyl transferase levels in crude extracts prepared from 'Topred' were higher than those from 'Starkrimson' (Fig. 3), demonstrating that the capacity for enzymatic ester synthesis remains, but substrates for the reaction are lacking. Generally, higher coloring mutations of 'Delicious' had lower levels of extractable AAT activity (Fig. 3).

Cultural practice

Fertilization. In addition to the study of light penetration through the tree canopy onto fruit surfaces, we examined how tree nitrogen status affected apple aroma. In a 2-year study with 'Redspur Delicious' apples, we observed a slight, but not statistically significant, influence of nitrogen nutrition on the level of 2-methylbutyl acetate in the apple flesh, but no marked effect on the other two volatiles was measured (Fig. 4).

Somogyi et al. (1964) found that poorly fertilized apple trees generally produced fruit with poor flavor. The authors concluded that nutrient balance is important for normal production of flavor and aroma volatiles. Of the three acetate esters measured in this study, we expected 2-methylbutyl acetate to be most responsive to rate/timing of N application, as its biosynthesis is thought to require branched-chain amino acid precursors (Hansen and Poll, 1993). However, we observed no marked effect of N application on the availability of amino-acid related precursors. Protein synthesis and enzyme production increase dramatically during fruit ripening (Drawert, 1974), hence increased catalysis of chloroplast components and associated macromolecules may create a large pool of amino acid residues for synthesis of branched-chain esters. Clearly, the concentration of free amino acids in ripening fruit need not be closely related to N availability in the tree. Ackermann et al. (1992) showed that, although concentrations of amino acids decreased with increased ripening of 'Glockenapfel'

apples, they were generally stable in storage. The supply of precursors arising from amino acid metabolism is apparently less variable than that derived from fatty acids via β -oxidation.

Ripening

Apple fruits are harvested when certain factors indicate physiological maturity has been reached, but prior to significant ethylene biosynthesis (Knee et al., 1989; Watada et al., 1984). These harvest indices include days elapsed from full bloom, internal and external fruit color, sugar content of the flesh (soluble solids), acidity of the juice, flesh firmness, and staining of cortical starch by iodine (Lau, 1989; Olsen, 1982). The combination of indices that are used to determine the correct harvest date is usually specific for a given variety. As starch, organic acids, procyanidins, pectins, and lipids are metabolized during ripening, there is a rapid increase in metabolites available for biosynthesis of the volatile molecules responsible for taste and smell. Concentrations of volatile compounds increase dramatically as ripening progresses (Fellman and Mattheis, 1995; Mattheis et al., 1991b; Romani and Ku, 1966), but we do not know whether the onset of biosynthesis of volatile compounds is concurrent with, or precedes and perhaps plays a role in the initiation of, the climacteric rise in fruit respiration. Our laboratories have performed many experiments to determine the relationship between maturity and volatile ester production. In 'Gala' apple fruit, acetate ester concentrations increased as harvest maturity advanced, especially if apples were ripened at room temperature for an additional 7 d (Fig. 5). We have observed similar results with 'Delicious', 'Rome' and 'Fuji' apples.

There is generally a sequence of flavor and aroma volatile production in apple fruit with advancement of maturity. Brown et al. (1966) believed that ethylene production and the climacteric rise in respiration were the key to production of volatiles. Flath et al. (1967) had noted that the taste and aroma of ripe apples was characterized by esters and aliphatic alcohols, and they associated ripening with biosynthesis of these classes of compounds. The temporal sequence of volatile biosynthesis suggested by these studies has since been examined in some detail. In studies with 'Golden Delicious', Paillard (1986) found that as ripening progressed, as measured by CO_2 evolution, degreening occurred. The color change was caused by a disorganization of chloroplasts, which then released membrane galactolipids rich in linolenic and linoleic acids. As these lipids were oxidized, presumably by lipoxygenase activity and/or β -oxidation, biosynthesis of the C_6 aldehydes hexanal and 2-hexenal was noted. Upon onset of the respiratory climacteric, aldehyde production decreased, and alcohol biosynthesis ensued, beginning with butanol, and followed by ethanol just prior to fruit senescence. Ester biosynthesis also closely followed

Fig. 3. Alcohol acetyl transferase (AAT) activity in mutations of the original 'Delicious' apple cultivar. (left) Direct mutations of 'Delicious'; (right) genetically related, yet high-coloring mutations of 'Starking Delicious'.

Fig. 4. Acetate ester concentration of 'Redspur Delicious' apples in response to nitrogen fertilization. Means within years over a 2-year experiment were not significantly different ($P \leq 0.05$).

Fig. 5. Principal acetate esters from 'Gala' apple flesh harvested over a 3-week period in late August-early September. (A) Ester content just after harvest; (B) and after a 7-d holding period at 25 °C. Harvest 1 fruit were preclimacteric, while harvest 3 fruit were postclimacteric.

advancement of the climacteric, beginning with butyl acetate, and followed by butyl butyrate $\approx$ 5 d later.

The orderly progression of volatile biosynthesis according to molecular class was also observed in 'Bisbee Delicious' (Mattheis et al., 1991b). Aldehydes were generally prominent in preclimacteric fruit. As ripening progressed, as monitored by internal ethylene concentration, C_4 to C_7 aldehyde levels decreased, whereas C_8 to C_{10} aldehyde levels did not change significantly. Ethanol was the predominant alcohol in preclimacteric fruit, but decreased with maturity. Emissions of other alcohols generally remained stable as fruits matured. Ethyl acetate was still present in fruit several weeks preclimacteric, but had all but disappeared from the ester profile by the time ethylene biosynthesis occurred. To a lesser extent, biosynthesis of hexyl acetate and 2-methylbutyl acetate also preceded the climacteric. Emissions of other esters were generally not noted until onset of ripening. In 'Rome' apples, we noted a similar progression in total acetate ester accumulation during ripening. As apples advanced from early to mid- and postclimacteric stages, the concentration of acetate esters progressively increased (Fellman and Mattheis, 1995). In 'Fuji' apples, acetate esters begin to accumulate as apples approach harvest maturity (Fig. 6), with 2-methylbutyl acetate the predominant compound.

Storage atmosphere

While a small percentage of apple fruits is marketed fresh, most are stored to keep fruit available to the market for an extended period of time (Knee, 1993). Storing apples in a low temperature, low oxygen (O_2), and high carbon dioxide (CO_2) environment is now a common practice (Smock, 1979). In general, CA storage delays the onset of biosynthesis of volatile flavor and aroma compounds and depresses the quantity produced (Patterson et al., 1974). Investigations of these effects of CA have centered on O_2 and CO_2 concentrations in the storage atmosphere. In 'Gala' apples the effect of CA storage on aroma

Fig. 6. 'Fuji' apple ester accumulation in relation to harvest maturity. Apples were harvested at 2-week intervals during September and October from a commercial orchard near Vantage, Wash. Internal ethylene concentrations were $<1 \mu\text{L}\cdot\text{L}^{-1}$ until week 5. The 50-g composite samples were juiced and analyzed via purge-and-trap capillary gas chromatography.

production is dramatic (Fig. 7). The concentrations of acetate esters within the fruit were significantly decreased by CA storage treatments, but a sequential increase in oxygen from 1% to 4% monthly seemed to increase the acetate content slightly (Fig. 7).

In a following study, 'Gala' fruit stored under dynamic CA conditions were exposed to ambient air 1, 2, or 3 d per week for 8 h, then returned to 1% O_2 . The dynamic treatments resulted in greater ester emission after 120 d of storage plus 7 d at 20 °C, in comparison with apples stored in static 1% O_2 . Production of the unique 'Gala' flavor note 1-methoxy-(2-propenyl) benzene was also higher after 120 d storage plus 7 d at 20 °C for the dynamic treatment (Mattheis et al., 1998).

When stored at ultra-low O_2 (ULO, 1% or lower O_2) CA conditions, 'McIntosh' apples emitted less ethanol and acetaldehyde (Lidster et al., 1983). This study also showed that 3 d after removal from ULO, fruit could no longer produce ethanol, acetaldehyde, ethyl butyrate, or hexanal. At higher O_2 levels, however, the ability to produce these volatiles was recovered within 3 d of removal from storage. Hexanol, ethyl acetate, and butanol emissions were all reduced in four apple cultivars after storage at ULO (Golias, 1984). However, we showed that ethyl acetate emitted from 'Bisbee Delicious' apples stored at 0.05% O_2 increased from 0 to $26.7 \text{ nL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, while butyl and 2-methylbutyl acetate concentrations decreased when compared to levels emitted from fresh fruit (Mattheis et al. 1991a).

In 'Law Rome' apples (a highly coloring strain) stored in RA or in

Fig. 7. The influence of storage conditions on acetate ester content of 'Gala' apple tissue after 4 months' storage at 1 °C. RA = refrigerated air; CA 1% = 1% oxygen; CA 1-4% = oxygen levels were raised 1% per month for the 4-month storage period.

CA at 0.5% O₂, when headspace volatiles were measured 6 d after removal from storage, total ester concentrations were similar to those of fresh fruit after 3 months, but were reduced by 6 months of CA storage (Fellman and Mattheis, 1995). Ester levels in '262 Rome' apples held under the same storage regimes as above had fallen after 3 months in storage. The ester profile also changed as a result of CA storage. Fresh 'Law Rome' fruit emitted primarily butyl and 2-methylbutyl acetate and fresh '262 Rome' emitted mostly 2-methylbutyl acetate; after 3 and 6 months of CA storage, however, ethyl acetate was the major ester measured in both cultivars 1 d after removal from storage.

Using 'Golden Delicious' at various CA atmospheres, Brackmann et al. (1993) confirmed the results of Strief and Bangerth (1988), showing that emissions of straight-chain esters, primarily butyl and hexyl acetate, were reduced by low O₂ (1%) and high CO₂ (3%). Emissions of branched chain esters, primarily 2-methylbutyl acetate, were suppressed by high CO₂, but not by low O₂. Brackmann et al. (1993) explained these results by relating the different CA conditions to the biosynthetic pathways of straight- and branched-chain esters: 1) straight-chain organic acid precursors are formed from β -oxidation of fatty acids and/or from lipoxygenase activity, both of which are O₂-requiring and are presumably slowed by ULO storage conditions, and 2) branched-chain organic acid precursors are formed from amino acid metabolism, which is presumably slowed by high CO₂ storage conditions, especially given that the tricarboxylic acid cycle produces the majority of amino acid precursors in plant cells and is known to be inhibited by elevated CO₂ concentrations (Frenkel and Patterson, 1973).

Ester synthesis

Alcohol acetyl-CoA transferase (AAT). The volatile profile of apple fruit may be modified by activity of the enzyme alcohol acetyl-CoA transferase. Paillard (1979) described the formation of volatile esters as being a condensation of an alcohol with a carboxylic acid. This reaction is shown CoA-dependent in banana (*Musa acuminata* Colla) (Ueda and Ogata, 1977), and apple (Fellman et al., 1991). Strawberry (*Fragaria xananassa* Duch.) AAT exhibited alcohol substrate specificity in the order hexyl > butyl > amyl > isoamyl >> 3-hexenyl (using acetyl-CoA as co-substrate), and acyl-CoA substrate specificity of acetyl > butyl >> propionyl (using butyl alcohol as co-substrate) (Pérez et al., 1993). This specificity is consistent with the volatile ester profile of strawberry fruit, in which the relative levels are hexyl acetate > butyl acetate > amyl and isoamyl acetate (Pérez et al., 1992). Apple AAT probably exhibits substrate specificity similar to the final products of the reaction; this specificity may vary among cultivars and help account for the differences in ester profiles among them. Activity of AAT appears to increase with the onset of ripening, followed by a subsequent decrease in extractable activity, and the pattern is similar in all apples tested, but the relative amount of AAT is usually cultivar-dependent (Fig. 8). Some apples, e.g., 'Fuji', have less extractable activity than others, e.g., 'Gala'. As mentioned in the previous section, the genome appears to influence the amount of AAT present, which may also reflect the ethylene-related ripening characteristics of the cultivar in question.

We previously investigated the response of apple AAT to RA and CA storage, where 'Law Rome' and '262 Rome' apples were held in RA vs. a CA storage regime with O₂ maintained at 0.5%. After 3 months of RA storage, AAT activity was about the same as that in fresh fruit whether measured 1 or 6 d after removal from storage. After 6 months, AAT activity was substantially reduced, although the activity after 6 d out of storage had recovered to nearly that in fresh fruit. The more highly coloring 'Law Rome' generally had lower AAT activities than did '262 Rome' at most storage durations, particularly 6 d after removal from storage. In CA storage (0.5% O₂), AAT activity of '262 Rome' was maintained at near-fresh levels for 3 months, but was reduced after 6 months of storage. In 'Law Rome', AAT activity was increased from that in fresh fruit after 3 months of storage, and did not change even after 6 months. Activity was much higher 6 d after removal from storage in 'Law Rome' and was slightly higher in '262 Rome'. We concluded that while CA storage beyond 3 months reduced

enzyme activity, AAT is reactivated after a short period out of storage, particularly in those fruit with low enzyme activity prior to storage (Fellman and Mattheis, 1995). In ongoing studies with 'Gala', no reactivation phenomenon was observed. Activity of AAT decreased markedly following 4 months of refrigerated air storage, with complete elimination of measurable activity after 4 months in 1% oxygen CA, even 7 d after removal from storage (Fig. 9). There appeared to be measurable activity in late-harvested apples placed in RA or in variable oxygen CA (where O₂ levels were raised 1% per month) when first removed from storage (Day 1 values), but the activity had fallen 90% or more by day 7 poststorage. This suggests that ester synthesis capacity of 'Gala' is connected to ripening, and inhibition of ripening-related events influences subsequent AAT activity after storage.

Substrate availability and other enzymes

Williams and Knee (1977) were among the first to suggest that a loss in flavor and aroma after storage resulted from an exhaustion of the supply of proper substrates for their biosynthesis. Knee and Hatfield (1981) showed that alcohols supplied as vapors to peel disks

Fig. 8. Alcohol acetyl transferase (AAT) activity in crude extracts of 'Gala' apples harvested over a 3-week period and ripened at 25 °C for 7 d. AAT was assayed via spectrophotometry. Data are from the same fruit used in Figure 5.

Fig. 9. Alcohol acetyl transferase (AAT) activity in extracts from 'Gala' apple fruit after 4 months storage at 1 °C. RA = refrigerated air; CA 1% = 1% oxygen; CA 1-4% = oxygen levels were raised 1% per month for the 4-month storage period. AAT was measured in crude extracts via spectrophotometry at day 1 poststorage and after 7 d at 25 °C.

and intact apples increased the emission of volatile esters. In later experiments (Bartley et al., 1985; Berger and Drawert, 1984; De Pooter et al., 1983) volatile production was increased by supplying apple fruit with exogenous organic acids, aldehydes, and alcohols. De Pooter et al. (1987) studied an observed decrease in aroma in 'Golden Delicious' apples resulting from high CO₂ CA storage conditions. By treating RA- and CA-stored apples with carboxylic acids, they determined that RA apples could produce esters rapidly, but aldehydes, not esters, increased in CA apples. From this, two conclusions were reached: 1) reduced ester synthesis in stored fruit was caused by a reduction in carboxylic acid metabolism, since RA-stored fruit responded to exogenous substrate application; and 2) high CO₂ CA storage reduces alcohol dehydrogenase activity, since these fruit seemed unable to reduce aldehydes to alcohol. Both conclusions point to a lack of adequate substrate for ester biosynthesis as causing a loss of flavor. Strief and Bangerth (1988) showed that after 7 to 9 months of CA storage, 'Golden Delicious' fruit no longer were able to produce volatile esters. In a study of 'Golden Delicious' fruits held under ULO CA conditions, however, fruits were again able to synthesize straight-chain esters if provided the proper precursor organic acids, aldehydes, and alcohols (Brackmann et al., 1993). This result led to the conclusion that suppression of volatile biosynthesis by ULO storage was caused by lack of substrate rather than by enzyme degradation. Our previous experiments also suggest that the loss of volatile esters in 'Rome' apples from 1 to 6 d after removal from cold storage results from lack of precursor alcohols (Fellman and Mattheis, 1995).

Esterase. The balance between acetate esters and their corresponding alcohols in the biosynthesis of volatile flavor and aroma compounds may be regulated by esterase activity. Williams et al. (1977) showed that while n-butyl acetate, n-hexyl acetate, and 2,3-methyl butyl acetate (isomer mix) were the primary esters in 'Cox's Orange Pippin', large amounts of hexanol, as well as butanol, pentanol, and isopentanol were also produced. Goodenough (1983) argued that the high concentration of volatile alcohols in ripe apples could be attributed to hydrolysis of excess acetate esters by esterase activity. In earlier work, Goodenough and Entwistle (1982) had isolated a 195 kd esterase tetramer (each subunit approximately 50 kd) from apple; hexyl and butyl acetate were the substrates most easily bound to its active site. The esterase also increased in activity with maturity, potentially increasing the conversion of substrate in ripening fruit (Goodenough and Riley, 1985). Later work by Knee et al. (1989), however, showed that activity of this esterase remained constant as fruit matured. Williams and Knee (1977) did not consider it likely that hydrolytic enzymes like esterase played a major role in production of volatile components, although Knee and Hatfield (1981) conceded that a proportion of the esters formed in ripening fruit were probably hydrolyzed by esterase activity. The resulting alcohols could then be metabolized further, producing a wide variety of volatile flavor and aroma molecules.

Lipoxygenase. Lipoxygenase may play a major role in determining the composition of volatile compounds in apple. De Pooter and Schamp (1989) noted that the largest volatile ester produced in apple is hexyl hexanoate, characterized by two C₆ units, one on either side of an ester bond. Further, many of the constituents of the volatile profile contain, at a maximum, C₆ units. One would expect some volatiles to consist of longer carbon chains, such as C₈ or C₁₀ units, if the β -oxidation pathway were the primary source of carbon substrate. Metabolism of linoleic or linolenic acid by lipoxygenase in apple peel forms 13-hydroperoxides, which are then cleaved by hydroperoxide lyase, resulting in the production of large quantities of hexanal (Kim and Grosch, 1979; Paillard and Rouri, 1984). Since hexanal is rarely found in intact fruit, further metabolism is indicated. Hexenyl acetate is found in apple volatile profiles, suggesting that some hexanal is used directly for ester biosynthesis (Dimick and Hoskin, 1983). De Pooter and Schamp (1989) suggest that hexanal is oxidized, perhaps by lipoxygenase action, to hexanoic acid, which is then available for synthesis of ethylene (Dumelin and Tappel, 1977), jasmonic acid (Vick and Zimmerman, 1984), or esters. As biosynthesis of volatile compounds is ripening-related, the possible connection between jasmonic acid and ethylene-mediated events during fruit ontogeny is pertinent (Fan, et al. 1998).

CONCLUSIONS

One of the most desirable consequences of ripening is the production of flavor and aroma volatiles that give apples their characteristic flavor and taste. From our continued studies with several apple cultivars, we can offer some general conclusions. Apparently, the influences of genome, growing conditions, harvest maturity and storage regime on compounds that serve as precursors for ester formation are critical factors that determine the ultimate levels of volatile esters in fresh and stored apples. The nature and amount of volatile compounds emitted from apples are cultivar- and strain-dependent. The diversity of the *Malus* genome may account for some of this variability, especially with regard to our discovery of the relationship between peel coloration and volatile production in 'Delicious' apples. Management of existing plantings for a color/flavor balance may be possible. Nitrogen has little or no effect on aroma production, but the effect of tree vigor on subsequent harvest maturity may be an influential factor. So too, the impact of other cultural practices on volatile biosynthesis remains practically unknown. Aroma production is closely linked to the onset of climacteric ripening, thus making harvest decisions critical. Prolonged low-oxygen storage is detrimental to volatile production in apples, yet some cultivars can regenerate the capability for ester biosynthesis. Although storage conditions are known to drastically modify the capacity of apples to produce organic acids, alcohols, and esters, much still remains to be discovered, particularly how this capacity can be maintained or regenerated. Further studies need to be directed at the role of other enzymes, such as lipoxygenase, esterase, and those in the short-chain acyl CoA synthesis pathway, in providing the precursors for subsequent esterification into characteristic aroma moieties. The climacteric ripening phenomenon results in a cascade of biochemical reactions that could result in increased precursor availability, and manipulation of ethylene-related responses may provide a more complete knowledge of the factors involved in the biosynthesis of apple flavor and aroma. Studies such as these are essential for the industry to better manage the ripening process, and to help growers provide a superior product to consumers over a longer period of time.

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