J. Agric. Food Chem. 1992, 40, 2232–2235

Aroma Components and Free Amino Acids in Strawberry Variety Chandler during Ripening

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Aroma components and free amino acids of strawberry *Fragaria ananassa* × *Duchesne* var. Chandler were studied at different ripening stages. Ethyl esters were the major volatile compounds in all ripening stages studied, ethyl butanoate and ethyl hexanoate being the two main esters identified in fully ripe berries. Asparagine, glutamine, and alanine were found to be the most prominent free amino acids in the HPLC profile. A comparison of ethyl ester concentration and alanine contents during Chandler ripening shows that, from 41 to 46 days after blooming, ester biosynthesis increased about 3-fold while alanine levels decreased from 16.7 to 1.6 mg/100 g.

INTRODUCTION

Typical flavor of most fruits, including soft fruits, is not present during early fruit formation but develops during a rather brief ripening period. During this period, metabolism changes to catabolism and volatile compounds are formed from major plants constituents (e.g., carbohydrates, lipids, and proteins) under genetic control.

To understand the aroma of a fruit, it is therefore necessary to know not only the nature of the constituents but how the pattern of the significant constituents changes in kind and quantity during the development of the fruit and also how each constituent arises and is in turn metabolized.

Strawberry aroma is mainly determined by a complex mixture of esters, aldehydes, alcohols, and sulfur compounds which have been extensively studied (McFadden et al., 1965; Drawert et al., 1973; Dirinck et al., 1977, 1981). 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (Sundt et al., 1970; Pickenhagen et al., 1981) and 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (Pyysalo et al., 1979) are considered to be among the most important volatile compounds reported in wild strawberries, although these two compounds have not been found in all cultivated varieties (Douillard and Guichard, 1989). Dirinck et al. (1981) worked on 30 *Fragaria ananassa* cultivars and concluded that esters were qualitatively and quantitatively the most important class of volatiles in strawberry flavor. The main components are those formed from volatile organic acids with an even carbon number such as acetic, butanoic, and hexanoic acid.

The biogenetic pathways of most of the aromatic components described are still not well understood. Among the plant nutrients that can be flavor precursors (Salunkhe and Do, 1976), we have focused our attention on free amino acids. Amino acid metabolism in relation to aroma biogenesis has been studied in fruits such as tomato (Yu et al., 1967) and banana (Myers et al., 1970; Tressl and Drawert, 1973). In strawberry, free amino acid composition could explain the distribution of different types of esters in its aroma.

Fragaria ananassa × *Duchesne* var. Chandler is a California strawberry cultivar, obtained by back-crossing var. Douglas with 55=72.361-105 (U.S. PL Pat. 4481), which has many advantages over Douglas such as firmer fruit and about 15–20% greater yield. Chandler is the strawberry cultivar most extensively cultivated in Spain. Berries from this cultivar have excellent organoleptic properties with strong aroma; however, to the best of our knowledge, no study of the volatile composition in Chandler has been reported. The aim of this work is to study the changes in the volatile components of strawberries (var. Chandler) and their potential precursors during ripening.

EXPERIMENTAL PROCEDURES

Materials. Strawberries var. Chandler were greenhouse grown and harvested at four ripening stages (30, 36, 41, and 46 days after blooming). Fresh fruits were used for chromatographic analysis of volatile compounds (250 g) and for ethanol extract (10 g).

Sampling Technique of Headspace Volatiles. Samples of headspace volatiles were obtained according to a method described by Olías et al. (1991). Strawberries were placed in desiccators (±5 L) housed within a thermostated water bath (25 °C). The vessel was continuously flushed with nitrogen (99.9% pure) (333 mL/min). For sampling, a standard charcoal tube (ORBO-32, Supelco) was attached to the outlet of the desiccator (sample time 4 h). Extraction of the trapped headspace volatiles was carried out with carbon disulfide (0.3 mL).

Gas Chromatography. The volatiles were separated in a HP5890 gas chromatograph equipped with a flame ionization detector, a programmable cool on-column inlet, and a 60 m × 0.25 mm i.d. fused silica capillary column SPB-1. Operating conditions were as follows: N_2 carrier gas, 30 cm s^{-1}; detector, 250 °C; injection volume, 0.5 μL. The column was held for 15 min at 40 °C and then programmed at 2 °C/min to 160 °C; inlet temperature followed oven temperature. Identification of compounds was made by means of gas chromatography–mass spectrometry using a MS-30/70-VG. Structures were assigned by comparison of the spectra with those of authentic standards. Quantitative determinations were obtained using methyl octanoate as external standard. Known quantities of this compound were added to carbon disulfide before extraction of the volatiles. Peak areas were expressed as nanograms of volatile per gram of strawberry per 80 L of headspace.

Ethanol Extract. Fruit sectors from 10 strawberries were blended with 20 mL of 95% ethanol. The residue was washed three times with 80% ethanol. Combined extracts were brought to a 50 mL final volume with 80% ethanol. Aliquots of this extract were used for the amino acid analysis.

Amino Acids Analysis. Ten milliliters of the ethanol extract was evaporated at 60 °C in an air-draft oven to remove ethanol. The aliquot was passed through a Dowex 50-X4 (H^+ form) to trap free amino acids. The column was eluted with 20 mL of 2 N NH_4OH. Eluent was evaporated, and the residues were dissolved in 1 mL of 0.1 M sodium bicarbonate buffer, pH 9. Amino acid derivatives were prepared according to a method

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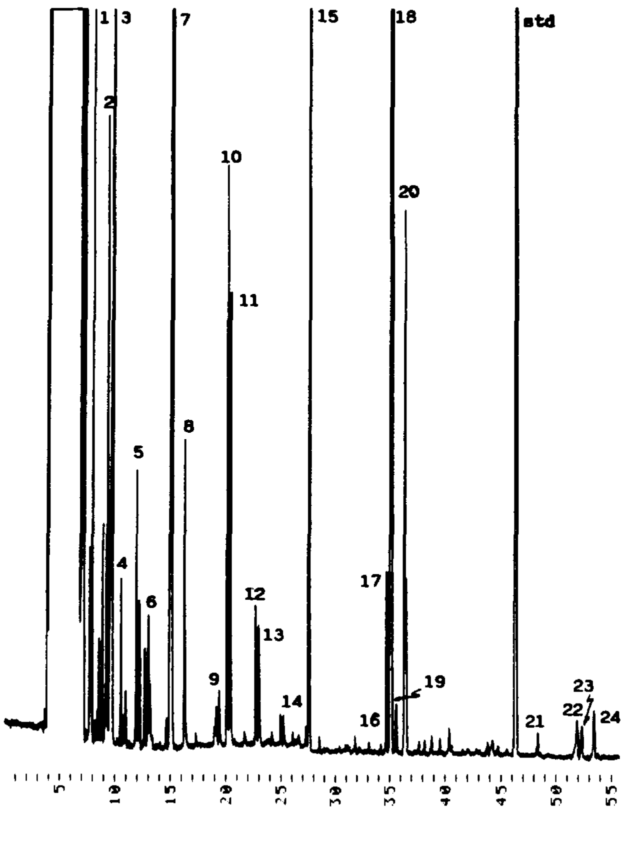

Figure 1. Typical gas chromatogram of strawberry var. Chandler volatiles collected by dynamic headspace.

described by Chang et al. (1981) with slight modifications. To 200 μL of amino acid solution was added 100 μL of 4-(dimethylamino)azobenzene-4'-sulfonyl chloride (DABS-Cl) solution. The mixture was heated at 70 °C for 12 min and dried, and the residue was dissolved in 1 mL of 70% (v/v) ethanol. Amino acid derivatives were then chromatographed into a high-performance liquid chromatograph (HPLC) HP 1090 equipped with a ODS 5-μm (2 mm × 250 mm) column and detection at 460 nm (Stocchi et al., 1985). The mobile phase utilized for the separation of DABS–amino acids consisted of two eluents: 25 mM potassium dihydrogen phosphate, pH 6.8 (solvent A), and acetonitrile/2-propanol (80:20) (solvent B). A 5-μL portion of the derivative was injected into the HPLC at constant flow of 0.5 mL/min. After 22 min of isocratic flow at 22% B, a linear gradient was begun that reached 34% B at 48 min, followed by a second gradient at 1% B/min to 84 min.

RESULTS AND DISCUSSION

A typical gas chromatogram of the dynamic headspace of the strawberry variety Chandler used in this work is depicted in Figure 1. The peak numbering is related to identification in Table I. Peaks 1, 16, and 21–24 are not included in further discussions but were identified as ethyl acetate, isopropyl pentanoate, hexyl butanoate, butyl hexanoate, ethyl octanoate, and octyl acetate, respectively. Dynamic headspace analysis allows the study of representative samples of primary aroma components (Dirinck et al., 1984), but with this technique it is possible to determine only the readily volatile compounds. Using this concentration method and working with intact fruits, we did not detect 2,5-dimethyl-4-methoxy-3-(2*H*)-furanone or the corresponding hydroxy compound. These compounds are, however, somewhat unstable (Hirvi et al., 1980), and different growth conditions (Hirvi and Honkanen, 1982) or cultivars (Douillard and Guichard, 1990) may cause variations in its concentration. Similarly, the only sulfur compound which was determined in Chandler was dimethyl disulfide (the FID detector is not very sensitive). Relative amounts of flavor components in the dynamic headspace of strawberries var. Chandler in four ripening stages are presented in Table I. Sulfur compounds are quantitatively minor constituents of fruit flavor, but because of their extremely low threshold values, they have to be taken into account in some strawberry cultivars, especially in "older" varieties (Dirinck et al., 1981).

Yamashita et al. (1977) reported that formation of esters in strawberry was found only at mature stages because of the lack of ester-forming enzyme activity at immature stages. As Table I shows, only when fruits reached a certain maturity stage, 36–41 days after blooming, was volatile biosynthesis enhanced. Although 30-day-old fruits did not have a significant aroma, they were included in Table I. We found that there were qualitative as well as quantitative differences. In 36-day-old fruits, esters with C_6 alcohols represented 25%, while in 46-day-old fruits these esters were only 4.8%. There was even a slight decrease in total volatile compounds from 36 to 41 days which was mainly caused by less production of 3-hexenyl acetate and hexyl acetate. This would explain why immature strawberries have a green odor very different from the rich and pleasant flavor of a fully ripe one. There

Table I. Volatile Compounds Identified in Strawberry Variety Chandler at Four Different Ripening Stages[a]

volatile compound (peak no.)	ripening stage: 30 DAB[b]	36 DAB	41 DAB	46 DAB
ethyl propionate (2)		9.9	7.7	30.2
methyl butanoate (3)	2.9	61.2	54.4	251.4
dimethyl disulfide (4)			1.0	8.1
isopropyl propanoate (5)		6.4	4.9	16.1
methyl 2-methylbutanoate (6)		4.0	5.2	9.9
ethyl butanoate (7)	18.1	81.9	88.4	317.2
butyl acetate (8)		9.4	9.7	26.2
isopropyl butanoate (9)				5.4
ethyl 2-methylbutanoate (10)	3.2	29.2	15.8	48.8
ethyl isopentanoate (11)		23.0	21.9	41.0
amyl acetate (12)		13.1	5.7	13.0
isoamyl acetate (13)		17.7	4.7	15.3
propyl propanoate (14)				3.3
methyl hexanoate (15)		30.5	32.6	116.8
sec-butyl butanoate (17)			2.0	17.7
ethyl hexanoate (18)	18.7	82.0	110.2	392.8
3-hexenyl acetate (19)		13.5	9.0	4.6
hexyl acetate (20)	7.5	107.4	26.8	61.5
sum	50.5	489.2	400.0	1379.4

[a] Relative amounts are expressed as nanograms per gram of fresh weight per 80 L. [b] DAB, days after blooming.

Table II. Free Amino Acids in Strawberry Variety Chandler during Ripening (Milligrams per 100 g of Fresh Weight)

amino acid	ripening stage: 30 DAB[a]	36 DAB	41 DAB	46 DAB
Asp	4.0	1.2	0.7	0.8
Glu	11.4	7.3	2.3	3.6
Asn	52.4	47.8	47.4	30.6
Gln	13.0	7.4	23.6	10.4
Ser	3.0	2.4	5.6	2.0
Ala	9.7	18.2	16.7	1.6
Pro	4.3	4.0	3.7	3.0
Val	2.0	1.3	1.5	1.0
Trp	2.2	1.7	*b*	*b*
His	2.4	*b*	1.5	1.4

[a] DAB, days after blooming. [b] Values lower than 0.25 mg/100 g of fruit.

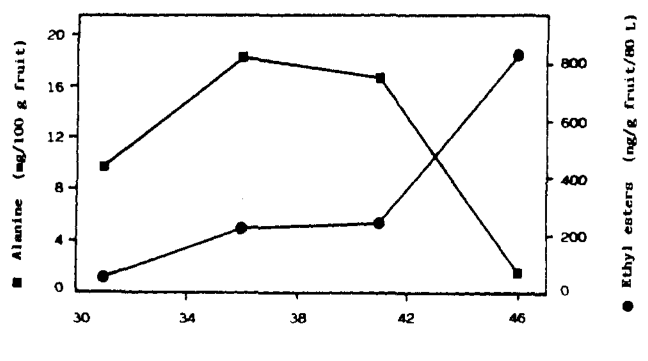

Figure 2. Free alanine and ethyl ester levels in strawberry var. Chandler during ripening.

were a few compounds, dimethyl disulfide, isopropyl butanoate, propyl butanoate, and *sec*-butyl butanoate, that were not found in the dynamic headspace of Chandler in the early stage harvested, isopropyl butanoate and propyl propanoate were only detected in fully ripe strawberry.

Ethyl esters were the major volatile components in all ripening stages studied and reached 60% in 41- and 46-day-old strawberries. The main esters identified in mature berries were ethyl butanoate and ethyl hexanoate. The fruits also contained high concentrations of methyl esters (ca. 25% at 46 days after blooming). In view of these results, ethyl and methyl esters could be considered the most significant components of strawberry aroma. Biosynthesis of both esters sharply increased when Chandler reached the optimum ripeness stage. The ratio of methyl/ethyl esters depends on the variety. Gorella, Jesco, Senge Gigana, and Sivetta have a volatile composition in which methyl esters represent 60–70% (Dirinck et al., 1981). Confitura, also studied by the same author, has higher levels of ethyl esters (ca. 50%) and lower levels of methyl esters (ca. 25%), similar to Chandler. Senga Sengana and Senga Litessa (Schreier, 1980) also have ethyl esters as main aroma components.

The current goal of aroma analysis is not only to identify the components but also to determine their importance to the flavor of the product under investigation. Using a gas chromatograph adapted with a sniffing detector (unpublished results), we found ethyl butanoate, ethyl 2-methylbutanoate, and ethyl hexanoate to be the major contributors to Chandler aroma, although the possibility of an underlying minor component strongly influencing the sensory results can never be ruled out. Data obtained agree with those reported in the literature for other cultivars. Dirinck et al. (1977) included ethyl butanoate and ethyl hexanoate among the organoleptically important compounds of var. Gorella. In a study of two new varieties, Annelie and Alaska Pioneer (Hirvi and Honkanen, 1982), obtained by back-crossing of cultivated strawberries with wild strawberries, the most important volatile compounds liberated while the fruit was macerated were found to be ethyl hexanoate, ethyl butanoate, 2,5-dimethyl-4-methoxy-3-(2*H*)-furanone, and *trans*-2-hexenal. Recently, ethyl butanoate, ethyl 3-methylbutanoate, and ethyl hexanoate were shown to be the most directly responsible for the strawberry-like aroma of *Pseudomonas fragi* (Cormier et al., 1991). However, a mixture of only these three compounds resulted in an artificial candy-like aroma. Results obtained suggest that ethyl butanoate and ethyl hexanoate are two of the most important volatiles in Chandler aroma for their abundance, observed odor impression, and low threshold, 0.001 and 0.003 ppm, (Pyysalo et al., 1979; Flath et al., 1967).

Amino acid metabolism generates aliphatic and branched-chain alcohols, acids, carbonyls, and esters. The earliest work was that of Hultin and Proctor (1961), who demonstrated that aroma production in banana puree was increased by the addition of valine. Conversion of alanine, leucine, and valine to volatile products by tomato enzyme extracts has also been described (Yu et al., 1968). Soft fruit are not rich sources of nitrogenous substances; total amino acid content is considerably lower than those of banana, peach, or orange (Drawert et al., 1970; Kuneman et al., 1988). The free amino acid profile of strawberry var. Chandler in four ripening stages is shown in Table II. Ten amino acids could be quantitatively determined, while threonine, glycine, leucine, isoleucine, phenylalanine, and lysine were found to be present in lower levels, less than 0.25 mg/100 fruit. Most of the common amino acids in fruits were present (Borroughs, 1970), asparagine, glutamine, and alanine being the three most prominent. Asparagine and glutamine are the major N-transport compounds found in plants and consequently in fruits (Atkins et al., 1975). In the ripening stages studied, asparagine represented about 50% of total amino acid content; in fact, strawberry might be distinguishable from other soft fruits by examining asparagine values (Kuneman et al., 1988).

There seem to be few studies on the changes of free amino acids in ripening fruits. Valine, leucine, and isoleucine have been studied as flavor precursors in banana tissue (Tressl and Drawert, 1973; Drawert, 1975; Drawert and Berger, 1981). Radioactive labeling studies have proved that these amino acids are transformed into branched-chain alcohols, 2-propanol, 3-methylbutanol, and 2-methylbutanol, respectively. The scheme of biosynthesis starts with an initial deamination of the amino acid followed by decarboxylation and various reductions and esterifications which lead to a great number of volatiles (Heath, 1986). Tressl and Drawert (1973) have shown that leucine and valine concentrations increase about 3-fold following the climateric rise in banana. Leucine was detected only in trace amounts in Chandler. Yamashita et al. (1975) found that leucine in strawberry was 0.4 mg/100 g; leucine in banana was 16.8 mg/100 g. These differences are responsible for the different content of isoamyl acetate in these fruit aromas. Isoamyl acetate was detected as a minor component in the dynamic headspace of Chandler (Table I); the low levels found in all ripening stages studied could be explained by the low amounts of free leucine available in the fruit. Phenylalanine also increases during ripening of banana. In our studies of strawberries there were not significative amounts of this amino acid the corresponding volatile compounds, phenolic alcohols and esters, which are supposed to be derived therefrom. Alanine was the amino acid that exhibited more significative changes during strawberry ripening. Alanine content sharply increased in fruits from 30 to 36 days, remained almost constant until 41 days, and decreased in the fully ripe stage. Slices from ripe strawberry fruit metabolize L-alanine, pyruvate, and 2-ketovalerate among other potential exogenous precursors of volatile compounds (Drawert and Berger, 1982). Feeding alanine and addition of ascorbic acid to strawberry segment cultures enhanced the formation of methyl hexanoate, ethyl hexanoate, ethyl butyrate, and ethyl decanoate (Drawert and Berger, 1981). The same authors concluded that ester biosynthesis was directly proportional to the concentration of alanine up to 3 mmol/L. A comparison of ethyl ester concentrations during Chandler ripening and alanine concentration at each maturity stage shows that ester biosynthesis increased when alanine reached its highest level. From 41 to 46 days berries developed maximum aroma, mainly formed by ethyl esters, while the free alanine level decreased from 16.7 to 1.6 mg/100 g (Figure 2). Changes in free amino acid content may reflect differences in volatile composition. Although amino acid composition could be influenced by several factors, such as deficiencies of particular mineral elements (Hewitt and Smith, 1975), differences in amino acid profile among varieties could explain different aroma patterns. Data obtained in this work allow us to suggest that high alanine content in strawberry var. Chandler could be primarily responsible for high ethyl ester levels in the volatile composition of this strawberry cultivar.

ACKNOWLEDGMENT

This work was supported by Proyecto de Investigación ALI88GE8371 from Plan Nacional de Ciencia y Tecnología de Alimentos. A.G.P. and L.C.S. are recipients of grants F.P.I. from Junta de Andalucía. We are indebted to Dr. D. G. Richardson for a critical reading of the manuscript.

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Received for review December 26, 1991. Revised manuscript received May 20, 1992. Accepted August 13, 1992.