Biochemical Changes during Fruit Development of Four Strawberry Cultivars

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ABSTRACT. As genetic factors affect strawberry (*Fragaria ×ananassa* Duch.) fruit development and quality, changes in metabolite concentrations were studied during fruit development of four strawberry cultivars grown in the field: three commercial cultivars (Capitola, Elsanta and Dover) and a genotype from Centre Interrégional de Recherche et d'Expérimentation de la Fraise, France ('CF1116'). Major and minor metabolites changed with development. The two strawberry cultivars with the highest starch content at early stages, 'Capitola' and 'Elsanta', also had the highest fruit weight at harvest. There was no correlation between strawberry weight and osmolarity. At maturity, significant differences were observed among cultivars for most of the metabolites studied. 'Capitola' and 'Elsanta' responded similarly for most measured variables. 'CF1116' was characterized by high juice osmolarity and high sucrose, inositol, glutamine, arginine and alanine concentrations, and low citrate and malate concentrations. 'Dover' was characterized by a high galactose concentration and low asparagine and alanine concentrations. Organic acid differences among cultivars appeared early during development, while differences in soluble sugars appeared during maturation. The developmental pattern of each amino acid varied among cultivars. Timing of the biochemical differences observed among cultivars provides some information on their metabolic origin.

Numerous breeding programs have been aimed at improving strawberry (*Fragaria ×ananassa*) taste and disease resistance (Hancock, 1999; Rosati, 1993). Three major components of fruit organoleptic quality are flavor, sweetness, and acidity. Several studies have been devoted to strawberry aroma (Hancock, 1999; Perkins-Veazie, 1995) and recently, a gene involved in strawberry flavor biogenesis was identified using DNA microarrays (Aharoni et al., 2000). Fruit with intense flavor also have high titratable acidity and high soluble solids (Kader, 1991). Numerous studies have addressed strawberry sweetness and acidity. Fruit soluble solids, sugars, titratable acidity, and organic acids at maturity are quantitatively inherited (Shaw, 1988; Shaw et al., 1987). Moreover, there appears to be genetic variability for these fruit quality traits (Ogiwara et al., 1998a; Shaw, 1988), thus making genetic gain possible.

Numerous biochemical changes are observed during strawberry development and especially during fruit ripening (Manning, 1994; Manning, 1998a). The major soluble constituents of

maturing and ripe strawberries are soluble sugars and organic acids (Hancock, 1999; Perkins-Veazie, 1995). The major soluble sugars in strawberries are glucose [1.4% to 3.1% fresh weight (FW)], fructose (1.7% to 3.5% FW), and sucrose (0.2% to 2.5% FW) (Perkins-Veazie, 1995). Glucose and fructose concentrations increase continuously during fruit development, while sucrose accumulates mostly during maturation (Hancock, 1999). The major organic acid is citrate the concentration of which ranges from 4 to 12 mg·g⁻¹ FW (Perkins-Veazie, 1995). This acid contributes greatly to fruit titratable acidity, which declines gradually during fruit development (Hancock, 1999). The sugar/organic acid ratio is a major parameter of strawberry taste (Perkins-Veazie, 1995).

Of the other soluble constituents of strawberries, amino acids may also directly affect fruit taste, as was shown by the sensory evaluation of another fleshy fruit, peach [*Prunus persica* (L.) Batsch] (Jia et al., 2000). Moreover, some amino acids are flavor precursors (Heath and Reineccius, 1986). The major amino acids in strawberries are asparagine, glutamine, and alanine (Perez et al., 1992). Anthocyanins (0.5 to 1.5 mg·g⁻¹ FW) are a major component of the fruit, while ascorbic acid (0.3 to 1.2 mg·g⁻¹ FW) makes an important contribution to the fruit nutritional value. Among the insoluble constituents, starch is present in young fruit and disappears before ripening (Knee et al., 1977).

While the major constituents of strawberries during maturation are well known, far fewer studies have been conducted on the minor constituents and earlier stages of development. The molecular events characterizing strawberry maturation are begin-

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ning to be elucidated (Manning, 1998b), but those concerning earlier stages of development have been less well studied. The objective of the present investigation was to compare four strawberry cultivars during fruit development for starch content, major and minor soluble sugars, and organic and amino acids. Anthocyanin levels were also estimated at maturity. Determination of the timing and nature of the biochemical differences observed among cultivars should provide information on the metabolic origin of these differences.

Materials and Methods

PLANT MATERIALS. Four strawberry genotypes were cultivated in the field at Centre Interrégional de Recherche et d'Expérimentation de la Fraise (CIREF) Prignonrieux near Bergerac, France: 'Dover' and 'Capitola' two American cultivars, 'Elsanta' a European cultivar and 'CF1116' ['Pajaro' x 'CF129' ('Earliglow' x 'Chandler')], a genotype from CIREF, France. Twenty cold-stored plants per cultivar were planted (0.4 x 0.3 m) in a sandy-loam soil in early July 1999. A plastic tunnel (0.7 m height x 0.8 m width) was installed in mid-February 2000. Standard fertilization and pest control measures for strawberry plants were applied. Twenty-five primary or secondary fruit (one or two fruit per plant), were collected at four stages of development: petal fall, 10 d after full bloom (DAB), turning (32 to 45 DAB depending on the cultivar) and maturity. The petal fall stage corresponded to the beginning of petal fall. The 10 DAB stage was determined by tagging primary and secondary flowers at anthesis and harvesting tagged young fruit 10 d later. The turning stage corresponded to the change between white and pink, with pink covering $\approx 1/8$ of the fruit surface (onset of maturation). Maturity corresponded to fully red fruit as for commercial picking. Since 'Capitola' is an everbearing cultivar, fruit were collected from its first harvest in the spring. The fruit were transported on ice from the field to the laboratory and stored for <5 h at 4 °C until analyses.

FWs of 25 individual fruit per stage were recorded and then five replications, each consisting of five fruit, were randomly selected for measurement of other variables. At the petal fall stage, each fruit (receptacle + achenes) was divided vertically into two equal parts: half was used for dry weight (DW) determination after drying at 60 °C until constant weight, and the other half was used for metabolite extraction. At the other stages, one-fourth of the fruit (receptacle + achenes) was used for DW determination, another fourth of the fruit was used for extraction of sugars, organic acids and amino acids, and the remaining half of the fruit was used for juice collection.

JUICE OSMOLARITY, pH, AND ACIDITY MEASUREMENTS. Fruit juice was analyzed at 10 DAB, at the turning stage, and at maturity. Five replications of five half-fruits were frozen in liquid nitrogen in a 5 or 10 mL syringe containing a small paper filter at the needle end. After thawing at 4 °C, the juice was extracted by hand pressing and stored on ice until analysis. Two hundred microliters of the juice was used for pH measurement with a LPH-330T pH meter (Tacussel, Villeurbanne, France) equipped with a micro-electrode. Titratable acidity was determined on 75 or 100 μ L of juice diluted with distilled water (1 juice : 5 water, v/v), using microtitration with NaOH at 0.1 mol·L⁻¹ to a pH of 7. Osmolarity was measured on 50 μ L of juice, diluted with pure water when necessary, using a micro-osmometer (Roebeling, Berlin). Osmolarity was converted to solute potential (25 °C) using 2.48 MPa per Osmol/kg (Pomper and Breen, 1995). To estimate solute

contribution to juice osmolarity at maturity, we used solute concentrations measured after extraction of fruit portions (see below) and the fruit water concentration.

ANTHOCYANINS. Total anthocyanins were determined at maturity. One gram FW of fruit obtained from portions of five fruit ground in liquid nitrogen, was extracted twice with 2 mL of 98% ethanol and total anthocyanins were estimated with a spectrophotometer at 517 nm after an 11 time dilution with ethanol containing 1.11% HCl (Sanz et al., 1999). The mean anthocyanin concentration of five replications was expressed as absorbance units/g DW.

SUGAR, ORGANIC, AND AMINO ACID ANALYSIS. Samples obtained from five fruit, were ground in liquid nitrogen. The entire sample (when the sample weighed <1 g) at petal fall, or 1 g FW at the other stages of development, was extracted for 20 min with 4 mL 8 ethanol : 2 water (v/v) at 80 °C. The mixture was centrifuged (3800 g_n for 10 min), and the pellet extracted again with 3 mL 1 ethanol : 1 water (v/v) at 80 °C. After a second centrifugation, the two supernatants were combined and the pellet was extracted again with 1 mL water at 80 °C. After a third centrifugation, the three supernatants were combined, dried under vacuum, and used for analysis of soluble sugars, organic acids, and free amino acids. The pellet was used for determination of starch.

Soluble sugars were purified using ion exchange resins (Moing et al., 1992) and analyzed by high-performance liquid chromatography (HPLC). Sucrose, fructose + inositol, and glucose were analyzed using a Ca column (PL Hi-Plex; Polymer Laboratories Ltd., Shropshire, United Kingdom) flushed with 0.5 mL·min⁻¹ water at 80 °C with a refractive index detector. This column does not allow separation of inositol from fructose. Therefore, inositol along with galactose, xylose, and arabinose were analyzed using anion exchange chromatography and pulsed amperometric detection (Moing et al., 1997). The fructose concentration was calculated as the difference between fructose + inositol obtained using the refractive HPLC method and inositol obtained using the amperometric HPLC method. The sum of soluble sugars refers to the sum of glucose, fructose, sucrose, inositol, galactose, xylose, and arabinose concentrations. After hydrolysis with amylo-glucosidase (Moing et al., 1992), starch was analyzed enzymatically (Kunst et al., 1984) using 96-well microplates and read at 340 nm with a microplate reader (MR 5000; Dynatech, St Cloud, France).

Organic acids were analyzed without sample purification, using anion exchange HPLC with conductivity detection (Moing et al., 1998). The sum of organic acids refers to the sum of citrate, malate, quinate, acetate, oxalate, succinate, shikimate, isocitrate, fumarate, and aconitate concentrations. Oxalate levels probably represent both soluble oxalate and ascorbate, since ascorbate was degraded to oxalate under our alkaline HPLC conditions.

Free amino acids were analyzed by HPLC, without sample purification, using the AccQ.Tag method from Waters, Milford, Mass., with fluorescence detection (Moing et al., 1998). Total amino acids is the sum of aspartic acid, glutamic acid, serine, asparagine, glycine, glutamine, histidine, threonine, arginine, alanine, γ -aminobutyric acid, proline, tyrosine, valine, methionine, isoleucine, leucine, lysine, and phenylalanine. The other amino acids were found as traces or were not detected.

Soluble sugar, organic acid, and amino acid quantification was carried out using Millenium software from Waters, Milford, Mass. HPLC peaks were identified by cochromatography with known standards for a few samples of each stage of development. The peak areas were calculated, and calibration was carried out using external standards of known quantities of sugar, organic, or amino acid from Sigma, St Quentin Fallavier, France.

SENSORY EVALUATION. A panel of 14 untrained individuals classified the four cultivars for preference, fragrance, aroma, sweetness, and acidity. For this evaluation, mature fruit from all cultivars were harvested on the morning of the same day in mid-May, transported to the laboratory on ice, and then allowed to equilibrate to room temperature just before tasting in the afternoon. Particular care was taken to eliminate overripe fruit at harvest. The cultivars were classified using a four-point hedonic scale (1 = high, 4 = low).

DATA ANALYSIS. Data on fruit weight ($n = 25$) and analysis ($n = 5$) are presented as means $\pm$ SD. Comparisons between cultivars were performed using Tukey's studentized range test at $P < 0.05$. For sensory evaluation, data are presented as means but comparisons were based on Friedman rank sums.

Results

FRUIT GROWTH, STARCH CONTENT, AND JUICE OSMOLARITY. The duration of fruit development between petal fall and maturity was 39 d for 'CF1116' and 'Elsanta', 42 d for 'Dover', and 49 d for 'Capitola'. Fruit FW increased by a factor of between 100 and 150 from petal fall to maturity (Fig. 1). At maturity, 'Capitola' had the highest FW, and 'CF1116' and 'Dover' the lowest. DW percentage was between 20% and 17% FW at petal fall and 10 DAB and decreased to 8% FW during maturation (data not presented).

Starch concentrations decreased between early stages of development and maturation (Fig. 2A). At petal fall, 'Elsanta' displayed the highest starch concentration and 'Dover' the lowest. At 10 DAB, starch concentrations in 'Capitola' and 'Elsanta' were significantly higher than those of the other cultivars. During maturation, 'CF1116' exhibited the highest starch concentration but differences among cultivars were less significant at maturity than at the turning stage.

Juice osmolality in all the genotypes (Fig. 2B) reached at least 400 mOsmol/kg at 10 DAB. Between 10 DAB and the turning stage, it increased in 'CF1116' and 'Dover' and decreased in 'Capitola' and 'Elsanta'. The highest value, 900 mOsmol/kg, was observed at the turning stage in 'Dover'. At maturity, juice osmolality of 'CF1116' was significantly higher than that of the other cultivars.

Fig. 1. Fruit fresh weight at four stages of fruit development for 'Capitola', 'CF1116', 'Dover', and 'Elsanta' strawberries. Vertical bars represent ($n = 25$). Mean separation for each growth stage by Tukey's studentized range test, $P < 0.05$.

ANTHOCYANIN CONCENTRATION. Total anthocyanin concentration at maturity was significantly higher in 'Capitola' (391 ± 36 absorbance units/g DW) and 'Dover' (389 ± 43 absorbance units/g DW) than in 'CF1116' (197 ± 43 absorbance units/g DW) and 'Elsanta' (236 ± 47 absorbance units/g DW) (mean $\pm$ SD of five replications, Tukey's studentized range test, $P < 0.05$).

SOLUBLE SUGAR CONCENTRATION. Concentrations of total soluble sugars (Fig. 3A) changed little between petal fall and 10 DAB, and then increased until maturity. Few differences in total soluble sugars were observed among cultivars except for 'CF1116' which had the highest concentration at 10 DAB and the turning stage.

The major soluble sugars, based on $\text{mg} \cdot \text{g}^{-1}$ DW, were glucose, fructose, and sucrose (Fig. 3B–D). The minor soluble sugars included inositol, xylose, galactose (Fig. 3E–G) and arabinose (data not presented). Concentrations of the major sugars increased with fruit development, whereas those of the minor sugars decreased.

At petal fall, glucose concentrations (Fig. 3B) in 'Capitola' and 'CF1116' were significantly higher than those in 'Dover' and 'Elsanta'. At the 10 DAB and turning stages, the glucose concentration in 'CF1116' was significantly higher than those of the other cultivars. At maturity, differences among cultivars decreased or became nonsignificant. No significant differences in fructose concentrations (Fig. 3C) were observed among cultivars at any stage but 10 DAB when 'CF1116' had a concentration significantly higher than the other cultivars, and at the turning

Fig. 2. (A) Starch concentration and (B) juice osmolality at four stages of fruit development for 'Capitola', 'CF1116', 'Dover', and 'Elsanta' strawberries. Vertical bars represent SD ($n = 5$). Mean separation for each growth stage by Tukey's studentized range test, $P < 0.05$.

Fig. 3. Soluble sugar concentrations at four stages of fruit development for 'Capitola', 'CF1116', 'Dover', and 'Elsanta' strawberries. Vertical bars represent SD (n = 5). Mean separation for each growth stage by Tukey's studentized range test, $P < 0.05$. (A) Total soluble sugars, (B) glucose, (C) fructose, (D) sucrose, (E) inositol, (F) xylose, and (G) galactose.

stage when 'Elsanta' had a fructose concentration significantly lower than the other cultivars. There were no significant differences in sucrose concentrations (Fig. 3D) among cultivars at petal fall. At 10 DAB, 'Capitola' and 'CF1116' had sucrose concentrations significantly higher than 'Dover' and 'Elsanta'. At the turning and maturity stages, the sucrose concentration in 'CF1116' was significantly higher than that in the other cultivars.

Inositol concentrations in 'CF1116' were significantly higher than those in the other cultivars at the petal fall, turning, and maturity stages (Fig. 3E). Few significant differences among cultivars were observed for xylose (Fig. 3F) or galactose (Fig. 3G) concentrations at petal fall and 10 DAB. At the turning and maturity stages, the xylose concentrations in 'Dover' and 'Elsanta' were significantly higher than those in 'Capitola' and 'CF1116',

and the galactose concentration in 'Dover' was significantly higher than those in the other cultivars. Arabinose concentrations (data not presented) were lower than $0.05 \text{ mg}\cdot\text{g}^{-1} \text{ DW}$ in all cultivars at all stages of development. We verified that these minor sugars were present in the receptacle, and not just in the receptacle + achenes, by analyzing some samples of fruit juice (data not presented). Sorbitol was not detected in extracts or juice from any cultivar at any stage, but the other minor sugars were also found in juice.

TITRATABLE ACIDITY AND ORGANIC ACID CONCENTRATION. Juice titratable acidity (Fig. 4A) increased from 10 DAB to the turning stage and then decreased until maturity. Few significant differences were observed among cultivars except at the turning stage, when juice titratable acidity for 'Dover' was significantly higher

Fig. 4. Juice titratable acidity and fruit organic acid concentrations, on a DW basis, at four stages of fruit development for 'Capitola', 'CF1116', 'Dover', and 'Elsanta' strawberries. Vertical bars represent SD (n = 5). Mean separation for each growth stage by Tukey's Studentized range test, $P < 0.05$. (A) Juice titratable acidity (TA), (B) total organic acids, (C) citrate, (D) malate, and (E) quinate.

Fig. 5. Concentration of the six major amino acids and their sum, on a DW basis, at four stages of fruit development for 'Capitola', 'CF1116', 'Dover' and 'Elsanta' strawberries. Vertical bars represent SD ($n = 5$). Mean separation for each growth stage by Tukey's studentized range test, $P < 0.05$. (A) Total amino acids, (B) asparagine, (C) glutamine, (D) arginine, (E) glutamate, (F) alanine, and (G) aspartate.

Table 1. Contribution of solutes to juice osmolarity at strawberry maturity. The solute concentrations measured after extraction were used to estimate their contribution (%) to osmolarity measured in juice expressed from the fruit (from Fig. 2). Values are means $\pm$ SD of five replications.

Cultivar	Osmolarity (mOsmol/kg)	Soluble sugars (%)	Organic acids (%)	Amino acids (%)	Sum (%)
Capitola	456	57.4 $\pm$ 5.4	13.5 $\pm$ 1.4	2.6 $\pm$ 0.6	73.5 $\pm$ 7.1
CF1116	758	56.8 $\pm$ 7.5	7.9 $\pm$ 1.2	2.9 $\pm$ 0.2	67.7 $\pm$ 8.7
Dover	525	48.3 $\pm$ 6.3	12.4 $\pm$ 2.0	1.5 $\pm$ 0.2	62.4 $\pm$ 8.0
Elsanta	607	43.2 $\pm$ 5.9	10.0 $\pm$ 0.6	2.5 $\pm$ 0.4	55.7 $\pm$ 6.3

Table 2. Sensory evaluation means at strawberry maturity. Fourteen untrained individuals classified the cultivars using a four-point hedonic scale (1 = high, 4 = low); n = 14.

Cultivar	Preference	Fragrance	Aroma	Sweetness	Acidity
'Capitola'	3.4 b ^z	3.3 b	3.4 b	3.1 b	2.0 ab
'CF1116'	1.5 a	2.4 ab	1.3 a	1.3 a	3.1 b
'Dover'	2.7 ab	2.6 ab	2.7 b	2.6 b	3.1 b
'Elsanta'	2.4 ab	1.8 a	2.6 b	3.0 b	1.8 a

^zMean separation within columns based on Friedman rank sums, $P < 0.05$.

than that in the other cultivars. No significant differences among cultivars were observed for juice pH which, in all cultivars, was close to pH 5 at 10 DAB, decreased to pH 3.7 at the turning stage and remained unchanged at maturity (data not presented).

Total organic acid concentration (Fig. 4B) increased by a factor of 4 or 5 between 10 DAB and the turning stage, and then decreased until maturity in all cultivars. At petal fall, total organic acid concentration in 'Dover' was significantly higher than that of the other cultivars. At 10 DAB, the turning stage, and maturity, total organic acid concentration in 'CF1116' was significantly lower than that in other cultivars.

The three major organic acids were citrate, malate, and quinate (Fig. 4C–E). The minor organic acids detected were acetate, oxalate, succinate, shikimate, isocitrate, fumarate, and aconitate (data not presented).

Citrate concentration (Fig. 4C) increased markedly between 10 DAB and the turning stage in all cultivars. During the four stages of development, the citrate concentration in 'CF1116' was significantly lower than that in other cultivars. The malate concentration (Fig. 4D) was low at petal fall and 10 DAB. It increased at the turning stage and then decreased to maturity in all cultivars. From 10 DAB to maturity, the malate concentration in 'CF1116' was significantly lower than that in other cultivars. The quinate concentration was high at petal fall, decreased at 10 DAB and at the turning stage and then remained stable until maturity in all cultivars (Fig. 4E). The quinate concentrations of 'Elsanta' and 'CF1116', were significantly lower than that of the other cultivars at petal fall and 10 DAB, respectively. No significant differences were observed among cultivars in terms of their quinate concentration at the turning stage and maturity.

AMINO ACID CONCENTRATION. Total amino acid concentration (Fig. 5A) increased between 10 DAB and the turning stage, and then remained stable or decreased slightly until maturity in all cultivars. At petal fall and 10 DAB, total amino acid concentrations in 'CF1116' and 'Elsanta' were significantly higher than those in 'Capitola' and 'Dover'. At the turning stage and maturity, total amino acid concentration in 'Dover' was the lowest but this difference was only significant at maturity. Asparagine and glutamine were the two major amino acids in all cultivars.

The concentration of each major or minor amino acid increased markedly between 10 DAB and the turning stage (Fig.

5B–G). Between the turning stage and maturity, asparagine and arginine concentrations decreased while those of glutamine, glutamate, and aspartate remained stable, and that of alanine increased. The significance of differences among cultivars was strongly dependent on the stage of development. The asparagine concentration in 'Dover' was significantly lower than that in the other cultivars at maturity (Fig. 5B). For the glutamine concentrations (Fig. 5C), no significant difference among cultivars was observed at petal fall, although 'CF1116' exhibited a glutamine concentration which was significantly higher than that in the other cultivars at the other stages of development. The arginine concentrations (Fig. 5D) in 'CF1116' and 'Elsanta' were significantly higher than those in 'Capitola' and 'Dover' at 10 DAB and the turning stage. At maturity, the arginine concentration in 'CF1116' was significantly higher than that in all the other cultivars. Although significant differences were observed among cultivars in terms of glutamate concentrations (Fig. 5E) at petal fall and 10 DAB, they were not sustained during later stages of development. The alanine concentration (Fig. 5F) in 'CF1116' was significantly higher than that in the other cultivars at the turning stage and maturity. As for aspartate (Fig. 5G), although 'Elsanta' exhibited a concentration which was significantly higher than that in the other cultivars at petal fall and 10 DAB, few or no significant differences among cultivars remained at the turning stage and maturity.

For the other amino acids (data not presented), the main features are summarized as follows. No significant differences were observed among cultivars in terms of their proline concentration. 'Dover' had a γ -aminobutyric acid concentration significantly lower than the other cultivars. 'CF1116' had a phenylalanine concentration significantly higher than the other cultivars at maturity. Lysine was only found in trace amounts in all cultivars.

CONTRIBUTION OF SOLUTES TO OSMOTIC POTENTIAL. The concentrations of soluble sugars, organic acids, and amino acids measured after extraction (Figs. 3–5) were used to estimate the contribution of these organic solutes (Table 1) to osmolarity measured in juice expressed from fruit (Fig. 2). The sum of soluble sugars, organic acids, and amino acids accounted for between 56% and 74% of juice osmolarity at maturity. The major contribution was that of soluble sugars (43% to 57%). Although their juice osmolarities were significantly different, the contribu-

tion of soluble sugars to osmolarity was the same in 'Capitola' and 'CF1116'.

SENSORY EVALUATION. After sensory evaluation by 14 individuals (Table 2), 'CF1116' ranked as the preferred cultivar, followed by 'Elsanta', 'Dover', and finally 'Capitola'. 'CF1116' was rated the sweetest and most aromatic cultivar, while 'Elsanta' was rated the most acid and fragrant cultivar.

Discussion

BIOCHEMICAL COMPONENTS OF STRAWBERRIES. Composition of the strawberries studied during the present investigation agrees with that reported in recent studies (Hancock, 1999; Perkins-Weazie, 1995). At maturity, data on juice osmolarity was in general agreement with those previously published (Pomper and Breen, 1995). However, we observed a smaller increase between the green and red stages than that reported by Pomper and Breen (1995). This may have resulted from differences in the technique employed for juice collection since Pomper and Breen (1995) used centrifugation instead of filtration after freezing and thawing. At early stages of development, juice osmolarity of strawberries is similar to that of grape (*Vitis vinifera* L.) berries. However, strawberry osmolarity changes little at maturity, whereas it increases 4-fold in grape berries (Diakou et al., 1997).

Few reports exist concerning the presence of starch in strawberries. At maturity, the mean starch concentration observed in the present study (0.2% DW) was in agreement with results obtained for 'Elsanta' strawberry (Chen et al., 1997). The changes observed during development are in agreement with data on histological observations and iodine staining after extraction (Knee et al., 1977). The fact that 'Capitola' and 'Elsanta', the two strawberry cultivars with the highest starch content at 10 DAB, also had the highest fruit weight at maturity supports the view that this polysaccharide could contribute transiently to the sink strength of young strawberries, as is the case with the tomato (*Lycopersicon esculentum* Mill) (Schaffer et al., 1999).

At maturity, total soluble sugar concentration in strawberries reaches about 500 mg·g⁻¹ DW corresponding to 40 mg·g⁻¹ FW, which is quite low compared with many other fleshy fruits (90 to 200 mg·g⁻¹ FW for grape, banana (*Musa coccinea* Colla), orange [*Citrus sinensis* (L.) Osbeck] and apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.], Coombe, 1976). During early stages of development, inositol can be considered an important sugar in strawberries, after glucose, fructose, and sucrose. At maturity it is a minor sugar as has been shown in several other cultivars (Ogiwara et al., 1998b). Inositol is usually a minor sugar in fleshy fruits, except in kiwi [*Actinidia arguta* (Sieb. et Zucc.) Planch. ex Miq.] (Klages et al., 1998).

Xylose, galactose, and arabinose are major components of cell wall polysaccharides in strawberry (Gross and Sams, 1984). However, we detected them only as minor soluble sugars, in line with certain other studies (Knee et al., 1977; Makinen and Soderling, 1980). Surprisingly, their concentrations were higher during early stages of development (may be resulting from a high cell-wall turnover) than during ripening, although ripening is usually accompanied by the release of galactose and arabinose residues from the side chains of cell wall pectic polysaccharides (Redgwell et al., 1997). We did not detect any maltose or raffinose, unlike studies using gas-liquid chromatography (Morvai et al., 1991). Previous data on sorbitol in strawberry are contradictory (Makinen et al., 1980; Morvai et al., 1991; Wrolstad and Shallenberger, 1981). We did not detect this sugar alcohol at any stage of development.

Citrate is a major organic acid in strawberries and in many other fleshy fruits (Ulrich, 1970). In addition to the two major organic acids, malate and citrate, we detected quinate, shikimate, succinate, and fumarate, in line with recent data (Fernandez-Trujillo et al., 1999; Mangas et al., 1998), and isocitrate in agreement with enzymatic data (Reyes et al., 1982). We did not detect tartrate or pyruvate.

The total concentration of free amino acids in strawberries was much higher during maturation than during earlier stages of development, which may indicate less demand for protein synthesis during maturation. At maturity, asparagine, glutamine, and alanine were the major fruit amino acids, in agreement with previous results (Perez et al., 1992). Asparagine and glutamine are nitrogen transport compounds. Although previous findings on proline detection in strawberries are contradictory (Gallander, 1979; Perez et al., 1992), we found this compound in significant quantities in all cultivars and at all stages.

DIFFERENCES AMONG CULTIVARS. At maturity, significant differences were observed among cultivars for most of the biochemical compounds studied. This genetic variability may result from the fact that the four cultivars arose from different breeding programs using different germplasm. 'Capitola' originated from the University of California (University of California, 1996), 'Dover' from the University of Florida (Howard and Albregts, 1980), 'Elsanta' from I.V.T., The Netherlands (Wassenaar et al., 1982), and 'CF1116' from C.I.R.E.F., France. 'Capitola' and 'Elsanta' behaved similarly with respect to most measured variables except anthocyanin and xylose concentrations. However, 'Capitola' was not preferred by the taste testers. 'CF1116', which was scored as the best and sweetest in the sensory evaluations, was characterized by high juice osmolarity, and high sucrose, inositol, glutamine, arginine, and alanine concentrations, and low citrate and malate concentrations. 'CF1116' was thus characterized by a high soluble sugar to organic acid ratio throughout fruit development. At maturity, the ratio for 'CF1116' was about twice that seen for the other cultivars. 'Dover' was characterized by high anthocyanin and galactose concentrations and low asparagine and alanine concentrations. Some of these differences appeared early during development, while others appeared during maturation.

Cultivar differences in terms of the major soluble sugar concentration at maturity have already been observed among strawberry cultivars (Ogiwara et al., 1998a). In the present study, differences among soluble sugar concentrations in the cultivars could be explained mainly by differences in sucrose concentrations. These differences appeared at the beginning of maturation but not during earlier stages. In wild tomato species, sucrose accumulation is associated with developmental loss of vacuolar acid invertase activity (Schaffer et al., 1999). It has also been proposed that soluble acid invertase regulates hexose and sucrose levels in developing strawberries (Ranwala et al., 1992). The differences observed among strawberry cultivars may also result from differences in sucrose synthase, or neutral invertase activities, an increase in sucrose concentration during development of strawberries was shown to be associated with increased activity of these two enzymes (Hubbard et al., 1991). Analysis of the enzymes at the molecular level should aid in verifying these hypotheses.

Although differences among strawberry cultivars in terms of their acidity (Ruiz-Nieto et al., 1997) and organic acid concentration (Morvai et al., 1991) have been described on several occasions, we observed few differences among cultivars regarding

titratable acidity at maturity. The major organic acid in strawberries, citrate, made an important contribution to titratable acidity during the present study. However, at the turning stage, citrate concentrations were not correlated with titratable acidity. The cultivar differences observed in terms of citrate at maturity paralleled those seen for malate and were already visible at petal fall or 10 DAB. Therefore, they may result from differences in synthesis or vacuolar storage capacity rather than in mobilization during maturation. Differences in mitochondrial aconitase activity may explain cultivar differences in citrate concentration, as has been proposed in the case of lemon [*Citrus limon* (L.) Burm, *Citrus limettioides* Tan.] (Sadka et al., 2000). The tonoplast transport mechanism involved in citrate and malate vacuolar storage (Canel et al., 1995) may also be affected.

Differences in amino acid concentrations have already been observed among strawberry cultivars (Temperli and Kunsch, 1976). Amino acids may constitute an important contributory factor to fruit taste, with arginine and asparagine increasing sweetness and aspartate increasing sourness (Jia et al., 2000). At maturity, 'CF1116' had the highest arginine concentration and slight differences were observed among cultivars for asparagine and aspartate. Some amino acids, including alanine, are precursors of aroma volatiles (Heath et al., 1986), and phenylalanine gives rise to anthocyanin synthesis. 'CF1116' was noted as the most aromatic cultivar and also had the highest alanine concentration during maturation. This agrees with the suggestion that a high alanine content may be responsible for high ethyl ester levels in the volatile composition of strawberry (Perez et al., 1992).

Conclusions

In strawberries, changes in concentrations of the major metabolites change with development. However, accumulation of organic acids, amino acids, and soluble sugars in the receptacle is strongly dependent on the cultivar. The cultivar differences characterized during this investigation suggest more in-depth studies should be undertaken, aimed at determining quantitative trait loci which may control fruit organoleptic quality in offspring arising from crosses between the cultivars we considered. This would constitute a first step towards development of marker-assisted selection for some parameters of strawberry quality.

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