JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Changes in Physicochemical Characteristics and Volatile Constituents of Strawberry (Cv. Cigaline) during Maturation

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Changes in the volatile composition of strawberries (cv. Cigaline) at six stages of maturity, from 28 to 44 days after anthesis, were investigated over two harvesting seasons. Volatiles were isolated by direct solvent extraction and analyzed by means of GC-FID and GC-MS, with special attention to the quantification of furanones. Firmness, skin color, soluble solids (SS), titratable acidity (TA), SS/TA ratio, organic acids, and sugars were also determined. With increasing maturity, soluble solids, SS/TA ratio, Minolta *a** value, and levels of sucrose, glucose, fructose, and malic acid increased, whereas Minolta *L* value, hue angle (Θ), titratable acidity, and levels of citric acid decreased. Furanones and esters were generally not detected before half-red fruits, whereas C_6 compounds were the main compounds in immature fruits. During maturation, levels of these so-called green components decreased drastically, whereas levels of key flavor compounds increased significantly and were closely correlated with skin color development. Maximum volatile production was observed in fully red fruits.

KEYWORDS: Strawberry; furanones; flavor; aroma; volatile compounds; maturation

INTRODUCTION

Strawberry plants, wild and cultivated, belong to the Rosacea family and *Fragaria* genus. The wild European strawberry is mainly from *F. vesca* L., whereas the cultivated varieties (*F. ananassa*) are hybrids from *F. chilosensis* and *F. virginiana*. The strawberry (*F. ananassa*) is cultivated worldwide and plays an important economic role. Strawberries are consumed as fresh fruits but are also frequently found in processed products such as liquor, syrup, jam, juice, ice cream, and concentrated flavor preparations.

Because of its typical aroma, the volatile components of strawberry have been extensively studied, and >360 volatiles have been reported (*1*−*3*). Different studies have shown that esters largely contribute to the strawberry aroma. Among them, ethyl 2-methylbutanoate, methyl and ethyl butanoates, methyl and ethyl hexanoates, and hexyl and (*E*)-hex-2-enyl acetates are considered to be important flavor-active components providing green and sweet fruity notes (*4*−*7*). Sulfur compounds (methanethiol, dimethyl sulfide, dimethyl disulfide, methylthiol acetate, and butanoate) are also considered to be important constituents, particularly in some "older" cultivars (cv. Souvenir de Charles Machiroux) (*8*). Due to their very low odor thresholds (respectively, 0.04 and 0.03 μg/kg), and their large amounts present in several cultivars, Furaneol [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone] (DHF) and mesifurane [2,5-dimethyl-4-methoxy-3(2*H*)-furanone] (DMF) are considered to be the two major flavor contributors to strawberry aroma (*4*, *5*, *9*−*11*). Nevertheless, due to its water-soluble nature and its thermal instability (*5*, *12*, *13*), Furaneol is rarely quantified. According to Ulrich et al. (*14*), methyl anthranilate (MA), which is characterized by an intensive spicy-aromatic and flowery note, is responsible for the typical character of the wild strawberry aroma. Douillard and Guichard (*5*) reported that lactones, particularly γ-decalactone, which are found in high concentrations in some cultivars, are among the key flavor compounds. Alcohols, even if numerous, are not thought to contribute significantly to strawberry flavor, except for linalool. The latter is present in large amounts in some cultivars (*4*, *7*, *9*) and possesses an intense and pleasant note and a low threshold value (6 μg/kg). Acids, generally present below their threshold values, have a small impact on strawberry flavor except for 2-methylbutanoic acid, which contributes to strawberry attributes at low concentrations (*15*, *16*).

Although strawberry volatiles have been intensively investigated, our knowledge of the changes in aroma composition during maturation is still limited (*13*, *17*−*19*). Moreover, except in ref *13*, Furaneol and mesifurane, considered by most of the authors to be among the most important volatile compounds in strawberry aroma, were not reported. The aim of this work was to study, over two harvesting seasons, changes in the physicochemical characteristics and volatile composition of the strawberry (cv. Cigaline) at six stages of maturity, with a special emphasis on the quantification of furanones. Cigaline is a new early cultivar, an improvement of Gariguette, with a very attractive and fruity flavor (*20*). In addition, no reports on the volatiles of Cigaline strawberries have appeared in the literature.

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10.1021/jf0350919 CCC: $27.50
Published on Web 02/06/2004

Table 1. Changes[a] in Firmness (F_d), Minolta *L*, *a**, *b** Values, and Hue Angle (Θ) of Strawberries (Cv. Cigaline) at Six Stages of Maturity

	white		pinky		red 1/2		red 3/4		red 4/4		dark red	
	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
F_d (ID_{50})	88.0y	70–100	95.3z	84–100	77.4x	36–100	63.7w	46–74	63.2w	38–75	64.9w	47–76
L	79.5z	61–98	64.6y	59–68	61.4y	41–74	43.9x	35–51	42.0x	33–48	42.5x	36–50
*a**	–6.0w	–13 to 3	–10.2v	–13 to 0	8.6x	–12 to 36	35.7y	32–40	38.2yz	29–43	40.2z	36–43
*b**	17.0y	1–33	32.0z	26–38	31.6y	23–39	31.6z	24–39	30.8z	22–38	32.0z	24–40
Θ (deg)	90.9y	27–122	107.8z	91–112	78.6x	41–109	41.6w	32–50	38.7w	31–45	38.5w	33–45

[a] Values are given as average of a 2-year-trial ($n = 40$). Values with different letters are significantly different (on the basis of Newman–Keuls test, $p < 0.05$).

MATERIALS AND METHODS

Solvent and Chemicals. Analytical grade dichloromethane, *n*-alkane (C_8−C_{40}) standards, and reference compounds were from Aldrich Chimie (Saint Quentin Fallavier, France).

Samples. Berries of the strawberry cv. Cigaline (origin: CIREF, France; Gariguette × Earliglow; cross made in 1989; selected in 1990; tested as CF 89.93.323; introduced in 1996; French plant patent CPOV 12 795; E.C. plant patent 96-1055] (*20*) were obtained from the experimental plots of the Ctifl of Lanxade (Dordogne, France). Over a period of two years (2001−2002), flowers at anthesis were tagged on a single day, and fruits were picked at six stages of maturity from 28 to 42 days after anthesis (DAA). Fruits were then characterized on the basis of fruit colors as established by Risser and Navatel (*21*): white (28 DAA), pinky (30 DAA), red 1/2 (35−36 DAA), red 3/4 (38−39 DAA), red 4/4 (physiological maturity) (42 DAA), and dark red (44 DAA). Immediately after harvesting, intact fruits were analyzed for skin color and firmness as described below. Fruits were then sepals dissected, frozen with liquid nitrogen, and stored at −25 °C until analysis.

Physical Analyses. Firmness and skin color were determined on two opposite sides of 20 strawberries using, respectively, an electronic Durofel (licensed by Copa-Ctifl) with a 0.50 cm^2 tip and a Minolta Chroma meter CR-200. The Durofel, a noninvasive dynamometer, provides a measure of the force used to press a spring-loaded pawl against the fruit surface (*22*−*24*). The measurements, ranging from 0 to 100, are expressed in ID_{50}. The Minolta Chroma meter was calibrated with a white reference plate, and measurements were recorded using *L*, *a**, and *b** color coordinates. Hue angle (Θ), a parameter that has been shown to be effective for predicting visual color appearance (*25*), was calculated using the formula $\tan^{-1}$ (*b**/*a**).

Chemical Analyses. The total soluble solids content (SSC) in juice (% Brix) was determined with an Atago PR-101 digital refractometer. Titratable acidity (TA) was determined by titrating 5 mL of juice to pH 8.1 with 0.1 N NaOH using an automatic titrator Crison Compact II with autosampler. The individual sugars (glucose, fructose, and sucrose) and organic acids (malic and citric) were determined in triplicate from 1 mL of juice using a single-injection HPLC technique as described by Doyon et al. (*26*). Fruit juice was obtained from 150 g of fruit after homogenization and centrifugation (8500*g*, 20 min, 4 °C).

Isolation and Concentration of the Volatiles. One hundred grams of frozen berries, 100 mL of saturated NaCl solution, and 10 μL of 4-nonanol (3.28 mg/mL) (internal standard) were homogenized in a Waring blender for 3 min. The pulp was then centrifuged (8500*g*, 20 min, 4 °C), and the clear juice was filtered through glass wool and immediately extracted in batches with 100 mL of analytical grade dichloromethane (further purified by distillation before use) for 30 min under magnetic stirring at 4 °C. Then, the mixture was centrifuged (8500*g*, 10 min, 4 °C) and transferred to a 250 mL separatory funnel. The aqueous phase was then re-extracted twice as mentioned above, according to the method of Moio et al. (*27*). After centrifugation and separation, both organic extracts were dried over anhydrous sodium sulfate, filtered through glass wool, and concentrated to ∼1 mL using a small Vigreux column at 45 °C. The extract was immediately injected in GC-MS and GC-FID. All analyses were performed in triplicate.

GC-FID Analysis. A Varian 3800 gas chromatograph equipped with a cold on-column injector was used. The flow of hydrogen N55 carrier gas was 1 mL/min. The oven was kept at 40 °C for 3 min, then programmed to 245 °C at 3 °C/min, and kept at 245 °C for 20 min. The injector was kept at 20 °C for 0.1 min, then programmed to 245 °C at 180 °C/min, and kept at 245 °C for 85 min. The FID was kept at 245 °C. One microliter of each sample was injected on a DB-Wax Etr (J&W Scientific, Folsom, CA) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The levels of the volatile compounds were expressed as 4-nonanol equivalent (assuming all response factors were 1). The concentrations are to be considered as relative data because recovery after extraction and calibration factors related to the standard were not determined.

GC-MS Analysis. A Varian 3800 gas chromatograph equipped with a cold on-column injector was used with the same DB-Wax Etr capillary column as above. The flow of helium N60 carrier gas was 1 mL/min. The oven and the injector temperature programs were as above. A Varian Saturn 2000 mass spectrometer with an ion trap was used. Mass spectra were recorded in electronic impact (EI) ionization mode. The ion trap, manifold, and transfer line temperatures were set, respectively, at 150, 45, and 250 °C. Mass spectra were scanned in the range from *m*/*z* 29 to 350 amu at 1 s/scan. Identifications were carried out by comparison of linear retention index and EI mass spectra with data from authentic compounds.

HPLC Conditions. A Waters 600 E liquid chromatograph equipped with a Waters 610 Fluid Unit pump was used. A variable-wavelengths UV−vis detector (Waters 486) set at 210 nm and a differential refractometer (Waters 410) were connected in series and used as detectors. Twenty microliters of each sample was injected on a 300 mm × 7.8 mm i.d. cation-exchange ICSep ICE-ION-300 column equipped with an ICSep ICE-GC-801/C guard column (Transgenomic, San Jose, CA). The column oven temperature was set at 30 °C, and the flow of mobile phase (0.008 N H_2SO_4) was 0.4 mL/min.

Statistical Analysis. Analyses of variance were performed using StatboxPro 5.0 (Grimmersoft). Significant differences were determined at $p < 0.05$.

RESULTS AND DISCUSSION

The effects of maturity on the physical and chemical constituents of cv. Cigaline strawberries were investigated over a two-year harvesting season. Fruits were picked at six stages of maturity, expressed in DAA, and characterized on the basis of fruit color: white (28 DAA), pinky (30 DAA), red 1/2 (35−36 DAA), red 3/4 (38−39 DAA), red 4/4 (physiological maturity) (42 DAA), and dark red (44 DAA). Firmness, skin color, SS, TA, SS/TA ratio, organic acids, sugars, and concentrations of main volatile compounds were determined for the different stages of maturity for the two harvests. The effects of maturity on the physical and chemical characteristics of cv. Cigaline strawberries are summarized in **Tables 1**−**5**. The data are given as average and minimum−maximum range on the two years of harvest. To follow the evolution of data and to compare differences between different stages of maturity, data were analyzed using a one-way analysis of variance with Newman−Keuls multiple-range test: experimental design, one factor (degree of maturity) with six levels (white, pinky, red 1/2, red 3/4, red 4/4, and dark red), complete randomization with 40 samples (20 fruits × 2 years) or 6 samples (3 replications × 2

Table 2. Changes[a] in Soluble Solids (SS), Titratable Acidity (TA), and SS/TA Ratio of Strawberries (Cv. Cigaline) at Six Stages of Maturity

	white		pinky		red 1/2		red 3/4		red 4/4		dark red	
	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
SS (% Brix)	6.7w	6.4–6.9	7.0w	6.6–7.5	8.4x	8.2–8.6	9.5y	9–10	9.9yz	9.1–10.8	10.4z	9.8–11
TA (mequiv/100 g)	16.0z	15–17	16.4z	15.7–17.2	13.7y	12.7–14.8	12.7x	12.4–13.2	11.2w	10.8–11.3	10.4v	10–10.9
SS/TA	0.5v	0.4–0.5	0.5v	0.4–0.5	0.7w	0.6–0.7	0.8x	0.7–0.8	0.9y	0.8–1	1.0z	1–1

[a] Values are given as average of a 2-year-trial ($n = 6$). Values with different letters are significantly different (on the basis of Newman–Keuls test, $p < 0.05$).

Table 3. Changes[a] in Sugar and Organic Acid Contents of Strawberries (Cv. Cigaline) at Six Stages of Maturity

	white		pinky		red 1/2		red 3/4		red 4/4		dark red	
	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
sucrose	0.4w	0.37–0.43	0.8x	0.46–1.11	1.4y	1.02–1.69	1.6yz	1.43–1.75	1.7z	1.59–1.88	1.7yz	1.63–1.7
glucose	1.9w	1.77–1.93	1.9w	1.75–1.9	2.2x	2.14–2.3	2.5y	2.36–2.75	2.6y	2.14–2.95	2.9z	2.82–2.97
fructose	1.9w	1.77–2.1	2.0w	1.9–2.01	2.4x	2.24–2.46	2.6y	2.5–2.87	2.8y	2.35–3.1	3.1z	2.96–3.26
citric acid	0.8z	0.78–0.85	0.8yz	0.7–0.8	0.7y	0.66–0.79	0.6x	0.59–0.66	0.6w	0.51–0.65	0.5w	0.52–0.55
malic acid	0.2x	0.2–0.25	0.3y	0.26–0.33	0.3yz	0.27–0.4	0.4yz	0.3–0.38	0.3yz	0.26–0.37	0.4z	0.34–0.42

[a] Values expressed in g/100 g are given as average of a 2-year-trial ($n = 6$). Values with different letters are significantly different (on the basis of Newman–Keuls test, $p < 0.05$).

Table 4. Relative Proportions[a] of the Main Volatile Classes of Strawberries (Cv. Cigaline) at Six Stages of Maturity

	white		pinky		red 1/2		red 3/4		red 4/4		dark red	
compound (*n*)[b]	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
furanones (2)		–[c]	0.2w	0–0.4	6.2x	3.7–8.8	13.8y	9.2–18.3	18.5z	16.5–20.3	18.5z	13.6–21.2
lactones (4)	0.1x	0–0.2	1.5x	0.3–2.8	6.7y	5.1–8.3	9.3z	6.9–12.4	6.9y	6–7.5	6.5y	4.7–8.7
carbonyls (2)		–	0.4w	0.1–0.8	0.8x	0.4–1.3	1.8z	1.5–2	1.8z	1.4–2	1.3y	1–1.6
acids (5)	6.4w	3.5–9.7	14.8x	10.3–21	57.5y	54–61.2	62.4yz	53.5–70.1	66.4z	63–69.4	68.5z	63.4–75.7
terpenes (4)	0.3y	0.1–0.6	1.2yz	0.6–1.8	1.9z	0.5–3.8	2.1z	1.8–2.3	1.3yz	0.9–1.7	1.0yz	0.7–1.4
C_6 compounds (6)	90.5z	87.2–93.3	79.0y	70.9–85.5	22.5x	17.1–28.1	5.9w	5.2–6.4	1.8w	1.5–2.2	1.5w	1–2
esters (7)	0.2x	0–0.4	0.4x	0.1–0.8	4.0yz	1.9–6.5	4.8z	4–5.6	3.3yz	2.4–4.3	2.6y	2.2–3.1
alcohols (1)	2.5z	1.9–3.3	2.4z	1.7–3.2	0.5y	0.5–0.6	0.1y	0.1–0.1	0.1y	0–0.1	0.1y	0–0.1

[a] Relative proportions of levels expressed as 4-nonanol equivalents are given as average of a 2-year-trial ($n = 6$). Values with different letters are significantly different (on the basis of Newman–Keuls test, $p < 0.05$). [b] Number of compounds identified in each class. [c] Not detected.

years), respectively, for physical analyses and for chemical and volatile analyses.

As shown in **Table 1**, firmness (F_d), Minolta *L* value, and hue angle (Θ) decreased from white to red 1/2 fruits and then appeared to level off. Minolta *a** values increased rapidly between red 1/2 and red 3/4 fruits, whereas Minolta *b** values were found to be very similar for the different stages of maturity, except for the first stage. Generally, firmness, Minolta *L*, *a**, *b** values, and hue angle were found to be not statistically significantly different for the last three stages, except for *a** values for red 3/4 and dark red fruits. Fruit firmness was ∼20% less in red 4/4 and dark red fruits than in red 1/2 fruits, which is in agreement with previous results reported by Forney et al. (*19*). Changes in *a** value and Θ show that strawberry ground color was changing from green to red as maturity increased (*25*). Rapid evolution of these parameters between red 1/2 and red 3/4 fruits indicates that strawberries ripen rapidly on plants in ∼2−3 days, as previously mentioned by Forney et al. (*19*). SS and sugar contents increased with increasing maturity (**Table 2**). The total sugar contents (sum of glucose, fructose, and sucrose) were correlated with SS ($r^2 = 0.95$; $p < 0.001$) (**Table 3**) The levels of fructose were slightly higher than those of glucose, and their ratio remained approximately the same during maturation. Sucrose levels were present at low levels in the first stages but increased ∼4-fold during maturity, whereas those of fructose and/or glucose increased only 1.5-fold during this period. These results agree with those previously reported by Forney and Breen (*28*). Nevertheless, some discrepancies were observed with those of Forney et al. (*19*), in particular with regard to the changes in soluble solids and glucose for the last three maturity stages. Levels of TA were found to be not statistically significantly different for the two first stages and then significantly decreased (**Table 2**). Citric acid showed a similar trend (**Table 3**). Contrary to previous observations in the strawberry (*19*), the levels of malic acid were found to increase slightly with increasing maturation (**Table 3**). Finally, the SS/TA ratios were found to be not statistically significantly different for the two first stages and then significantly increased with increasing maturity (**Table 2**).

The relative proportions of the main volatile classes and the main volatile compounds quantified in Cigaline strawberries are reported in **Tables 4** and **5**. In this study, special attention was paid to the quantification of furanones. Various methods have been developed for the quantitative analysis of furanones in foods. Except for the work done by Sanz et al. (*13*), in which mesifurane, Furaneol, and its glucoside were determined simultaneously by HPLC, GC is the most frequently used method for the separation of these compounds. Probably the most reliable and accurate method for the quantitative analysis of DHF and its methyl ether is the stable isotope dilution analysis described by Sen et al. (*29*). Not having the facilities for this method, we used solvent extraction in agreement with previous studies (*2*, *5*, *9*, *30*, *31*), and because Furaneol has proved to be difficult to isolate by dynamic headspace, simultaneous distillation−extraction or solid phase microextraction methods (*8*, *17*−*19*, *32*−*36*). Dichloromethane was chosen in agreement

Table 5. Changes in the Concentrations[a] of Volatiles of Strawberries (Cv. Cigaline) at Six Stages of Maturity

compound	assignment[b]	RI[c]	white		pinky		red 1/2		red 3/4		red 4/4		dark red	
			mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
furanones														
mesifurane	A	1580		–[d]		–	77x	18–148	1152y	503–1850	1917z	1462–2435	1782z	1558–2010
Furaneol	A	2015		–		+[e]	710w	521–1002	3227x	2257–4152	6217z	5707–6841	4440y	2918–5114
lactones														
γ-hexalactone	A	1682		–		–	10y	0–22	18y	8–25	16y	4–30	27z	21–31
γ-octalactone	A	1885		–	12	0–25	17	4–20	23	16–30	21	8–35	25	17–35
γ-decalactone	A	2131		+	29w	15–39	814x	750–920	2846z	2085–3896	2887z	2540–3279	2146y	1545–2987
γ–dodecalactone	A	2357		–		+	26y	9–43	46yz	17–75	58z	37–71	51yz	32–87
carbonyls														
pentan-2-one	B	985		–	11w	3–21	83w	45–133	542y	413–652	704z	558–812	381x	314–469
heptan-2-one	A	1174		–		–	11x	5–17	32y	23–43	58z	43–83	37y	27–47
acids														
2-methylpropanoic acid	A	1556	18v	14–25	23v	4–46	412w	121–760	961x	425–1594	2130z	1698–2562	1457y	1098–1858
butanoic acid	A	1607		+	15w	11–24	505x	150–881	1633y	1198–2139	4103z	3905–4560	3602z	2151–4870
2-methylbutanoic acid	A	1657	71v	55–84	265w	192–330	3262x	2875–3781	6959y	4890–9052	9810z	8750–10450	8995z	6596–12540
hexanoic acid	A	1834	203w	116–330	228w	205–284	3449x	1916–4985	10240y	9654–11443	12744z	11240–14414	9230y	6840–11217
octanoic acid	B	2046		–		–	25w	14–34	99y	85–121	115z	81–145	75x	65–87
terpenes														
(*E*)-furan linalool oxide	A	1429		–		+		+		+	30	15–47	20	16–25
(*Z*)-furan linalool oxide	A	1457		+		+	27	9–49	48	4–95	37	24–52	35	21–51
linalool	A	1538	11x	8–13	16x	9–27	80x	29–131	278z	210–345	281z	194–375	185x	107–265
nerolidol	A	2037		–	12w	0–29	115x	25–255	320z	260–378	220y	140–280	114x	78–174
C_6 compounds														
hexanal	A	1075	447yz	228–645	490z	114–880	445yz	211–670	157xy	124–193	43x	19–71	36x	32–40
(*Z*)-hex-3-enal	A	1142	122z	16–234	79yz	29–145	45y	28–65	34y	27–47		+		+
(*E*)-hex-2-enal	A	1218	2244z	1848–2987	1441y	1323–1624	1364y	1154–1754	982x	740–1194	379w	299–462	326w	235–415
hexanol	A	1347	620	385–874	319	29–625	583	57–1250	355	87–654	211	39–410	93	18–187
(*Z*)-hex-3-en-1-ol	A	1372	372z	198–545	330yz	8–680	273yz	23–545	137yz	17–287	15y	7–26		+
(*E*)–2-hexen-1-ol	A	1394	681z	599–876	590z	357–845	336y	201–475	198xy	65–333	129x	98–152	37x	11–60
esters														
methyl butanoate	A	998		–		–	330w	245–520	863z	676–1013	755y	625–887	501x	421–593
methyl 2-methylbutanoate	A	1009		–		–	28x	4–57	178yz	100–264	209z	54–321	112y	36–201
butyl acetate	A	1067		–		–	47	11–97	47	29–65	61	41–90	45	28–59
isoamyl acetate	A	1118		–		–	14x	4–25	82z	42–125	56y	27–87	47y	25–71
methyl hexanoate	A	1186		–		+		+	111z	10–164	105z	23–145	58y	20–97
hexyl acetate	A	1270		+		+	16x	4–29	46z	29–63	48z	41–57	25y	17–35
(*E*)-hex-2-enyl acetate	A	1325		+		+	61x	15–116	190z	131–238	210z	96–321	106y	37–187
methyl anthranilate	A	2206		–		–		–		–		+	12	8–15
alcohols														
benzyl alcohol	A	1854	126z	80–187	105yz	43–174	70yx	49–89	33x	23–40	40x	19–61	17x	11–24

[a] Values expressed in μg/kg equivalents of 4-nonanol are given as average of a 2-year trial ($n = 6$). Values with different letters are significantly different (on the basis of Newman–Keuls test, $p < 0.05$). [b] A, identified by linear retention index and mass spectra of authentic standards; B, tentatively identified. [c] Linear retention index based on a series of *n*-hydrocarbons. [d] Not detected. [e] Concentration < 10 ppb.

with Douillard and Guichard (*5*) and because of its ability to extract compounds with an enolone structure (*37*) or methyl anthranilate (*38*). Hirvi et al. (*12*) reported that decomposition of Furaneol is dependent on pH and temperature. In accordance with these findings, extractions were performed at 4 °C and at pH ~3.5. In **Table 5**, the concentrations of volatiles are given as average and minimum−maximum range of the two years of harvest. With an average of 12.7% over the years of harvest, the coefficients of variation are relatively good, particularly those of Furaneol and mesifurane, respectively, 10.1% (range = 3.5−26,1%) and 7.4% (range = 2.9−17.2%).

As shown in **Table 4**, the maturation of cv. Cigaline strawberries is characterized by opposite changes in three major groups of volatiles: C_6 compounds, acids, and furanones. C_6 aldehydes and alcohols, products of the enzymatic breakdown of unsaturated fatty acids (*39*, *40*), are the main volatile components isolated in immature strawberries (white−pinky). The relative proportion of these so-called green components decreased drastically with maturation, from 90.5 to 1.5% in white and dark red fruits, respectively, whereas the relative proportions of furanones and acids increased as the fruit ripened. As shown in **Table 5**, (*E*)-hex-2-enal was the main C_6 aldehyde found in all stages compared to the levels of (Z)-hex-3-enal. This is consistent with the results previously reported by Pérez et al. (*41*). As previously mentioned, in most plants, compounds with a (3*Z*)-enal structure are rapidly isomerized by a (3*Z*,2*E*)-enal isomerase to the (2*E*)-enal form (*42*−*45*). Due to the isolation method chosen [enzyme deactivation with saturated NaCl solution during homogenization] (*46*−*48*), it seems reasonable to suppose that the concentrations of C_6 compounds, given in **Table 5**, are representative of the endogenous levels of the fruit. These results are similar to those previously reported in cv. Chandler strawberries (*17*) or in various fruits (*49*−53) and confirm that C_6 compounds certainly account for the "green" note of immature strawberries.

With decreasing concentrations of C_6 compounds, acids become the major volatiles in strawberries. 2-Methylpropanoic, butanoic, 2-methylbutanoic, and hexanoic acids are present already in white fruits, and their concentrations increase significantly during maturation, reaching their highest amounts

in red 4/4 fruits and then slightly decreasing. Octanoic acid, not detected before red 1/2 stage fruits, showed a similar trend. Although the acids, because of their high threshold values, are regarded as irrelevant compounds, 2-methylbutanoic acid was found to have a characteristic strawberry flavor (*15*). This compound was present in the last three stages of maturity at a concentration far above (respectively, 38, 54, and 49 odor units) its odor threshold in water (180 ppb) (*14*).

Furaneol (DHF) and mesifurane (DMF) are considered to be among the most important volatiles reported in strawberry aroma (*1*, *2*, *10*, *11*). In this study, these two compounds were not detected before the stage red 1/2, except for DHF at trace levels in pinky fruits. With increasing maturity, these two compounds showed a similar pattern; levels of these compounds increased rapidly with increased maturity, reaching their highest amounts for red 4/4 fruits and then slightly decreased. These results are consistent with those previously reported by Sanz et al. (*13*), who assessed levels of these compounds in different strawberry varieties by HPLC at four ripening stages (white, pinky, ripe, and over-ripe). Although they account for <20% of all compounds identified in mature fruits (**Table 4**), DHF and DMF are present at concentrations far above (respectively, 155000 and 64000 odor units) their odor thresholds (respectively, 0.04 and 0.03 ppb), and it is obvious that these compounds play an essential role in the Cigaline aroma.

In this study, four γ-lactones were identified at the different stages of maturity. Among these compounds, γ-decalactone, previously reported as an important component in strawberry aroma (*5*), was the main lactone. These compounds, well-known in other fruits, are responsible for the spicy, floral, and fruity characteristics of peach, apricot, or plum aroma (*52*, *54*−*57*). In general, they are not detected before pinky fruits, except for γ-decalactone at trace levels in white fruits. With increasing maturity, their levels increased from early stages to red 3/4 fruits and then were found to be very similar. Except γ-hexalactone, these compounds are present, particularly γ-decalactone, at a concentration above their odor threshold (γ-hexalactone, 1600 ppb; γ-octalactone, 7 ppb; γ-decalactone, 11 ppb; γ-dodecalactone, 7 ppb) (*55*). Therefore, they are possible contributors to the aroma of Cigaline strawberry.

Among the esters, except hexyl and (*E*)-hex-2-enyl acetates, most were not detected until red 1/2 fruits. This is consistent with the results previously reported by Pérez et al. (*17*) and Yamashita et al. (*58*). The levels of these compounds increased rapidly with increasing maturity, reaching their highest amounts for red 3/4 and red 4/4 fruits, and then slightly decreased. Methyl butanoate, methyl 2-methylbutanoate, and (*E*)-hex-2-enyl acetate were the major volatile esters identified in this study. Methyl anthranilate was detected only at low levels in the two last stages.

With regard to the terpenes, the levels of (*E*)- and (*Z*)-furan linalool oxide, linalool, and nerolidol significantly increased with increased maturity. Linalool, widely reported in a number of fruits and particularly in Muscat grapes (*59*), was present at a low levels in early stages, reaching its highest amounts for red 3/4 and red 4/4 stages, and then slightly decreased. In all stages of maturity, and in particular for mature stages, linalool was present at a concentration far above its odor threshold (6 ppb). Due to its intense and pleasant note, this compound participates very probably in Cigaline aroma. Nerolidol, first described in the strawberry by Schreier (*31*), was not detected in white fruits but showed a trend similar to that of linalool.

With regard to the carbonyl compounds, the levels of pentan-2-one and heptan-2-one, previously described as important components in strawberry aroma (*14*), increased with increasing maturity and then slightly decreased. Nevertheless, heptan-2-one was present at close to its odor threshold level (50 ppb), and only pentan-2-one, present far above its odor threshold (50 ppb) (*14*), could play a role in Cigaline aroma.

Finally, the levels of benzyl alcohol decreased with increased maturity. Nevertheless, due to its odor threshold (620 μg/L) (*9*), this compound probably has no influence on the aroma of Cigaline.

These results confirm that the formation of aroma compounds in fruit is a dynamic process, during which concentrations of volatiles change both qualitatively and quantitatively. In immature fruits, C_6 compounds, in particular (*E*)-hexen-2-al, were the main components, whereas furanones and esters were generally not detected before red 1/2 fruits. During fruit maturation, levels of C_6 compounds decreased drastically, whereas those of furanones, acids, lactones, and esters increased significantly and are closely correlated with skin color. Maximum volatile production was observed at complete maturity, that is, for full-red fruits. Nevertheless, results clearly indicate that most of the changes in the levels of flavor-active compounds occurred between red 1/2 and red 3/4 stages. For that reason, and because strawberries are often, for commercial reasons, harvested between these two latter stages, the changes in volatiles and physicochemical characteristics of strawberries subjected to a maturation "off plant" will be the subject of further investigations.

ACKNOWLEDGMENT

We thank Catherine Abaglo, Cathy Guérineau, Florence Verpont, and Pierre Vaysse for technical assistance and Amy Boiron for fruitful discussion.

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Received for review September 25, 2003. Revised manuscript received December 24, 2003. Accepted January 11, 2004.

JF0350919