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Glycosidically Bound Aroma Compounds and Impact Odorants of Four Strawberry Varieties

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ABSTRACT: This paper reports the determination of glycosidically bound aroma compounds and the olfactometric analysis in four strawberry varieties (Fuentepina, Camarosa, Candonga and Sabrina). Different hydrolytic strategies were also studied. The results showed significant differences between acid and enzymatic hydrolysis. In general terms, the greater the duration of acid hydrolysis, the higher was the content of norisoprenoids, volatile phenols, benzenes, lactones, Furaneol, and mesifurane. A total of 51 aglycones were identified, 38 of them unreported in strawberry. Olfactometric analyses revealed that the odorants with higher modified frequencies were Furaneol, γ-decalactone, ethyl butanoate, ethyl hexanoate, ethyl 3-methylbutanoate, diacetyl, hexanoic acid, and (*Z*)-1,5-octadien-3-one. This last compound, described as geranium/green/pepper/lettuce (linear retention index = 1378), was identified for the first time. Differences with regard to fruity, sweet, floral, and green aroma characters were observed among varieties. In Candonga and Fuentepina, the green character overpowered the sweet. In the other two strawberry varieties sweet attributes were stronger than the rest.

KEYWORDS: *glycosides, strawberry, aroma, flavor, olfactometry*

INTRODUCTION

Strawberry is a much appreciated fruit due to its aroma, taste, and health properties. It is usually consumed fresh (75% of total production) but is also used in the food industry as an important ingredient in jam, yogurt, syrup, tea, juice, ice cream, and other food products (25% of overall production).[1] Aroma is one of the most valued attributes of strawberry. The aroma of this fruit includes volatile compounds, both in their free form and as nonvolatile compounds, present mainly as glycoconjugates formed by a sugar and an aglycone.

There are numerous studies concerning the free volatile compounds of strawberry, with more than 360 volatile flavor compounds[2] identified. To learn more about the volatile composition of strawberry, several olfactometric studies have been undertaken using gas chromatography−olfactometry (GC-O).[3−5]

Nonvolatile compounds are, moreover, potential natural sources of aroma because hydrolysis of the bonds between the sugar and the aglycone turns this molecule into an aromatic compound. As ripening proceeds, the increase in these soluble sugars results in an increase in the availability of precursors capable of producing aroma compounds.[6]

These nonvolatile compounds have been extensively studied in grapes[7−9] and in other fruits such as lychee, acerola, blackberry, pineapple, and mango,[10−14] among others. Strawberry precursors have hardly been studied. After the description of the presence of 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one β-D-glucopyranoside in strawberry,[15] Wintoch et al.[16] analyzed the glycosidical aroma compounds from two strawberry species using Amberlite XAD-2. Other research groups have focused their studies on one aglycone, Furaneol (2,5-dimethyl-4-hydroxy-2*H*-furan-3-one),[17] due to its high influence on the overall flavor. In addition, there have been some studies concerning the evolution of these nonaromatic precursors during ripening. These studies show an increase in their aglycones during the above-mentioned stage.[18] Knowledge of the strawberry aromatic precursors is important because it enables us to predict the final aroma of new strawberry-based products. As a result, there are several different groups studying the production process of strawberry fermentation products.[19,20] Such analyses would enable us to estimate the aromatic potential and therefore select the best raw material. The aim of this study was to determine the aromatic potential of different strawberry varieties with the aim of selecting the most suitable varieties for producing several fermented strawberry-based food products. Therefore, the aroma compounds released by acid hydrolysis of glycosidic precursors isolated from four different varieties have been determined. Free aromas were also studied by GC-O analyses to determine the most important compounds, from a sensory point of view, in these varieties.

MATERIALS AND METHODS

Reagents and Standards. Dichloromethane, ethanol, and methanol were supplied by Merck (Darmstadt, Germany) and ethyl acetate and sodium fluoride by Fluka (Buchs, Switzerland). Sodium dihydrogen phosphate 1-hydrate, L-(+)-ascorbic acid, and citric acid were purchased from Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). LiChrolut EN resins were purchased from Merck. An alkane solution (C8−C28), 20 mg/L in dichloromethane, was used to calculate the linear retention index (LRI) of each analyte. The chemical standards used for the identification and quantification of volatile

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Table 1. Concentration (Micrograms per Kilogram of Strawberries Except Where Indicated) of Volatile Compounds Released after Harsh Acid and Enzymatic Hydrolysis of the Strawberry Precursors Pool[a]

	0 min	15 min	1 h	4 h	1 week, 45 °C	enzymatic
terpenes						
α-terpinolene	nd	0.62 ± 0.10 a	2.94 ± 0.36 b	4.51 ± 0.14 c	0.27 ± 0.01 d	1.17 ± 0.09 f
(*Z*)-rose oxide	nd	nd	0.02 ± 0.00 a	0.25 ± 0.01 b	nd	nd
(*R*/*S*)-linalool	nd	75 ± 2 a	3.50 ± 0.30 b	nd	5.07 ± 0.13 c	105 ± 2 d
α-terpineol	nd	27 ± 1 a	111 ± 13 b	50 ± 1 c	77 ± 2 d	1.28 ± 0.14 e
nerol	nd	6.20 ± 0.79 a	12.72 ± 0.39 b	nd	nd	2.18 ± 0.22 c
geraniol	4.46 ± 0.36 a	29 ± 1 b	4.91 ± 0.63 a	nd	3.74 ± 0.35 a	5.95 ± 0.56 a
farnesol	nd	12 ± 1	nd	nd	nd	nd
linalool acetate	nd	0.23 ± 0.04	nd	nd	nd	nd
terpinen-4-ol[b]	nd	nd	3.01 ± 0.27 a	2.84 ± 0.09 a	0.68 ± 0.04 b	nd
δ-terpineol[b]	nd	nd	6.74 ± 0.41 a	6.05 ± 0.38 a	3.93 ± 0.05 b	nd
neric acid	nd	nd	0.20 ± 0.02 a	0.50 ± 0.04 b	nd	1.01 ± 0.03 c
norisoprenoids						
β-damascenone	nd	0.46 ± 0.02 a	1.30 ± 0.11 b	2.28 ± 0.08 c	0.59 ± 0.02 d	nd
β-ionone	0.15 ± 0.01 a	nd	nd	nd	nd	0.25 ± 0.01 b
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)[b]	nd	0.28 ± 0.01 a	1.68 ± 0.01 b	2.77 ± 0.09 c	0.59 ± 0.02 d	1.11 ± 0.01 e
tert-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB)[b]	nd	0.34 ± 0.01 a	6.35 ± 0.09 b	7.51 ± 0.44 b	2.13 ± 0.04 c	0.50 ± 0.00 d
3-oxo-β-ionone[b]	nd	1.33 ± 0.03 a	4.51 ± 0.42 b	4.65 ± 0.21 b	2.77 ± 0.14 c	nd
actinidols[b]	nd	0.24 ± 0.02 a	5.81 ± 0.52 b	6.62 ± 0.23 b	4.09 ± 0.15 c	0.25 ± 0.02 a
norisoprenoid 1[b]	nd	nd	2.81 ± 0.22 a	4.15 ± 0.10 b	0.27 ± 0.03 c	nd
3-oxo-α-ionol	nd	nd	0.63 ± 0.07 a	nd	nd	75 ± 2 b
volatile phenols						
guaiacol	nd	nd	nd	0.70 ± 0.09 a	nd	0.91 ± 0.04 a
m-cresol	nd	nd	nd	nd	nd	0.22 ± 0.01
eugenol	0.62 ± 0.01 a	0.70 ± 0.08 a	1.35 ± 0.17 b	6.06 ± 0.86 c	1.07 ± 0.02 b	18 ± 1 d
4-ethylphenol	nd	0.08 ± 0.00 a	nd	nd	nd	1.11 ± 0.14 b
4-vinylguaiacol	4.38 ± 0.08 a	5.73 ± 0.79 a	116 ± 11 b	151 ± 14 b	35 ± 2 c	352 ± 8 d
(*E*)-isoeugenol	1.79 ± 0.09 a	1.38 ± 0.07 a	0.91 ± 0.14 [b,c]	1.33 ± 0.05 ab	0.68 ± 0.01 c	3.70 ± 0.57 d
4-vinylphenol	121 ± 2 a	247 ± 16 b	12606 ± 1440 c	20904 ± 3263 ce	6231 ± 120 d	27863 ± 2764 e
vanillin derivatives						
vanillin	0.50 ± 0.01 a	1.08 ± 0.01 b	2.22 ± 0.23 c	3.81 ± 0.10	2.36 ± 0.06	8.21 ± 0.68
methyl vanillate	0.10 ± 0.00 a	nd	nd	nd	nd	1.16 ± 0.09 b
acetovanillone	nd	nd	nd	0.56 ± 0.09 a	nd	2.19 ± 0.00 b
homovanillyl alcohol	nd	nd	nd	nd	1.18 ± 0.00	nd
homovanillinic acid[b]	5.21 ± 0.10 a	4.10 ± 0.24 b	nd	nd	nd	83 ± 4 c
benzenes						
benzaldehyde	0.74 ± 0.06 a	1.86 ± 0.01 b	3.69 ± 0.35 c	8.13 ± 0.68 d	3.07 ± 0.12 c	11 ± 1 e
phenylacetaldehyde	0.67 ± 0.01 a	0.87 ± 0.10 a	3.24 ± 0.19 b	4.35 ± 0.27 c	nd	4.46 ± 0.43 c
benzyl alcohol	1.69 ± 0.21 a	3.14 ± 0.37 b	21 ± 1 c	59 ± 1 d	10 ± 1 e	1361 ± 40 f
β-phenylethanol	nd	1.90 ± 0.08 a	4.54 ± 0.05 b	9.61 ± 0.27 c	3.21 ± 0.19 d	97 ± 4 e
ethyl cinamate	nd	nd	7.09 ± 0.09 a	23 ± 1 b	18 ± 1 c	3.21 ± 0.19 d
2-phenoxyethanol	1.03 ± 0.04 a	1.38 ± 0.24 ac	0.64 ± 0.11 b	0.96 ± 0.01 a	0.54 ± 0.06 b	1.85 ± 0.14 c
benzoic acid	7.10 ± 0.93 a	10 ± 1 b	113 ± 17 c	210 ± 19 d	44 ± 3 e	240 ± 7 d
dihydromethyleugenol[b]	nd	nd	0.20 ± 0.03 a	0.41 ± 0.01 b	0.18 ± 0.01 a	3.20 ± 0.17 c
lactones						
δ-octalactone[c]	nd	0.47 ± 0.01 a	1.09 ± 0.01 bc	1.47 ± 0.01 b	1.08 ± 0.00 c	nd
γ-nonalactone[c]	nd	0.68 ± 0.01 a	nd	nd	nd	0.86 ± 0.00 b
γ-decalactone[c]	nd	0.10 ± 0.01 a	7.54 ± 0.00 b	17 ± 1 c	nd	1.08 ± 0.03 d
pantolactone	2.49 ± 0.03 a	1.18 ± 0.11 b	6.49 ± 0.49 c	8.47 ± 0.98 c	nd	nd
miscellaneous						
(*Z*)-3-hexen-1-ol	nd	1.16 ± 0.01 a	2.03 ± 0.01 b	2.19 ± 0.09 b	2.17 ± 0.12 b	13 ± 1 c
(*E*)-2-hexen-1-ol	4.37 ± 0.52 a	4.95 ± 0.52 a	2.87 ± 0.15 b	2.90 ± 0.10 b	3.00 ± 0.17 b	5.80 ± 0.17 c
ethyl decanoate	4.34 ± 0.05 a	4.38 ± 0.02 a	4.36 ± 0.02 a	nd	4.28 ± 0.00 a	nd
2-ethylhexanoic acid	1.30 ± 0.22 a	1.20 ± 0.06 a	1.13 ± 0.01 a	1.07 ± 0.01 a	1.87 ± 0.22 ab	2.19 ± 0.09 b
4-methoxy-2,5-dimethyl-3(2*H*)-furanone (mesifurane)[b]	50 ± 5 a	338 ± 4 b	339 ± 19 b	315 ± 23 c	251 ± 6 d	307 ± 9 c
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol)[b]	23 ± 1 a	58 ± 2 b	74 ± 1 c	102 ± 3 d	60 ± 1 b	74 ± 3 c
cinnamic acid[b]	338 ± 18 a	586 ± 61 b	2917 ± 226 c	7657 ± 555 d	1828 ± 80 e	6209 ± 119 d

[a]Concentrations of the same compound with different letters show significant differences ($p < 0.05$). nd, not detected. [b]Chemical standard not available; tentatively identified. Data are relative areas (to 4-methyl-2-pentanol × 1000). [c]Data are the relative areas (to 4-methyl-2-pentanol × 1000). Chemical standard available, but the degradation of the products did not allow quantification.

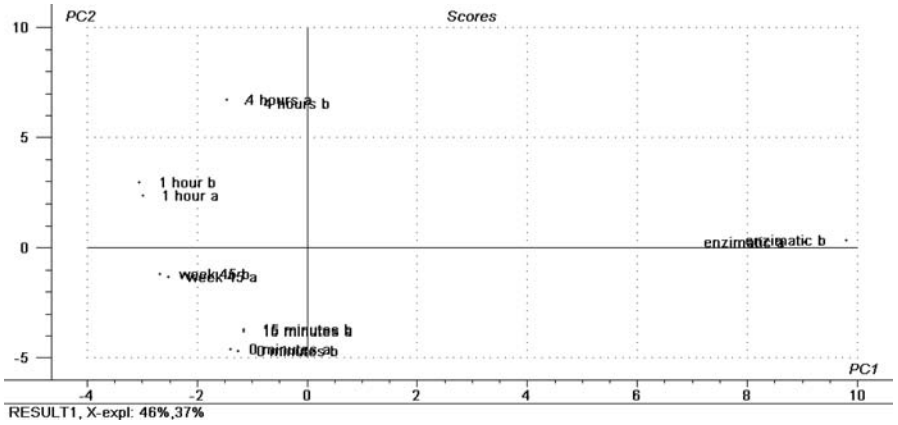

Figure 1. Principal component plot showing the scores for the samples of acid and enzymatic hydrolysis.

compounds were as follows: (*Z*)-rose oxide, linalool, α-terpineol, nerol, geraniol, benzaldehyde, β-phenylethanol, and 2-phenoxyethanol were purchased from Fluka. β-Ionone was sourced from Sigma (St. Louis, MO, USA), and guaiacol, *m*-cresol, eugenol, 4-ethylphenol, 4-vinylguaiacol, methyl vanillate, acetovanillone, zingerone, homovanillyl alcohol, phenylacetaldehyde, benzyl alcohol, ethyl cinnamate, γ-nonalactone, γ-decalactone, (*Z*)-3-hexen-1-ol were from Aldrich (Gillingham, U.K.). (*E*)-Isoeugenol, 4-vinylphenol, δ-octalactone, and δ-decalactone were purchased from Lancaster (Strasbourg, France). Finally, β-damascenone and vanillin were supplied by Firmenich (Geneva, Switzerland) and Panreac (Barcelona, Spain), respectively.

Samples. We employed freshly purchased *Fragaria ananassa* var. Camarosa strawberries to optimize the extraction method and to obtain the aroma precursors extract. Aromatic precursors were then determined in four different varieties of strawberry: Fuentepina, Camarosa, Candonga, and Sabrina. These strawberries were also employed for the olfactometric studies.

Extraction of Aroma Precursors. To study the effects of different kinds of hydrolysis, we prepared a precursors pool from strawberries of Camarosa variety acquired in the market. The preparation procedure was based on that of Ibarz et al.[7] We used an Ultra Turrax T25 Basic mixer (Ika, Labortechnik) to crush and homogenize 2 kg of strawberries with 1 L of cold Milli-Q water in the presence of 0.13 M NaF, to prevent microbial growth, and 50 mg/L of ascorbic acid (as an antioxidant). This mixture was then centrifuged and filtered, obtaining a strawberry must, which was placed in Pyrex flasks to which 2 g of LiChrolut resins (previously preconditioned with dichloromethane, methanol, and Milli-Q water) per kilogram of strawberry was added. The oxygen of the flasks was evacuated using nitrogen. We left the must in contact with the resins for 16 h in a Heidolph Promax 1020 shaker (Schwabach, Germany) at 90 rpm. We packaged the resin, and each cartridge of 500 mg was washed with 50 mL of water. It was then completely vacuum-dried, and free aromas were extracted with 50 mL of dichloromethane and discarded. Thirty milliliters of an ethyl acetate/methanol solution (9:1) was subsequently percolated through the resin. The solvents were evaporated under vacuum, resuspended in a 50:50 ethanol/water solution, and kept at −20 °C.

To analyze the four different strawberry varieties, we followed the same technique as that utilized for obtaining the precursors pool. In this case we processed 10 g of strawberry because we obtained the best results in previous studies using that quantity (data not shown). The must was percolated through a 200 mg LiChrolut EN cartridge (previously preconditioned with 10 mL of dichloromethane, 10 mL of methanol, and 10 mL of Milli-Q water). After that, the column was washed with 20 mL of Milli-Q water and then was completely dried. To eliminate all free aromatic compounds, we passed 20 mL of dichloromethane through the cartridge. To recover the precursors from the resin we employed 20 mL of a solution of ethyl acetate/methanol (9:1). This eluate was concentrated to 1 mL under vacuum at 40 °C and then taken to dryness under a gentle nitrogen stream. Each sample was extracted in duplicate.

Acid and Enzymatic Hydrolysis. Different hydrolytic conditions were performed to study their influence on the aromatic profile of strawberry using the precursors pool previously obtained. The acid hydrolyses assayed were 15 min and 1 and 4 h at 100 °C and 1 week at 45 °C. For this hydrolysis we mixed 8 mL of citric buffer (0.2 M, pH 2.5), 1 mL of the precursor extract, and 1 mL of an ethanol/water solution (50:50) (to maintain the same concentration of ethanol in all of the acid hydrolysis assays in a 20 mL vial). After this, the vial was sealed and placed in the oven. Moreover, an enzymatic hydrolysis was performed during 16 h at 38 °C. In this case we used 8.7 mL of citrate (0.1 M)/phosphate (0.2 M) buffer solution at pH 5, 1 mL of the precursor extract, which was subjected to vacuum to remove the ethanol, and 800 μL of a pectinase enzyme solution with 200 mg/mL of AR 2000.

Otherwise, for the analysis of the four varieties of strawberry, the dry extract was reconstituted in 10 mL of citric buffer (0.2 M, pH 2.5, 10% EtOH) and was subjected to hydrolysis at 100 °C for 1 h. Before any hydrolysis was undertaken, the remaining oxygen was displaced from the vial with nitrogen to prevent oxidation of the compounds during the process. Each hydrolysis was done in duplicate.

Extraction of Volatiles Released in the Hydrolysis. After the hydrolysis, the solution was percolated through a 50 mg LiChrolut EN cartridge (previously preconditioned with 6 mL of dichloromethane, 2 mL of methanol, and 2 mL of citric buffer solution) and then was washed with 1 mL of Milli-Q water and dried. To elute the aromatic compounds, 700 μL of dichloromethane was passed through the column and collected in a Kuderna Danish (Supelco, Bellefonte, PA, USA); 14 μL of the internal standard 4-methyl-2-pentanol (402.6 μg/g) was added. Finally, the solution was concentrated to 100 μL with a gentle nitrogen stream.

Preparation of the Olfactometry Extract. To obtain a representative extract of each strawberry variety for the olfactometry analyses, we followed the method used by Ferreira et al.[21] Eighty grams of the fruit was crushed and placed in a purge and trap system.[22] A Lichrolut EN cartridge was placed on top of the bubbler flask. A nitrogen stream of 500 mL/min was applied to the sample for 100 min, releasing the free volatile compounds of strawberry in the headspace being trapped by the cartridge. Finally, these compounds were eluted with 3.2 mL of dichloromethane containing 5% methanol. The extract was concentrated to a final volume of 200 μL.

GC-MS and GC-O Analytical Conditions. GC analysis of the volatiles released in the hydrolysis was performed with a CP-3800 chromatograph coupled to a Saturn 2200 ion trap mass spectrometric detection system from Varian (Sunnyvale, CA, USA). A DB-WAXetr capillary column (J&W Scientific, Folsom, CA, USA) (60 m × 0.25 mm i.d., film thickness = 0.5 μm) preceded by a 3 m × 0.25 mm uncoated (deactivated, intermediate polarity) precolumn from Supelco was used. Helium was the carrier gas at a flow rate of 1 mL/min. The oven temperature program was 3 min at 40 °C, ramped at 10 °C/min to 90 °C, ramped at 2 °C/min to 230 °C, and finally held at this temperature for 37 min. Initially, the injector was kept at 35 °C for

Table 2. Concentration (Micrograms per Kilogram of Strawberries Except Where Indicated) of Volatile Compounds Released after Harsh Acid Hydrolysis of the Precursor Extract from Each Strawberry Variety[a]

	Fuentepina	Camarosa	Candonga	Sabrina
terpenes				
α-terpinolene (1)[b]	0.58 ± 0.05 a	0.39 ± 0.01 b	0.24 ± 0.01 c	0.19 ± 0.01 d
(*Z*)-rose oxide (2)	0.02 ± 0.00	nd	nd	nd
(*Z*)-linalool oxide[c] (3)	1.16 ± 0.13 a	nd	nd	7.68 ± 0.34 b
(*E*)-linalool oxide[c] (4)	1.02 ± 0.03 a	nd	nd	4.81 ± 0.46 b
(*R*/*S*)-linalool (5)	9.21 ± 0.23 a	13 ± 1 a	32 ± 3 b	48 ± 2 c
α-terpineol (6)	100 ± 4 a	63 ± 6 b	89 ± 10 b	78 ± 5 b
nerol (7)	0.82 ± 0.09 a	0.93 ± 0.13 a	3.83 ± 0.43 b	6.03 ± 0.42 c
geraniol (8)	18 ± 2 a	22 ± 1 ab	28 ± 2 b	45 ± 5 c
farnesol	nd	nd	9 ± 1 a	18 ± 2 b
δ-terpineol[c] (9)	1.19 ± 0.09 a	0.48 ± 0.03 b	0.59 ± 0.01 b	0.34 ± 0.01 c
norisoprenoids				
β-damascenone (10)	2.00 ± 0.18 a	1.75 ± 0.00 a	1.14 ± 0.14 b	0.65 ± 0.01 c
β-ionone	nd	0.92 ± 0.01 a	nd	0.67 ± 0.04 b
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)[c] (11)	1.09 ± 0.08 a	0.46 ± 0.03 b	0.42 ± 0.01 b	0.08 ± 0.01 c
tert-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB)[c] (12)	3.84 ± 0.07 a	4.34 ± 0.45 a	0.96 ± 0.09 b	0.58 ± 0.02 c
3-oxo-β-ionone[c] (13)	2.01 ± 0.18 a	1.48 ± 0.03 a	0.74 ± 0.04 b	0.44 ± 0.01 c
actinidols[c] (14)	2.84 ± 0.32 a	2.24 ± 0.04 a	0.91 ± 0.01 b	0.75 ± 0.01 c
norisoprenoid 1[c] (15)	0.69 ± 0.04 a	0.73 ± 0.01 a	0.27 ± 0.01 b	0.05 ± 0.01 c
volatile phenols				
m-cresol	nd	0.65 ± 0.02 a	0.47 ± 0.03 b	nd
eugenol	nd	0.91 ± 0.01 a	0.17 ± 0.01 b	0.27 ± 0.03 c
4-vinylguaiacol (16)	76 ± 1 a	31 ± 2 b	31 ± 2 b	26 ± 1 b
4-vinylphenol (17)	8565 ± 92 a	994 ± 73 b	9602 ± 90 c	2426 ± 242 d
vanillin derivatives				
vanillin (18)	2.96 ± 0.08 a	4.12 ± 0.03 b	1.46 ± 0.10 c	1.75 ± 0.19 c
zingerone (19)	0.76 ± 0.01 a	nd	nd	1.07 ± 0.07 b
benzenes				
benzaldehyde (20)	6.82 ± 0.11 a	4.94 ± 0.17 b	3.80 ± 0.35 b	4.74 ± 0.30 b
phenylacetaldehyde (21)	3.60 ± 0.28 a	2.66 ± 0.08 b	2.16 ± 0.03 c	2.16 ± 0.03 c
benzyl alcohol (22)	37 ± 1 a	20 ± 1 b	14 ± 1 c	8.45 ± 0.49 d
β-phenylethanol (23)	9.39 ± 0.62 a	7.55 ± 0.18 a	6.09 ± 0.29 b	6.17 ± 0.37 b
ethyl cinamate (24)	8.71 ± 0.69	nd	nd	nd
2-phenoxyethanol (25)	5.20 ± 0.42 a	7.95 ± 0.67 b	3.29 ± 0.42 c	5.42 ± 0.42 a
benzoic acid (26)	131 ± 12 a	80 ± 7 b	129 ± 3 a	116 ± 5 a
lactones				
δ-octalactone[d] (27)	2.89 ± 0.15 a	2.10 ± 0.18 b	14 ± 0 c	7.65 ± 0.93 d
γ-nonalactone[d] (28)	1.89 ± 0.13 a	1.94 ± 0.16 a	1.42 ± 0.07 b	1.45 ± 0.16 b
γ-decalactone[d] (29)	12 ± 1 a	5.55 ± 0.45 b	23 ± 1 c	26 ± 2 c
pantolactone (30)	1.66 ± 0.01 a	1.28 ± 0.01 a	0.93 ± 0.01 b	0.84 ± 0.01 c
miscellaneous				
(*Z*)-3-hexen-1-ol (31)	5.26 ± 0.41 a	5.21 ± 0.08 a	4.85 ± 0.04 b	4.10 ± 0.00 c
(*E*)-2-hexen-1-ol (32)	19 ± 2 ab	24 ± 1 a	17 ± 1 b	18 ± 1 b
ethyl decanoate (33)	16 ± 1 a	17 ± 0 a	16 ± 0 a	16 ± 0 a
2-ethylhexanoic acid (34)	13 ± 1 a	14 ± 1 ab	13 ± 1 a	15 ± 1 b
4-methoxy-2,5-dimethyl-3(2*H*)-furanone (mesifurane)[c] (35)	5.07 ± 0.02 a	22 ± 1 b	34 ± 1 c	42 ± 1 d
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol)[c] (36)	8.15 ± 0.01 a	39 ± 1 b	16 ± 1 c	19 ± 1 c
cinnamic acid[c] (37)	1678 ± 36 a	178 ± 24 b	850 ± 49 c	877 ± 61 c

[a]Concentrations of the same compound with different letters show significant differences ($p < 0.05$). nd, not detected. [b]Peak number in Figure 2 is given in parentheses. [c]Chemical standard not available. Tentatively identified. Data are relative areas (to 4-methyl-2-pentanol × 1000). [d]Data are the relative areas (to 4-methyl-2-pentanol × 1000). Chemical standard available, but the degradation of the products did not allow quantification.

0.3 min and a pressure pulse of 25 psi for 2.60 min was applied. The injector was then heated to 250 °C at a rate of 200 °C/min. The splitless time was 2.60 min. The injection volume was 4 μL. The global run time was recorded in full scan mode (m/z 40−220 mass range). The chromatographic data were analyzed by Varian Saturn GC-MS version 6.3 software.[23]

To carry out the olfactometric analyses, we followed the protocol described in Ferreira et al.[21] The sensory panel was composed of six expert sniffers. Each strawberry extract was smelled once a day by each panelist. Sniffing time was approximately 30 min. The experiments were carried out in a Thermo 8000 series GC equipped with a flame ionization detector (FID) and a sniffing port (ODO-1 from SGE) connected by a flow splitter to the column exit. The chromatographic conditions were the same as described in Campo et al.[22] Tasters were asked to score the intensity of each aromatic stimulus using a 4-point scale (0 = not detected, 1 = weak, 2 = clear but not intense note,

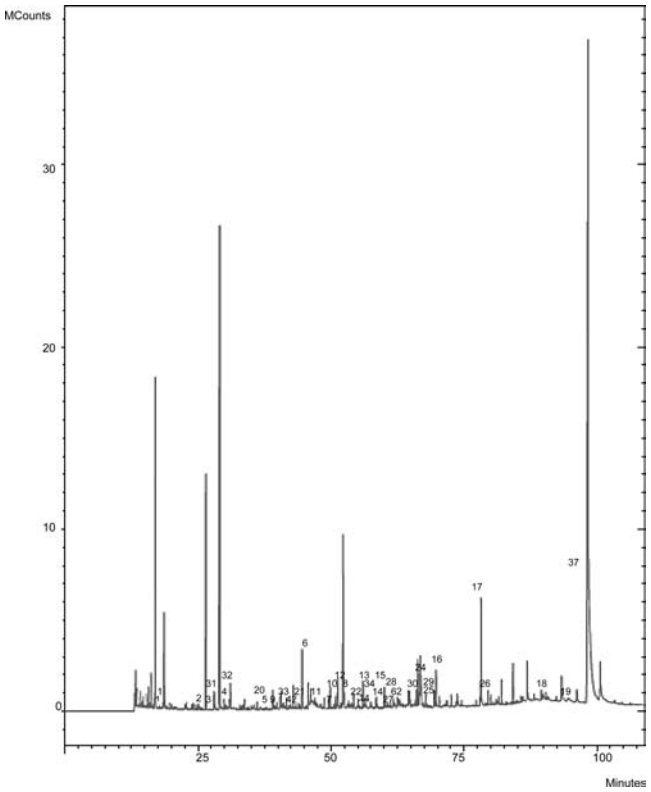

Figure 2. GC-MS chromatographic profile of the strawberry variety Fuentepina.

3 = intense note). Results were expressed as "modified frequency" (MF), calculated with the formula proposed by Dravnieks.[24] The identification of the odorants was done by comparison of their odors, chromatographic retention indices, and MS spectra with those of pure reference compounds.

Data Treatment. Analysis of variance (ANOVA) was performed using the Statistica (version 7.0) software package (Statsoft, Tulsa, OK, USA). Principal component analysis (PCA) was carried out using Unscrambler vs 9.7 from Camo (Oslo, Norway).

RESULTS AND DISCUSSION

Influence of Type of Hydrolysis. In general, the concentrations of the released compounds were very different depending on the type of hydrolysis (Table 1 and Figure 1). PCA was performed to observe which conditions were related to the release of the different compounds. As can be seen in Figure 1, PC1, which explains 47% of the variance, clearly separates the acid hydrolyses from the enzymatic ones. Also, PC2 (35% of the variance) groups the samples as a function of time. As the time of hydrolysis increased, the concentrations of norisoprenoids, volatile phenols, benzenes, and lactones were higher. The behavior of terpenes was heterogeneous. The amounts of α-terpinolene, (*Z*)-rose oxide, and neric acid increased during the harsh hydrolysis, reaching the highest amount after 4 h of the hydrolytic assay. However, the remaining terpenes reached their maximum concentration between 15 min and 1 h of hydrolysis. In the case of vanillin derivatives, each compound followed a different trend. With respect to the miscellaneous group, it is important to mention the cases of Furaneol and cinnamic acid, which increased during hydrolysis, reaching their maximum after 4 h.

Results after leaving the precursors pool for 1 week at 45 °C in citric buffer did not show great differences from the aforementioned hydrolysis. However, hydrolysates from the enzymatic assay were very rich in linalool, 3-oxo-α-ionol, and some volatile phenols such as eugenol, 4-vinylguaiacol, and 4-vinylphenol. Vanillin derivatives were also released more effectively. Moreover, this hydrolysis resulted in an extract with high amounts of benzyl alcohol and β-phenylethanol. With regard to Furaneol, there were no significant differences between 1 h of acid hydrolysis and 1 h of enzymatic hydrolysis. On the other hand, when harsh acid hydrolysis was applied, the release of terpenes, with the exception of linalool, was greater. These results are in accordance with previous studies[8] in which different hydrolytic strategies have been compared. The enzymatic hydrolysis was much more efficient for releasing volatile phenols, vanillin derivatives, and benzenes such as β-phenylethanol and benzyl alcohol than acid hydrolysis.

Despite these results, we decided to apply acid hydrolysis to perform the assays in each strawberry extract due to its similarity with alcoholic fermentation.[8] This was done to compare the results with a hypothetical strawberry fermentation. The time period chosen was 1 h as a compromise between compounds that are degraded after 4 h and those that are not formed earlier than this.

Study of the Aglycones Released from Hydrolysis of Four Strawberry Varieties. With the results obtained after testing the selected strategies taken into account, 1 h of harsh acid hydrolysis was applied for the analysis of minor aromatic compounds released from nonvolatile precursors of the four strawberry varieties.

As can be observed in Table 2, within the analyzed varieties, Fuentepina (Figure 2) proved to have the highest quantity of aromatic compounds present as precursors. After this, Camarosa and Sabrina varieties presented high levels, the Candonga variety being the poorest in these nonaromatic molecules.

In general, among the aglycones quantified, the major ones were linalool, α-terpineol, geraniol, 4-vinylguaiacol, 4-vinylphenol, benzyl alcohol, benzoic acid, γ-decalactone, and cinnamic acid. The presence of 4-vinylphenol in strawberries, especially in Candonga variety, is remarkable because it reached values between 0.9 and 9.6 mg/kg of fruit. This is in agreement with the results obtained by Groyne,[18] who observed a great amount of variability of this compound related to the strawberry variety.

The Sabrina variety was characterized by high amounts of terpenes, presenting discrete values for the rest of the aglycones with respect to the other varieties tested.

One of the most important components of strawberry flavor is 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one (Furaneol),[6] which is responsible for the sweet, caramel, and burnt sugar notes at high concentrations and fruity at lower concentrations. This compound reached the highest levels in Camarosa variety. Another important compound of this fruit is mesifurane, which is described with similar descriptors. In this case, Sabrina showed the highest levels of mesifurane as a glycosidically bound aroma form.

Finally, it is important to remark that XAD-2 Amberlite was the adsorbent employed for the determination of strawberry aromatic precursors in previously published works. In this work we tested the effectiveness of LiChrolut EN cartridges. This resin has been demonstrated as being more efficient that the Amberlite used in previous works by other authors. We identified a total of 51 aglycones with LiChrolut EN resins, 38 of which had previously not been reported in strawberry. Knowing the aromatic potential of the strawberries gives us an idea of the overall final aroma of a product made from this fruit, and therefore we could select the best variety as starting substrate.

Odor Active Compounds Determined Using GC-O. We performed olfactometric analyses of the free aroma compounds of four varieties of strawberry. This extraction technique enables

Table 3. Odor Active Compounds of the Four Strawberry Varieties Analyzed

			% modified frequency[a]			
LRI VF5-MSDBWax	odor descriptor	identity	Fue	Cam	Cdo	Sab
918	solvent, gas, glue	not identified	0	0	31	0
972	dairy product, sweet, buttery	diacetyl	55	61	55	78
1007	fruity, strawberry, sweet	isobutyl acetate	0	48	33	24
1033	fruity, strawberry, sweet	ethyl butanoate	69	59	75	73
1052	fruity, sweet, anise, cream	ethyl 2-methylbutanoate	50	46	29	0
1066	fruity, apple, anise, green, metallic	ethyl 3-methylbutanoate	61	33	69	73
1180	rubber, moisture, gas	not identified	0	0	0	34
1191	fruity, anis	methyl hexanoate	17	0	0	33
1236	fruity, raspberry, strawberry, anise	ethyl hexanoate	43	33	62	55
1303	mushroom, metallic, chlorine, cucumber	1-octen-3-one	51	33	45	53
1312	spicy, green, barbecue, yeast	2-methyl-3-furanthiol	55	0	50	36
1346	floral, sweet, strawberry	(*Z*)-rose oxide	38	0	0	0
1378	geranium, green, pepper, lettuce	(*Z*)-1,5-octadien-3-one	82	51	80	29
1380	tropical, pineapple, citrus, green	methyloctanoate[b]	0	0	61	0
1458	vinegar	acetic acid	67	38	48	38
1548	green, grass, sweet, cucumber	(*E*)-2-nonenal	41	0	0	0
1552	garbage, sulfur, peanuts, barbecue	not identified	0	0	49	0
1563	floral, lemon	(*R*/*S*)-linalool	33	0	0	0
1570	unpleasant, fatty acid, vomit, vinegar	not identified	0	0	0	40
1597	tropical, sweet, caramel, cotton candy	mesifurane[b]	43	33	35	45
1609	strawberry	not identified	31	0	0	0
1626	burnt hair	2-acetylpyrazine	73	61	75	53
1631	cheese, vomit, feet	butyric acid	27	17	35	43
1676	cheese, feet, sweat, milk	isovaleric acid	59	67	61	61
1730	fruity, honey, berry, tropical, sweet, floral	phenyl acetate[b]	0	31	22	38
1826	sweet, floral, rose	*β*-damascenone	0	26	0	41
1850	soil, green, spicy, pepper, peanuts, dry grass	hexanoic acid[b]	65	54	66	58
1865	camphor, barbecue, spicy	guaiacol	45	76	35	59
2052	caramel, strawberry, sweet	Furaneol	82	82	80	85
2100	leather, animal, stable	*p*/*m*-cresol	31	47	33	36
2170	peach, sweet, strawberry	*γ*-decalactone	80	26	85	83
2221	animal, spicy, licorice	sotolon	45	76	0	31
2294	latex, spicy, burnt	not identified	0	0	0	53
2420	coconut, vanillin	*γ*/*δ*-dodecalactone	0	29	25	33

[a]Fue, Fuentepina; Cam, Camarosa; Cdo, Candonga; Sab, Sabrina. [b]Tentatively identified by lineal retention index and odor descriptor.

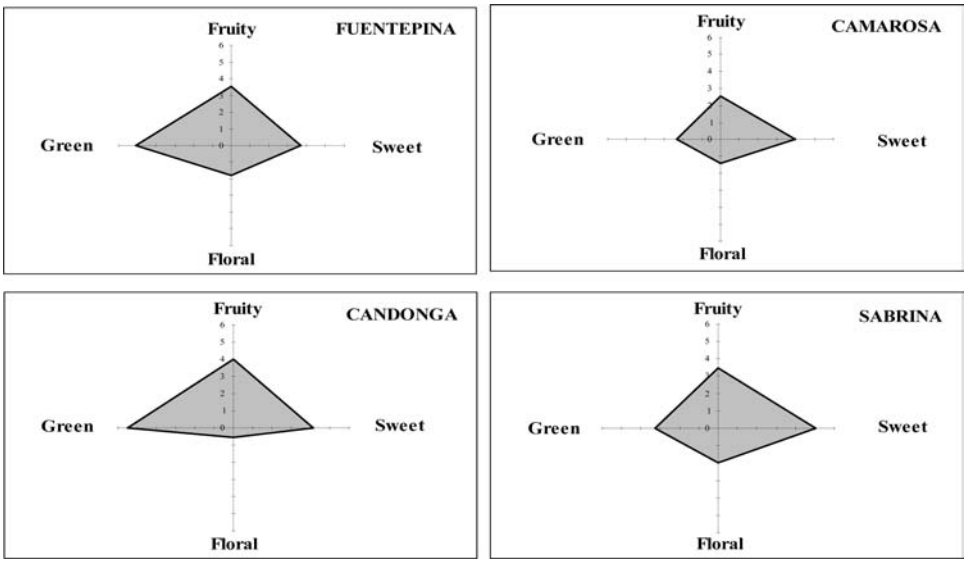

Figure 3. Sensory profile plot of Fuentepina, Camarosa, Candonga, and Sabrina varieties considering fruity, sweet, green, and floral characteres.

us to obtain a more representative extract than other techniques, and therefore it provides a more realistic idea of the overall sample flavor. Thirty-four important odor zones were perceived in the headspace extract. Table 3 shows the modified frequency (MF) of all the perceived odorants; only those with MF > 30 in at least one sample (odor active compounds) are included. Among these perceived aromatic zones, six were not identified.

Within the odor zones that had the greatest impact in the majority of the strawberry varieties, we identified Furaneol, γ-decalactone, ethyl butanoate, ethyl hexanoate, ethyl 3-methylbutanoate, diacetyl, and hexanoic acid, in agreement with other studies.[25,3,4] These compounds, therefore, seem to be responsible for the overall impact aroma of strawberries. They provide caramel-like, fruity, buttery, and sour notes. Furthermore, other odor zones with high MF were perceived in most of the varieties, with unpleasant notes such as cheese/feet/sweat/milk or burnt hair. We identified them as isovaleric acid and 2-acetylpyrazine. Panelists also perceived an odor zone described as geranium/green/pepper/lettuce (LRI = 1378) with an MF > 80 in Fuentepina and Candonga varieties, identified as (*Z*)-1,5-octadien-3-one. This odor zone had been observed by other authors but, to our knowledge, it had not been identified. There are some odor zones that clearly differ one variety from the others. This is the case of the floral/sweet/strawberry (LRI = 1346) and floral/lemon (LRI = 1563) notes identified as (*Z*)-rose oxide and (*R*/*S*)-linalool and which are present in only Fuentepina strawberry. In the Candonga variety tasters perceived a tropical/pineapple/citrus/green (LRI = 1380) odor zone with a high MF (61), tentatively identified as methyl octanoate, which was not perceived in the other strawberries.

As expected, Furaneol reached a high MF (≥80) but mesifurane MF values hovered at 33−45. These compounds, like the rest of the aglycones, are released during the fruit ripening stage, their presence increasing as a free form in ripe strawberry.[18] Depending on the fruit developmental stage, different aglycones will appear. This explains why some data from the precursors analysis (Table 2) do not match the olfactometric results. (*Z*)-Rose oxide is present only as a precursor in the Fuentepina variety and was perceived only in this variety during the olfactometric analysis. Additionally, panelists perceived the peach/sweet/strawberry (LRI = 2170) odor zone identified as γ-decalactone with a very high MF (≥80) in all varieties except for Camarosa. This odor zone reached a low MF (26), a similar situation occurring in the precursors determinations. However, the results obtained in olfactometric and precursors assays for linalool and β-damascenone do not match. As mentioned above, this confirms the staggered release of the aglycones. In conclusion, we could say that there were some odor zones that clearly differ among varieties, being present in only one of the varieties.

We used spider webs to have a general visual comparison of the four strawberry varieties considering fruity, sweet, floral, and green aroma characters (Figure 3). For that purpose, we added the MF of the odor zones of each character type of every strawberry (divided by 10) and then divided by the total of odor zones found for that character during the olfactometric analysis. Differences can be observed among the different strawberry varieties. The Camarosa variety was the least aromatic one because its aromatic zones reached the lowest MF. Green character predominates over sweet in Fuentepina and Candonga; however, in the other two varieties the sweet character is stronger than the other attributes.

In the case of Candonga, the figure shows that the floral character is almost imperceptible compared to the fruity character, which is very high.

In summary, the results suggest that this method is suitable for the determination of glycosidically bound aroma compounds of strawberry. There were several significant differences among varieties with respect to the content in precursors, Fuentepina being the variety that had the highest quantity of aromatic compounds present as precursors. A total of 38 aglycones have been described for the first time in strawberry.

In general, the key odorants were Furaneol, γ-decalactone, ethyl butanoate, ethyl hexanoate, ethyl 3-methylbutanoate, diacetyl, and hexanoic acid. In addition, we could state that the presence of some odor zones clearly differs among varieties. On the other hand, if we consider fruity, sweet, floral, and green aroma characters, the overall aroma of Fuentepina and Candonga varieties presented mainly green notes; however, in the case of Camarosa and Sabrina varieties the aromatic notes were mainly sweet.

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Notes

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ABBREVIATIONS USED

LRI, linear retention index; FID, flame ionization detector; MF, modified frequency; PCA, principal component analysis; ANOVA, analysis of variance; GC-O, gas chromatography−olfactometry; GC-MS, gas chromatography−mass spectrometry.

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