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 non-volatile compounds, present mainly as glycoconjugates formed by a sugar and an aglycone.

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

Non-volatile compounds are, moreover, potential natural sources of aroma because the 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 non-volatile 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-2H-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-2H-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 non-aromatic 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 of 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 4 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), ethyl acetate and sodium fluoride by Fluka (Buchs, Switzerland). Sodium dihydrogen-phosphate 1-hydrate, L (+) ascorbic and citric acids were purchased from Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, U.S.). 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 compounds were as follows: (Z)-rose oxide, linalool, α-terpineol, nerol, geraniol, benzaldehyde, β-phenylethanol, 2-phenoxyethanol were purchased from Fluka (Buchs, Switzerland). β-ionone was sourced from Sigma (St. Louis, MO) 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 from Aldrich (Gillingham, UK). (E)-isoeugenol, 4-vinylphenol, δ-octalactone, 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 in order 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

In order to study the effects of different kinds of hydrolysis we prepared a precursors pool from strawberries of *Camarosa* variety adquired in the market. The preparation procedure was based on 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, adding 2 gr of LiChrolut resins (previously pre-conditioned with dichloromethane, methanol and Milli-Q water) per kg of strawberry. The oxygen of the flasks was evacuated using nitrogen. We left the must in contact with the resins for 16h 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. 30 mL of an ethyl

acetate/methanol solution (9:1) were subsequently percolated through the resin. The solvents were evaporated under vacuum, resuspended in a 50:50 ethanol/water solution and kept at -20ºC.

To analyze the 4 different strawberry varieties, we followed the same technique as that utilized for obtaining the precursors pool. In this case we processed 10 gr of strawberry since 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 pre-conditioned with 10 mL dichloromethane, 10 mL methanol and 10 ml Milli-Q water). After that, the column was washed with 20 mL of Milli-Q water and then was completely dried. In order 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 ºC 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 in order to study their influence on the aromatic profile of strawberry using the precursors pool previously obtained. The acid hydrolyses assayed were: 15 min, 1 h and 4 h at 100 °C and one 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 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 ºC for one hour. Before any hydrolysis was undertaken, the remaining oxygen was displaced from the vial with nitrogen in order 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 pre-conditioned with 6 mL dichloromethane, 2 mL methanol and 2 mL of citric buffer solution). Then was washed with 1 mL Milli–Q water and dried. To elute the aromatic compounds, 700 µL of dichloromethane were passed through the column and collected in a Kuderna Danish Supelco (Bellefonte, PA, USA), adding 14 µL of the internal standard 4-methyl-2-pentanol (402.6 µg/g). Finally 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]. 80 g of the fruit were crushed and placed in a purge and trap system[22]. A Lichrolut EN cartridge was placed on the 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

Gas chromatographic 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 (Bellefonte, PA, USA) 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, 10 °C/min up to 90 °C, 2 °C/min up to 230 °C and finally held at this temperature for 37 min. Initially the injector was kept at 35 °C for 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 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 (40–220 m/z 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., (2009). 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., (2005). 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, 3=intense note). Results were expressed as "modified frequency" (MF), calculated with the formula proposed by Dravnieks[24]. The identification of the odorants

199 was done by comparison of their odors, chromatographic retention index and MS
200 spectra with those of pure reference compounds.

201 **Data treatment**

202 Analysis of variance (ANOVA) was performed using Statistica (version 7.0) software
203 package (Statsoft, Tulsa, USA). Principal component analysis (PCA) was carried out
204 using Unscramble vs. 9.7 from Camo (Norway).

205 **RESULTS AND DISCUSSION**

206 **Influence of type of hydrolysis**

207 In general, the concentrations of the released compounds were very different depending
208 on the type of hydrolysis (Table 1 and Figure 1). Principal component analysis (PCA)
209 was performed in order to observe which conditions were related to the release of the
210 different compounds. As can be seen in Figure 1, PC1 which explains 47% of the
211 variance, clearly separates the acid hydrolyses from the enzymatic ones. Also, PC2
212 (35% of the variance) groups the samples in function of time. As the time of hydrolysis
213 increased, the concentration of norisoprenoids, volatile phenols, benzenes and lactones
214 was higher. The behavior of terpenes was heterogeneous. The amounts of α-terpinolene,
215 (Z)-rose oxide and neric acid increased during the harsh hydrolysis, reaching the highest
216 amount after four hours of the hydrolytic assay. However, the remaining terpenes
217 reached their maximum concentration between 15 min. and 1 h of hydrolysis. In the
218 case of vanillin derivatives, each compound followed a different trend. With respect to
219 the miscellaneous group, it is important to mention the cases of furaneol and cinnamic
220 acid, which increased during hydrolysis, reaching their maximum after 4 h.

221 Results after leaving the precursors pool one week at 45 ºC in citric buffer did not show
222 great differences over the aforementioned hydrolysis. However, hydrolysates from the
223 enzymatic assay were very rich in linalool, 3-oxo-α-ionol and some volatile phenols

224 such as eugenol, 4-vinylguaiacol and 4-vinylphenol. Vanillin derivatives were also
225 released more effectively. Moreover, this hydrolysis resulted in an extract with high
226 amounts of benzyl alcohol and β-phenylethanol. With regard to furaneol there were no
227 significant differences between 1 h of acid or enzymatic hydrolysis. On the other hand,
228 when applying harsh acid hydrolysis the release of terpenes with the exception of
229 linalool, was greater. These results are in accordance with previous studies[8] in which
230 different hydrolytic strategies have been compared. The enzymatic hydrolysis was much
231 more efficient for releasing volatile phenols, vanillin derivatives and benzenes such as
232 β-phenylethanol and benzyl alcohol than acid hydrolysis.
233 Despite these results, we decided to apply acid hydrolysis to perform the assays in each
234 strawberry extract due to its similarity with alcoholic fermentation[8]. This was done in
235 order to compare the results with a hypothetical strawberry fermentation. The time
236 period chosen was one hour as a compromise between compounds which are degraded
237 after 4 h and those which are not formed earlier than this.
238 **Study of the aglycones released from hydrolysis of four strawberry varieties**
239 Taking into account the results obtained after testing the selected strategies, 1 h of harsh
240 acid hydrolysis was applied for the analysis of minor aromatic compounds released
241 from non-volatile precursors of the four strawberry varieties.
242 As can be observed in Table 2, within the analyzed varieties, *Fuentepina* (Figure 2)
243 proved to have the highest quantity of aromatic compounds present as precursors. After
244 this, *Camarosa* and *Sabrina* varieties presented high levels, the *Candonga* variety being
245 the poorest in these non-aromatic molecules.
246 In general, among the aglycones quantified, the major ones were linalool, α-terpineol,
247 geraniol, 4-vinylguaiacol, 4-vinylphenol, benzyl alcohol, benzoic acid, γ-decalactone
248 and cinnamic acid. The presence of 4-vinylphenol in strawberries, especially in

249 *Candonga* variety, is remarkable because reached values between 0.9-9.6 mg/kg of
250 fruit. This is in agreement with the results obtained by Groyne[18], which observed a great
251 amount and variability of this compound related to the strawberry variety.
252 The Sabrina variety was characterized by high amounts of terpenes, presenting discrete
253 values for the rest of the aglycones with respect to the other varieties tested.
254 One of the most important components of strawberry flavor is 2,5-dimethyl-4-hydroxy-
255 2H-furan-3-one (furaneol)[6], responsible of the sweet, caramel, burnt sugar notes at high
256 concentrations and fruity at lower concentrations. This compound reached the highest
257 levels in *Camarosa* variety. Another important compound of this fruit is mesifurane,
258 which is described with similar descriptors. In this case, *Sabrina* accounted the highest
259 levels of mesifurane as glycosidically bound aroma form.
260 Finally, it is important to remark that XAD-2 Amberlite was the adsorbent employed for
261 the determination of strawberry aromatic precursors in previously published works. In
262 this work we tested the effectiveness of LiChrolut EN cartridges. This resin has been
263 demonstrated as being more efficient that the Amberlite used in previous works by other
264 authors. We identified a total of 51 aglycones with LiChrolut EN resins, 38 of which
265 had previously not been reported in strawberry. Knowing the aromatic potential of the
266 strawberries gives us an idea of the overall final aroma of a product made from this fruit
267 and therefore we could select the best variety as starting substrate.
268 **Odor active compounds determined using GC-O**
269 We performed olfactometric analyses of the free aroma compounds of 4 varieties of
270 strawberry. This extraction technique enables us to obtain a more representative extract
271 than other techniques and therefore it provides a more realistic idea of the overall
272 sample flavor. Thirty-four important odor zones were perceived in the headspace
273 extract. Table 3 shows the modified frequency (MF) of all the perceived odorants, only

274 those with MF higher than 30 in at least one sample (odor active compounds) being
275 included. Among these perceived aromatic zones, 6 were not identified.
276 Within the odor zones which had the greatest impact in the majority of the strawberry
277 varieties, we identified furaneol, γ-decalactone, ethyl butanoate, ethyl hexanoate, ethyl-
278 3-methylbutanoate, diacetyl and hexanoic acid, in agreement with other studies[25,3,4].
279 These compounds, therefore, seem to be responsible for the overall impact aroma of
280 strawberries. They provide caramel-like, fruity, buttery and sour notes. Furthermore,
281 other odor zones with high MF were perceived in most of the varieties, with unpleasant
282 notes such as cheese/feet/sweat/milk or burnt hair. We identified them as isovaleric acid
283 and 2-acetylpyrazine. Panelists also perceived an odor zone described as
284 geranium/green/pepper/lettuce (LRI=1378) with an MF higher than 80 in *Fuentepina*
285 and *Candonga* varieties, identified as (Z)-1,5-octadien-3-one. This odor zone had been
286 observed by other authors but, to our knowledge, it had not been identified. There are
287 some odor zones that clearly differ one variety from the others. This is the case of the
288 floral/sweet/strawberry (LRI=1346) and floral/lemon (LRI=1563) notes identified as
289 (Z)-rose oxide and (R/S)-linalool and which are only present in *Fuentepina* strawberry.
290 In the *Candonga* variety tasters perceived a tropical/pineapple/citrus/green (LRI=1380)
291 odor zone with a high MF (61), tentatively identified as methyloctanoate, which was not
292 perceived in the other strawberries.
293 As expected, furaneol reached a high MF (≥80) but mesifurane MF values hovered at
294 33-45. These compounds, like the rest of the aglycones, are released during the fruit
295 ripening stage, their presence increasing as a free form in ripe strawberry[18]. So,
296 depending on the fruit developmental stage, different aglycones will appear. This
297 explains why some data from the precursors analysis (Table 2) does not match with the
298 olfactometric results. (Z)-Rose oxide is only present as a precursor in the *Fuentepina*

299 variety and was only perceived in this variety during the olfactometric analysis.
300 Additionally, panelists perceived the peach/sweet/strawberry (LRI=2170) odor zone
301 identified as γ-decalactone, with a very high MF (≥80) in all varieties except for
302 *Camarosa*. This odor zone reached a low MF (26), a similar situation occurring in the
303 precursors determinations. However, the results obtained in olfactometric and
304 precursors assays for linalool and β-damascenone do not match. As mentioned above,
305 this confirms the staggered release of the aglycones. In conclusion we could say that
306 there were some odor zones that clearly differ among varieties, being only present in
307 one of the varieties.
308 We used spider webs to have a general visual comparison of the four strawberry
309 varieties considering fruity, sweet, floral and green aroma characters (Figure 3). For that
310 purpose, we added the MF of the odor zones of each character type of every strawberry
311 (divided by 10), and then divided by the total of odor zones found of that character
312 during the olfactometric analysis. Differences can be observed among the different
313 strawberry varieties. The *Camarosa* variety was the least aromatic one since their
314 aromatic zones reached the lowest MF. Green character predominates over sweet in
315 *Fuentepina* and *Candonga*; however in the other two varieties the sweet character is
316 stronger than the other attributes.
317 In the case of *Candonga*, the figure shows that the floral character is almost
318 imperceptible compared to the fruity character, which is very high.
319 In summary, the results suggest that this method is suitable for the determination of
320 glycosidically bound aroma compounds of strawberry. There were several significant
321 differences among varieties with respect to the content in precursors, *Fuentepina* being
322 the variety which had the highest quantity of aromatic compounds present as precursors.
323 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 differ 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.

Abbreviations Used

Linear retention index (LRI)

Flame ionization detector (FID)

Modified frequency" (MF),

Principal component analysis (PCA)

Analysis of variance (ANOVA)

Gas chromatography olfactometry (GC-O)

Gas chromatography mass spectrometry (GC-MS)

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FIGURE CAPTIONS

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

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

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

Table 1. Concentration (μg/kg of Strawberries Except Where Indicated [x]) of Volatile Compounds Released After Harsh Acid and Enzymatic Hydrolysis of the Strawberry Precursors Pool.

	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 [x]	nd	nd	3.01±0.27 [a]	2.84±0.09 [a]	0.68±0.04 [b]	nd
δ-terpineol [x]	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) [x]	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) [x]	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 [x]	nd	1.33±0.03 [a]	4.51±0.42 [b]	4.65±0.21 [b]	2.77±0.14 [c]	nd
actinidols [x]	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 [x]	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 [a,b]	0.68±0.01 [c]	3.70±0.57 [d]
4-vinylphenol	121± 2 [a]	247±16 [b]	12606±1440 [c]	20904±3263 [c,e]	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 [x]	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 [a,c]	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]
dihydromethyl-eugenol [x]	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 [y]	nd	0.47±0.01 [a]	1.09±0.01 [b,c]	1.47±0.01 [b]	1.08±0.00 [c]	nd
γ-nonalactone [y]	nd	0.68±0.01 [a]	nd	nd	nd	0.86±0.00 [b]
γ-decalactone [y]	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 [a,b]	2.19±0.09 [b]
4-methoxy-2,5-dimethyl-3(2H)furanone (mesifurane) [x]	50±5 [a]	338±4 [b]	339±19 [b]	315±23 [c]	251±6 [d]	307±9 [c]
4-hydroxy-2,5-dimethyl-3(2H)furanone (furaneol) [x]	23±1 [a]	58±2 [b]	74±1 [c]	102±3 [d]	60±1 [b]	74±3 [c]
cinnamic acid [x]	338±18 [a]	586±61 [b]	2917±226 [c]	7657±555 [d]	1828±80 [e]	6209±119 [d]

nd: non detected.

[x] Chemical standard not available. Tentatively identified. Data are relative areas (to 4-Methyl-2-pentanol x 1000).

[y] Data are the relative areas (to 4-Methyl-2-pentanol x 1000). Chemical standard available,but the degradation of the products did not allow quantification.

Concentrations of the same compound with different letter show significant differences ($p < 0.05$).

Table 2. Concentration (μg/kg of Strawberries Except Where Indicated [x]) of Volatile Compounds Released After Harsh Acid Hydrolysis of the Precursor Extract from Each Strawberry Variety.

	Fuentepina	Camarosa	Candonga	Sabrina
Terpenes				
α-terpinolene (1)	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 [x] (3)	1.16±0.13 [a]	nd	nd	7.68±0.34 [b]
(E)-linalool oxide [x] (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 [a,b]	28±2 [b]	45±5 [c]
Farnesol	nd	nd	9±1 [a]	18±2 [b]
δ-terpineol [x] (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) [x] (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) [x] (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 [x] (13)	2.01±0.18 [a]	1.48±0.03 [a]	0.74±0.04 [b]	0.44±0.01 [c]
Actinidols [x] (14)	2.84±0.32 [a]	2.24±0.04 [a]	0.91±0.01 [b]	0.75±0.01 [c]
norisoprenoid 1 [x] (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 [y] (27)	2.89±0.15 [a]	2.10±0.18 [b]	14±0 [c]	7.65±0.93 [d]
γ-nonalactone [y] (28)	1.89±0.13 [a]	1.94±0.16 [a]	1.42±0.07 [b]	1.45±0.16 [b]
γ-decalactone [y] (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 [a,b]	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 [a,b]	13±1 [a]	15±1 [b]
4-methoxy-2,5-dimethyl-3(2H)furanone (mesifurane) [x] (35)	5.07±0.02 [a]	22±1 [b]	34±1 [c]	42±1 [d]

4-hydroxy-2,5-dimethyl-3(2H)furanone (furaneol) [x] (36)	8.15±0.01 [a]	39±1 [b]	16±1 [c]	19±1 [c]
cinnamic acid [x] (37)	1678±36 [a]	178±24 [b]	850±49 [c]	877±61 [c]

nd: non detected.
(num): peak number in Figure 2.
[x] Chemical standard not available. Tentatively identified. Data are relative areas (to 4-Methyl-2-pentanol x 1000).
[y] Data are the relative areas (to 4-Methyl-2-pentanol x 1000). Chemical standard available,but the degradation of the products did not allow quantification.
Concentrations of the same compound with different letter show significant differences ($p < 0.05$).

Table 3. Odour Active Compounds of the Four Strawberry Varieties Analysed.

LRI VF5-MSDBWax	Odour descriptor	Identity	% Modified frequency			
			Fue	Cam	Cdo	Sab
918	solvent, gas, glue	n.i	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, anis, cream	ethyl 2-methylbutanoate	50	46	29	0
1066	fruity, apple, anis, green, metallic	ethyl-3-methylbutanoate	61	33	69	73
1180	rubber, moisture, gas	n.i	0	0	0	34
1191	fruity, anis	methylhexanoate	17	0	0	33
1236	fruity, raspberry, strawberry, anis	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 *	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	n.i	0	0	49	0
1563	floral, lemon	(R/S)-linalool	33	0	0	0
1570	unpleasant, fatty acid, vomit, vinegar	n.i	0	0	0	40
1597	tropical, sweet, caramel, cotton candy	mesifurane *	43	33	35	45
1609	strawberry	n.i	31	0	0	0
1626	burnt hair	2-acetyl pyrazine	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 *	0	31	22	38
1826	sweet, floral, rose	β-damascenone	0	26	0	41
1850	soil, green, spicy, pepper, peanuts, dry grass	hexanoic acid *	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	n.i	0	0	0	53
2420	coconut, vanillin	γ/δ-dodecalactone	0	29	25	33

n.i: not identified
* Tentatively identified by Lineal Retention Index and odour descriptor.
Fue: Fuentepina; Cam: Camarosa; Cdo: Candonga; Sab: Sabrina.

Figure 1.

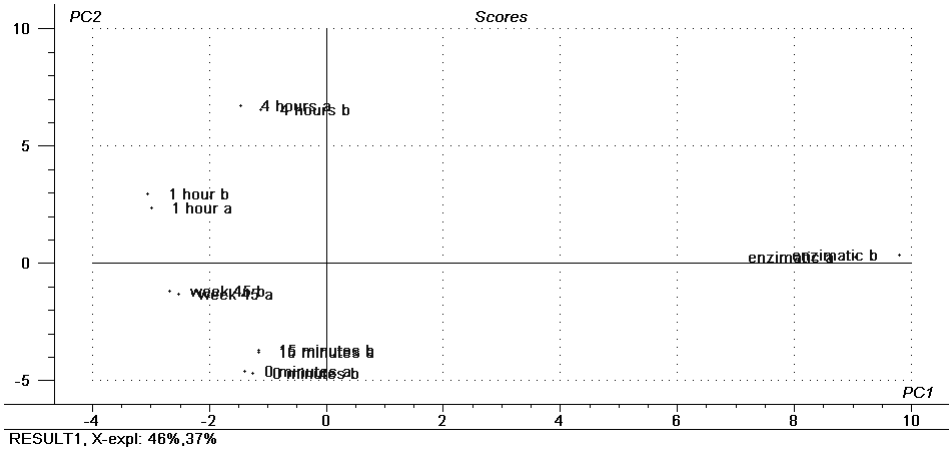
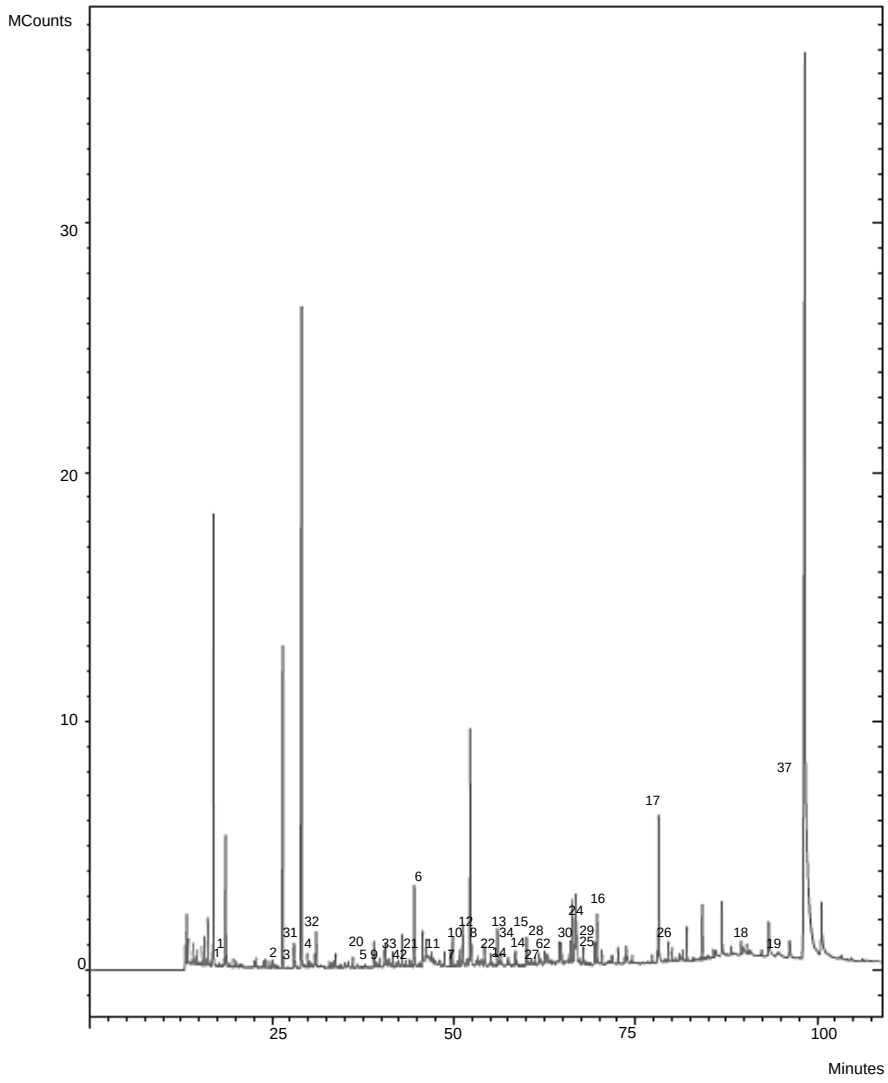

PC2
Scores
10
5
0
-5
4 hours a
4 hours b
1 hour b
1 hour a
enzimatic b
week 15 a
15 minutes b
-4
-2
0
2
4
6
8
10
PC1
RESULT1, X-expl: 46%,37%

Figure 2.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10
0
25
50
75
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Minutes
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2
3
31
32
4
20
5
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21
42
11
6
7
10
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12
22
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62
27
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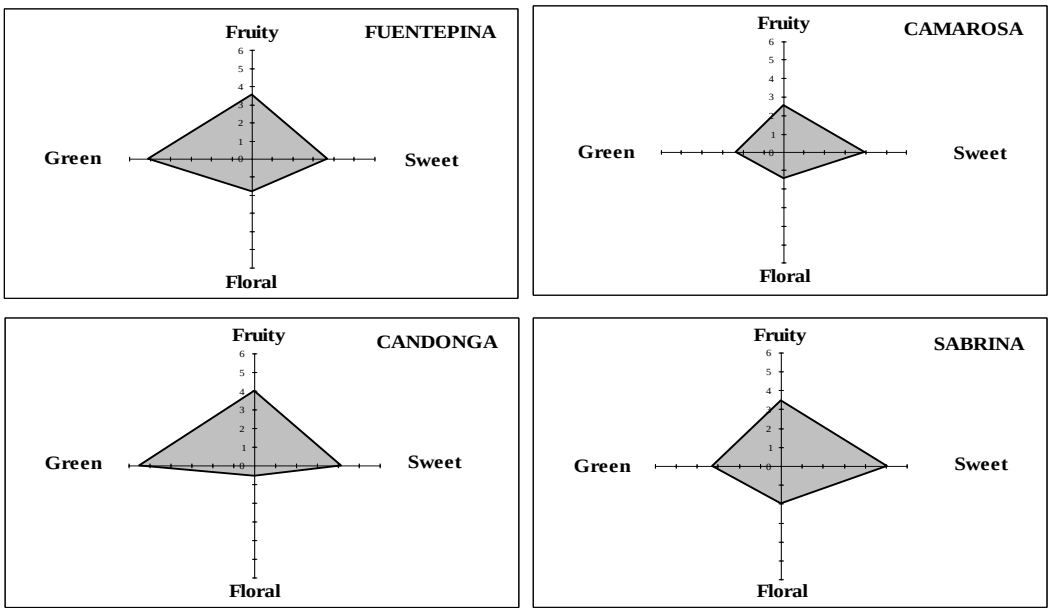
37

Figure 3.


Fruity
FUENTEPINA
Green
Sweet
Floral
Fruity
CAMAROSA
Green
Sweet
Floral
Fruity
CANDONGA
Green
Sweet
Floral
Fruity
SABRINA
Green
Sweet
Floral

TOC Graphic