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1 **Title:** Variability of free and glycosylated volatiles from strawberries destined for the fresh  
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19 **Abstract:** Free- and glycosylated-volatile profiles of 14 strawberry varieties, 9 for industrial  
20 processing ('Darselect', 'Clery', 'Honey', 'Honeoye', 'Siabel', 'FCMO060', 'Fraise19', 2  
21 'Senga Sangana') and 5 for fresh market ('Gariguet', 'Charlotte', 'CIR121', 2 'Clery' (full-  
22 field and hydroponic)), were compared. All volatiles were analysed by GC-MS. Volatiles  
23 from glycosides were first released by direct enzymatic hydrolysis. The extraction method  
24 was optimised for furaneol, a key component of strawberry aroma. More than 60 volatile  
25 compounds were identified, the most abundant being butyl acetate (average: 17 mg/kg),  
26 furaneol (average: 2 mg/kg) and free hexanoic acid (average: 3 mg/kg). Free-volatile profiles  
27 showed a split between fresh market strawberries, distinguished by esters and carbonyl  
28 molecules like isobutyl acetate or hexanal, and strawberries for processing, distinguished by  
29 molecule like 3-penten-2-one and 1-butanol. The three 'Clery' profiles were different notably  
30 in their hexanal, 4-vinylguaiacol and 3-penten-2-one concentrations. The glycosylated volatile  
31 profiles were similar among most strawberry varieties with, as major glycosylated volatiles,  
32 hexanoic acid (average: 1.7 mg/kg), benzyl alcohol (average: 0.5 mg/kg), gamma-decalactone  
33 (average: 0.5 mg/kg) and coumaran (average: 2.5 mg/kg). The potential for volatile  
34 enhancement by deconjugation was different. Potentially fresh market strawberries had a  
35 volatile increase of 6% against 50% for strawberries for processing.

36 **Keywords:** glycoside, glycosidase, GC-MS, aroma, *Fragaria x ananassa*

## 37 1. Introduction

38 Over 4.3 millions of tons of strawberries are produced each year, out of which 80% to  
39 90% are destined for the fresh market (López-Aranda et al., 2011) and the rest for processing.  
40 Strawberries are found in juices, in jams and in semi-processed food products, which can be  
41 incorporated in yogurt, ice cream or pastry. Strawberry is also the most appreciated fruit by  
42 French consumers and 92% of them (Bhat, Geppert, Funken, & Stamminger, 2015) like  
43 strawberries because of their specific aroma. Therefore, aromatic potential of strawberries for  
44 processing interest for future process optimisation to preserve or enhance the aromatic power  
45 of strawberries.

46 The molecules most impacting strawberry aroma (Nuzzi, Lo Scalzo, Testoni, &  
47 Rizzolo, 2008) are furaneol, mesifuran, ethyl butanoate, ethyl hexanoate,  $\beta$ -linalool and  
48 hexanal. They are responsible for “caramel”, “apple-like”, “fruity” and “green” notes,  
49 respectively, in strawberries. Several molecules are not ubiquitous but are important for the  
50 aroma of strawberries in which they are found. This is the case of gamma-decalactone with  
51 “peach-like” note found in ‘Elsanta’ or ‘Senga Sangana’ varieties or trans-nerolidol with  
52 “floral” note found in ‘Elvira’ or ‘Pandera’ varieties (Larsen, Poll, & Olsen, 1992). Despite its  
53 complexity, scientists agree that furaneol is the key molecule (Wein et al., 2002; Ulrich et al.,  
54 1997). This furanone is obtained by several successive enzymatic transformations of D-  
55 fructose-1,6-diphosphate, the last one of which is a reduction by a quinone oxidoreductase  
56 (Raab, 2006).

57 Various parameters can influence the aroma of strawberries (Perez, Rios, Sanz, &  
58 Olias, 1992; Miszczak, Forney, & Prange, 1995). The first one is genetic. Indeed Aharoni  
59 (2004) compared the aroma profile of wild strawberries (*Fragaria vesca*) and cultivated  
60 strawberries (*Fragaria x ananassa*). Nerolidol dominates among terpenes in cultivated  
61 strawberries, while, in wild strawberries, monoterpenes like  $\beta$ -myrcene and  $\alpha$ -pinene,

62 responsible of the characteristic musky aroma of this species, are more prevalent (Bianchi,  
63 Lovazzano, Lanubile, & Marocco, 2014). Maturity also impacts the strawberry aroma:  
64 Forney, Kalt, & Jordan (2000) showed an increase of methyl butanoate and methyl hexanoate  
65 with maturity and Darbellay, Luisier, Villettaz, Amadò, & Azodanlou (2004) a decrease of  
66 (Z)-3-hexenyl acetate and an increase of ethyl hexanoate during ripening in 'Carezza',  
67 'Darselect' and 'Marmolada' varieties. The atmosphere during packaging also impacts  
68 volatile production: Nielsen & Leufven (2008) showed an increase of ethyl acetate (fermented  
69 molecules) with CO<sub>2</sub>-enriched atmosphere in the 'Korona' variety.

70 However not all volatiles exist as such in the intact raw fruit. Some are formed during  
71 eating or processing by endogenous enzymes acting on precursors. A well-known reaction  
72 cascade is that of lipoxygenase leading to "green" notes from fatty acids (Pérez, Sanz, Olías,  
73 & Olías, 1999). Another major route is that of glycosides, where the flavour-bearing  
74 molecules (aglycones which become volatiles if liberated) are bound to sugars for storage.  
75 The sugar moiety can be a mono- or disaccharide and more rarely a trisaccharide, the first  
76 aglycone-bound sugar always being  $\beta$ -D-glucose (Sarry & Gunata, 2004). These precursors  
77 are present in all fruits, including strawberries. They were studied by Stahl-Biskup, Intert,  
78 Holthuijzen, Stengele, & Schulz (1993) in apples, apricots, blackberries, mangoes, plums and  
79 tomatoes or Gunata, Bayonove, Baumes, & Cordonnier (1985) in grapes. In strawberries,  
80 furaneol, mesifuran, benzyl alcohol, 2-phenylethyl alcohol, hydroxylinalool, 2-  
81 methylbutanoic acid or hexanoic acid can be found as glycosides (Groyne, Lognay, &  
82 Marlier, 1999). For example furaneol is predominantly conjugated to malonyl-D-glucose  
83 (Raab, 2006).

84 These glycosides are traditionally identified and quantified after isolation by solid  
85 phase extraction (SPE) (Young & Paterson, 1995). SPE consists in injecting the fruit extract  
86 on an XAD-2 or a C18-column, which binds the aglycone moieties of the glycosides. The

87 sugars and free volatiles are first eluted by water and dichloromethane, respectively; the  
88 glycosides are thereafter eluted with another solvent (generally methanol) (Ubeda et al., 2012;  
89 Roscher, Herderich, Steffen, Schreier, & Schwab, 1996). This method allows to eliminate  
90 matrix interactions. However, it is not adapted to compare free volatiles to bound volatiles  
91 because of the discrimination that is generated by affinity extraction, and because the natural  
92 evolution of volatiles in fruit matrices is not considered. Indeed, Hampel, Robinson, Johnson,  
93 & Ebeler (2014) showed that this pre-treatment leads to a loss and a discrimination of many  
94 compounds, and can not be used to compare free volatile to glycoconjugated volatiles. They  
95 advised direct hydrolysis to compare quantitatively free and bound volatiles.

96 The aim of this study is to establish direct hydrolysis by AR2000 glycosidase as a  
97 method to compare aroma profiles and aromatic potentials of processing and fresh market  
98 strawberries. Fourteen strawberry batches, 9 for processing (obtained from industrial sources)  
99 and 5 for fresh market (obtained from CIREF (Centre Interrégional de Recherche et  
100 d'Expérimentation de la Fraise, Douville (France))) were characterised. Direct enzymatic  
101 hydrolysis was combined with GC-MS analysis and descriptive statistics to evaluate aromatic  
102 potential of these strawberries.

103

## 104 **2. Material & method**

### 105 **2.1. Plant material**

106 Strawberries for processing were commercial samples harvested in 2015, individually  
107 quick frozen (IQF), and stored at -20°C. Among them, 'FCMO' and 'Fraise19' are a mix of  
108 several strawberry varieties used by the French food industry. Fresh market strawberries were  
109 from CIREF, harvested in 2016, and were frozen at -20°C (Table 1).

110

### 111 **2.2. Reagents and solvents**

112 Pentane (Normapur purity  $\geq 99.9\%$ ) and Ethyl acetate (purity  $\geq 99.9\%$ ) were purchased  
113 from VWR International (Darmstadt, Germany). Enzymatic kits to measure D-  
114 glucose/fructose (ref: E 0139106), D-glucose (ref: E 0716251), L-malic acid (ref: E 0139 068)  
115 and citric acid (ref: E 0139076) were from R-Biopharm (Darmstadt, Germany).  
116 Hydromatrix™ (diatomaceous earth) was from Agilent Technologies (Les Ulis, France). The  
117 internal standard was 4-nonanol (purity  $> 97\%$ ) from Merck Schuchardt (Hohenbrunn,  
118 Germany). The AR2000 enzyme to measure the aromatic potential came from Oenobrand  
119 (Montferrier-sur-Lez, France) as freeze-dried powder (6.17 nkat/mg of powder). For retention  
120 index (RI) calculations in GC-MS, a solution of C7-C30 saturated alkane 1000  $\mu\text{g/mL}$  (ref:  
121 49451-U) from Supelco (Bellefonte, Pennsylvania, USA) was used.

122

## 123 2.3. Fruit characterisation

### 124 2.3.1. Brix and pH

125 Strawberries (about 100 g) were ground and used for measurement of Brix degree  
126 using a digital refractometer PR-101 from Agato (Tokyo, Japan) and of pH using pHmeter  
127 FE20 FireEasy from Mettler Toledo (Viroflay, France).

### 128 2.3.2 Sugars and Acids

129 Sugars (glucose, fructose, sucrose) and main organic acids (citric acid and malic acid)  
130 were quantified by colorimetric enzymatic measurements with kits for food analysis from R-  
131 Biopharm (Darmstadt, Germany) and expressed in  $\text{g.kg}^{-1}$  FW. The measurements were  
132 performed on a 96 well-microplate with a SAFAS (FLX-Xenius, SAFAS, Monaco) equipped  
133 with an automatic injection device (Garcia & Renard, 2014).

134

## 135 2.4 Characterization of Volatiles Compounds

### 136 2.4.1 Sample preparation:



137 A dozen of frozen strawberries (around 150 g) were mashed for 1 min in a blender  
138 (Waring-Nova, Grosseron, St. Herblain, France) (Figure 1). An aliquot (40 g) was sampled  
139 for enzymatic hydrolysis with 40 mg of AR2000. Sodium chloride was added to the rest of  
140 the mashed strawberries (75 g/100 g of strawberry) to inhibit endogenous enzymes. After  
141 another mixing, this was divided: one aliquot of 40 g used for incubation (hydrolysis negative  
142 control) and an aliquot for direct extraction (determination of free volatiles). After incubation  
143 (24 h, 40°C with stirring at 120 rpm), salt was added to the hydrolysed aliquots to have the  
144 same salting-out effect during extraction of volatile compounds by accelerated solvent  
145 extraction.

#### 146 2.4.2 Accelerated solvent extraction (ASE)

147 Hydromatrix™ (13 g) and 4-nonanol (11.24 µg diluted in 100 µL of methanol), as  
148 internal standard, were mixed with 7.5 g of mashed fruit to obtain a homogeneous powder  
149 while inactivating enzymes. The powder was rapidly transferred to a 33 mL pressurized  
150 extraction cell for immediate extraction. The extractor was an ASE 200 system (Dionex,  
151 Sunnyvale, CA). Extraction conditions were as follows: pentane and ethyl acetate (1:2,  
152 mL:mL) as solvent, 10<sup>7</sup> Pa, 40 °C, 5 min preheating then 5 min static incubation. The extract  
153 was concentrated to 1 mL by distillation under vacuum (300 Pa, 25°C, using a Multivapor  
154 R12, Büchi, Rungis, France) then by nitrogen flux prior to gas chromatography.

#### 155 2.4.3 GC-MS

156 Samples (1 µL) were injected into a GC-MS system (Trace1300-ISQ LT; Thermo  
157 scientific, USA) equipped with a TG-WAXMS capillary column [30 m, 0.25 mm i.d., 0.5 µm  
158 film thickness] (Thermo scientific).

159 Injection was in splitless mode at 250 °C. The carrier gas was helium with a constant flow of  
160 1.2 mL.min<sup>-1</sup>. Oven temperature program was 35 °C for 2 min ramped at 5°C.min<sup>-1</sup> to 230°C

161 then held for 5 min. Mass spectra were obtained by electron ionization at 70 eV, with  
162 scanning from  $m/z$  35 to 250 at 2 scans. $s^{-1}$ .

163 Volatile levels were expressed in micrograms per kilograms of juice (ppb) in 4-  
164 nonanol equivalent. Aromatic potential was calculated by difference between hydrolysed  
165 samples and incubated control samples. This aromatic potential was compared to the initial  
166 free volatile profile to observe molecules increase or decrease. Aromatic potential  
167 corresponds to glycoconjugates released by AR2000 and the evolution of these molecules and  
168 original free volatiles under action of endogenous enzymes.

169 Data were collected with GC-MS Solution software Chromeleon 7.2 and, when it was  
170 possible, the major compounds were identified by their retention index and their mass spectra  
171 using the mass spectral database NIST 14 (US National Institute of Standards and Technology  
172 (NIST), Gaithersburg, MD, USA)].

173

## 174 **2.5 Statistics**

175 For each batch, three samples of twelve strawberries each were analyzed. Principal  
176 Component Analysis (PCA) was performed with XLSTAT software (Addinsoft, France) to  
177 compare the 14 strawberry varieties with quantified volatiles as variables. Correlation factor  
178 was calculated by Pearson's method.

179

## 180 **3. Results**

### 181 **3.1 General characteristics of the strawberries**

182 Global characteristics of the strawberry are summarized in Table 1. The average pH  
183 was 3.5 and the average Brix degree was between 8~9. This is coherent with previous studies  
184 (Schwieterman et al., 2014). The main acid was citric acid but with a high proportion (~30%)  
185 of malic acid. Acid concentrations were similar among all strawberries (fresh market or for

186 processing). Glucose, fructose and sucrose are present in similar proportions (around 30% for  
187 glucose, 45% for fructose and 25% for sucrose) among the strawberries for processing while  
188 table strawberries had no sucrose but had higher fructose and glucose concentrations. In  
189 strawberries, sucrose does not vary during ripening (Sturm, Koron, & Stampar, 2003) so this  
190 difference can not be explained by a simple difference of maturity. Interestingly, the three  
191 Clery samples had similar pH but different Brix degree (7.8 for Clery; 6.9 for Clery PC; 8.6  
192 for Clery HS) with an absence of sucrose for Clery PC and Clery HS from CIREF.

193

### 194 **3.2 Optimisation of volatile extraction**

195 The extraction solvent was optimized for furaneol, being a key molecule for  
196 strawberry aroma. In a pre-test, a synthetic solution of furaneol (0.5 g/L) was extracted by n-  
197 pentane/dichloromethane (2:1 mL:mL), dichloromethane or n-pentane/ethyl acetate (1:2  
198 mL:mL). One millilitre of this standard solution was extracted with 1 mL of solvent three  
199 times and extracts were injected after concentration. Extraction yields were of 27% with n-  
200 pentane/dichloromethane, 65% with dichloromethane and 90% with n-pentane/ethyl acetate,  
201 which was therefore retained as solvent for all subsequent extractions. This comparison was  
202 completed by ASE on strawberry purees and out of 34 quantified compounds (Figure 2), 30  
203 compounds had a better extraction yield with n-pentane/ethyl acetate (1:2 mL:mL) compared  
204 to 4 compounds (isobutyl acetate, 3-penten-2-ol, acetoin and 4-methylpentan-2-ol) for  
205 dichloromethane. N-pentane/dichloromethane (2:1 mL:mL) seemed to be the worst solvent  
206 for strawberry volatile extraction. N-pentane/ethyl acetate was thus selected to extract  
207 volatiles from strawberries for this study.

208

### 209 **3.3 Identification of volatiles**

210 More than 60 volatile compounds were detected and identified (Table 2). Most  
211 representative compounds of a strawberry aroma were found: furaneol, mesifuran, ethyl  
212 butanoate, gamma-decalactone,  $\beta$ -linalool, trans-nerolidol, butyl acetate, ethyl hexanoate,  
213 butanoic acid, isobutanoic acid and hexanoic acid (Larsen et al., 1992 ; Ménager, Jost, &  
214 Aubert, 2004 ; Schwieterman et al., 2014 ; Ubeda, Callejón, Troncoso, Morales, & Garcia-  
215 Parrilla, 2014 ; Lambert, Demazeau, Largeteau, & Bouvier, 1999 ; Darbellay et al., 2004).  
216 Some molecules, like 2-phenylethyl alcohol (rose aroma) or 1-terpineol (citrus aroma), were  
217 only detected after glycoconjugate release. Cinnamic acid, another characteristic molecule of  
218 strawberry, was also found but it had a variability too large to be considered in interpreting  
219 the results. In the same triplicate, it can be absent or present in a quantity higher than 10  
220 mg/kg.

221

### 222 **3.4 Free volatiles profile**

223 Free volatiles profiles (Suppl. data) were analyzed by PCA (Figure 3) and their  
224 repartition by chemical class is presented in Table 3. The sample map (Figure 3.a.) showed  
225 two groups of samples. The first principal component clearly separated strawberries for  
226 processing from fresh market strawberries while the second principal component  
227 differentiated 'Darselect' from all others. Each initial batch was distinct from the others  
228 except 'Fraise19' and 'FCM0', which were co-located. These two "varieties" come from a  
229 mix used by food industry, which may explain the similitudes between them. The correlation  
230 circle (Figure 3.b.) indicated that strawberries for processing are differentiated by volatiles  
231 like 2,3-butanediol, pantolactone, acetoin, 3-phenylpropyl alcohol or butanoic acid. Molecules  
232 like benzyl alcohol, caprolactones, octanoic acid and 5-(hydroxymethyl)-2-furfural with,  
233 respectively, floral, fruity, acidic and caramel notes, seemed responsible for the 'Darselect'  
234 differentiation. Fresh market strawberries were differentiated by carbonyls and esters like

235 hexanal, 3-methylbutyl acetate, isobutyl acetate, 2-hexanone. Again the three 'Clery' batches  
236 were distinct, indicating that their volatile profile would not depend only on genetics but also  
237 on cultivation method and pedoclimatic conditions.

238 The variety 'Gariguette' was distinguished by methyl hexanoate, 2-methylbutanoic  
239 acid and isobutyric acid.

240 Fresh market strawberries (Table 3) had on average three times as much volatile  
241 compounds as strawberries for processing, and esters as the most abundant molecules,  
242 whereas strawberries for processing were rich in acids, except for 'Darselect,' which was rich  
243 in esters.

244

### 245 **3.5 Glycoconjugated volatile profile**

246 Glycoconjugated volatile profiles (Suppl. data) were analyzed by PCA (Figure 4) and  
247 their repartition by chemical class is presented in Table 4.

248 Alcohols and acids were preponderantly present as glycoconjugates rather than free  
249 volatiles. They represent on average 50% of the glycosides. 'Senga Sangana' (Bulgaria) had  
250 the highest glycoside concentration and also the highest concentration of glycoconjugated  
251 ketones. The highest concentration of glycoconjugated furans, of which furaneol glycoside  
252 was the most abundant, was found in 'Siabel' (45% of total glycoconjugates). 'Darselect' was  
253 also different from other varieties in glycoconjugates, as it had a very low concentration of  
254 coumaran (in "other compounds") and a high level of lactones (25% of total glycoconjugates).

255 The PCA sample map (Figure 4.a) revealed another trend. All strawberries, except  
256 'Darselect', 'Senga Sangana' (Bulgaria) and 'Gariguette' (but this last with very high  
257 variability), were clustered in the center of the PCA sample map, indicating that their  
258 glycoconjugated pools were qualitatively similar. 'Darselect' differed in having higher  
259 concentrations of 1-terpineol, gamma-caprolactone and ethyl 3-hydroxybutanoate than the

260 others. 'Senga Sangana' (Bulgaria) was differentiated by 2- and 3-hexenoic acid, 1,2-  
261 cyclopentanedione and phenol. Finally, 2-hexanone, propanoic acid, acetoin, 2-  
262 hydroxyfuraneol and isobutanoic acid were glycoconjugates specific to 'Gariguette'.

263 Although strawberries for processing and fresh market strawberries had generally  
264 similar glycoconjugate profiles, the ratio "glycoconjugates/volatile compounds" was higher in  
265 strawberries for processing. Indeed, strawberries for fresh market had an average of 6% of  
266 potential volatile increase, except for "Gariguette" (36%), as opposed to strawberries for  
267 processing, which had an average volatile increase of 50%. The highest increase (87 % ) was  
268 found for "Senga Sangana" (Bulgaria). So strawberries for processing seem to have a higher  
269 aromatic potential.

270

## 271 **4 Discussion**

### 272 **4.1 Methodology**

273 Glycosides are generally quantified using a SPE pre-treatment but this separation  
274 technique, by affinity, may not be optimal to assess quantitatively free volatiles and  
275 glycosides. By this method, Roscher, Koch, Herderich, Schreier, & Schwab (1997) estimated  
276 the glycosylated furaneol to be between 66% and 750% of the free furaneol in strawberries,  
277 whereas in this study it represented on average 14% of free furaneol (except in 'Gariguette'  
278 where bound furaneol was more than twice the amount of free furaneol). Hampel et al. (2014)  
279 confirmed obtaining differing results depending on the method with many molecules, like for  
280 1-nonanol in 'Cabernet Sauvignon', which is multiplied by approximately 7 after direct  
281 hydrolysis and by more than 22 using the SPE method. The differences are also qualitative.  
282 They identified 95 new compounds using direct hydrolysis by glycosidases, to be compared to  
283 only 67 using the SPE method in the Chardonnay grapes. Furthermore, they observed that 24  
284 volatiles are significantly increased and 5 decreased using direct hydrolysis as opposed to

285 SPE, which showed an increase for 17 compounds and a decrease for 13 compounds. Hampel  
286 et al. (2014) assumed that glycosides of terpenes are more retained on SPE columns, which is  
287 confirmed by Baek & Cadwallader (1999). Hampel et al. (2014) have a similar result on  
288 melon. Voirin's thesis (1990) confirms these observations on grape. He obtained an SPE yield  
289 of 15% for glycosides with phenyl-type aglycone and 70% to 100% for glycosides of terpene  
290 aglycones.

291

#### 292 **4.2 Volatile molecules of strawberries**

293 Although furaneol is the key molecule for strawberry aroma, it was not qualitatively  
294 discriminant to compare volatile profile, as expected because it is ubiquitous in strawberries.  
295 This molecule is already found when strawberry volatiles are analyzed by liquid extraction  
296 (Larsen et al., 1992; Lambert et al., 1999) and sometimes found with solid phase  
297 microextraction (SPME)/headspace (Darbellay et al., 2004) which confirms this conclusion.  
298 The comparisons have to rely on other molecules.

299 In strawberries, the 2-methylbutanoate and 2-methylbutyl esters are present in all  
300 reference articles but only 3-methylbutyl acetate and 2-methylbutanoic acid were found here  
301 (as identified by their retention index and MS spectra). These molecules come from two  
302 different amino acids, L-leucine (for 3-methylbutyl/butanoate) and L-isoleucine (for 2-  
303 methylbutyl/butanoate) by the same pathway (Pérez, Olías, Luaces, & Sanz, 2002). The  
304 nature of amino acid supplies could explain the absence or presence of 2-methyl and 3-methyl  
305 butyl compounds. The C6 carbonyls 3-hexenal and 2-hexenal with their green odor types,  
306 other important molecules of strawberry aroma, were absent. This was expected in the  
307 aliquots which were salted during mashing as this treatment aims to inhibit the endogenous  
308 enzymes of the lipoxygenase (LOX) pathway. This absence could also be explained by their  
309 further degradation to hexenol by action of alcohol deshydrogenase (ADH) (Speirs et al.,

1998) or hexenoic acid by oxidation in AR2000-treated samples. Indeed, although 2- and 3-hexenal were not found, 2- and 3-hexenoic acid (quantifiable) and 3-hexenol (not quantifiable) were present. The identified volatiles had mainly fruity or floral odor type. These differences could arise from extraction with ethyl acetate/n-pentane (2:1), which is more polar than dichloromethane (the usual solvent) and the long incubation time (overnight) allowing chemical degradation. Between control samples with and without incubation, decreases of molecules like 2-methylbutanoic acid and hexanoic acid and increases of molecules like 2-hexenoic acid and 2-hydroxy-4-pyranone were observed and some molecules became undetectable like ethyl cinnamate and ethyl hexanoate.

319

### 4.3 Comparison of industrial and fresh-market strawberries

#### *Free volatiles*

The differentiation between industrial and fresh market strawberry aroma profiles may explain their different uses, resulting from distinct selection goals. Strawberries for processing need to keep their texture and color after treatment especially in dairy products. Fresh market strawberries, which are produced to be eaten as-is, need to have a good instant aromatic quality to attract consumers. This is reflected in the present results by generally higher contents of free volatiles in the fresh-market strawberries. Zorrilla-Fontanesi et al., (2012) studied the natural genetic variability of aroma in strawberry. They compared genomes of plants stem from 2 parents and associated them to aroma differences. This genetic approach probably could be interesting to complete this comparison between strawberries for process and for fresh market. An interesting point was that the 3 studied 'Clery' have different volatiles profiles. These differences can not be explained only by genetics especially considering that the two samples from CIREF come from the same cultivar and were grown at the same location. The only difference was that one sample was in full-field and the other in



335 hydroponics. The cultivation method also appeared to have a non-negligible effect on aroma  
336 profile.

337

### 338 Glycoconjugated volatiles

339 However limited differences were observed between fresh-market and industrial  
340 strawberries in the case of glycoconjugated volatiles. Indeed previous studies (Ubeda et al.,  
341 2012; Groyne et al., 1999) found as main glycoconjugated volatiles in strawberries furaneol,  
342 benzyl alcohol, 2-phenylethyl alcohol, 4-vinylphenol, 4-vinylguaiacol and hexanoic acid.  
343 Results are consistent with literature for ‘Gariguette’ where Lambert et al. (1999) found also  
344 methyl hexanoate, 2-methylbutanoic acid as well as hexanoic acid as main glycoconjugated  
345 volatiles. Except for 4-vinylphenol, all were also found in this study. The importance of  
346 glycoconjugates of benzyl alcohol and 2-phenylethyl alcohol in strawberries was confirmed  
347 by the low discriminant effect of these molecules in the glycoconjugates profiles. Among  
348 released aglycones some molecules are interesting for flavor thanks their low thresholds, like  
349 2-phenylethyl alcohol (rose note) with a threshold of 4 µg/kg (4 parts per billion (ppb)  
350 (Fenaroli’s Handbbok, 2010), furaneol (caramelic note) with a threshold of 0.03 µg/kg or γ-  
351 decalactone (peach note) with a threshold of 11 µg/kg. These increases show that  
352 deglycosylation could be a way to enrich natural aroma extracts.

353

## 354 **5. Conclusion**

355 Direct enzymatic hydrolysis allowed to reexamine the free to glycoconjugated volatile  
356 ratios in strawberries. Free volatiles were more abundant than glycoconjugated volatiles,  
357 especially in fresh market strawberries, and that the glycosylated/free volatiles ratio was  
358 higher in strawberries for processing. All tested strawberries had similar glycoconjugates  
359 profiles (except for “Darselect”, “Senga Sangana” (Bulgaria) and “Gariguette”). Indeed, the

360 release of glycoconjugated volatiles would enhance aroma in a similar manner for all  
361 varieties. It can therefore be an interesting way to enhance the aromatic power of strawberry  
362 preparations in industry. Besides fresh market strawberries had a lower aromatic reserve,  
363 which indicates that strawberries for processing would be better candidates for future  
364 experiments on glycoside hydrolysis during process.

365

366

367

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374

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**Table 1: Physico-chemical characteristics and origin of strawberries material**

	<i>Origin</i>	<i>Harvest year</i>	<i>Glucose<sup>a</sup></i>	<i>Fructose<sup>a</sup></i>	<i>Sucrose<sup>a</sup></i>	<i>Citric acid<sup>a</sup></i>	<i>Malic acid<sup>a</sup></i>	<i>Brix</i>	<i>pH</i>
<b>FCMO060</b>	Spain	2015	1.59±0.11	2.26±0.08	1.94±0.37	0.50±0.05	0.28±0.01	6.1±0.2	3.6±0.2
<b>Siabel</b>	Bulgaria	2015	1.75±0.25	2.49±0.08	1.41±0.35	0.70±0.08	0.31±0.03	ND	3.5±0.2
<b>Clery</b>	?	2015	2.12±0.31	3.19±0.46	1.88±0.32	0.40±0.01	0.18±0.01	7.8±0.2	3.7±0.2
<b>Honeoye</b>	Bulgaria	2015	2.30±0.28	2.92±0.33	1.91±0.40	0.62±0.09	0.27±0.03	8.6±0.3	3.8±0.2
<b>Honey</b>	China	2015	1.37±0.28	2.20±0.26	0.96±0.02	0.46±0.02	0.18±0.03	6.2±0.2	3.7±0.2
<b>Senga sangana</b>	Bulgaria	2015	2.49±0.31	2.83±0.34	2.32±0.27	0.70±0.20	0.37±0.01	9.7±0.3	3.5±0.2
<b>Darselect</b>	Germany	2015	2.36±0.62	2.84±0.17	1.43±0.30	0.66±0.21	0.18±0.01	8.4±0.3	3.5±0.2
<b>Senga sangana</b>	Poland	2015	1.59±0.48	2.40±0.37	1.17±0.13	0.50±0.02	0.25±0.05	7.7±0.3	3.4±0.2
<b>Fraise 19</b>	Morocco	2016	1.42±0.17	1.97±0.35	0.73±0.38	0.36±0.22	0.19±0.01	6.4±0.2	3.6±0.2
<b>CIR121</b>	France (CIREF)	2016	3.00±0.04	2.94±0.12	0.00	0.81±0.02	0.29±0.00	9.0±0.3	3.7±0.2
<b>Clery PC</b>	France (CIREF)	2016	2.39±0.03	2.39±0.11	0.00	0.59±0.03	0.19±0.00	6.9±0.2	3.5±0.2
<b>Clery HS</b>	France (CIREF)	2016	2.91±0.07	2.83±0.35	0.00	0.94±0.03	0.25±0.01	8.6±0.3	3.5±0.2
<b>Charlotte</b>	France (CIREF)	2016	3.60±0.04	3.67±0.19	0.00	0.73±0.02	0.21±0.00	11.1±0.3	3.9±0.2
<b>Gariguette</b>	France (CIREF)	2016	2.80±0.15	2.76±0.26	0.00	0.96±0.04	0.35±0.01	9.0±0.3	3.4±0.2

<sup>a</sup> in g per 100 g of fresh material ; PC : Full-field ; HS : Hydroponis

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**Table 2 : Identified<sup>a</sup> volatile compound in GC-MS analyses of strawberries samples**

	Calculated RI <sup>b</sup>	Perception threshold in water (µg/L)	Odortype	Ref <sup>c,d</sup>
<i>Acids</i>				
(E)-2-Hexenoic acid	1980	NA	fruity	7
2-Mehtylbutanoic acid	1662	180	acidic	1;2;3;6;7
3-Hexenoic acid	1954	NA	cheesy	
3-Methylhexanoic acid	1916	NA	-	7
Butanoic acid	1625	240	cheesy	1;2;5;7
Citraconic / itaconic acid*	1040	NA	-	
Formic acid	1503	NA	acetic	7
Hexanoic acid	1846	3000	fatty	1;2;5;7
Isobutanoic acid	1570	8100	acidic	1;2;5;7
Nonanoic acid	2171	3000	fatty	5;7
Octanoic acid	2060	3000	waxy	2;5;7
Propanoic acid	1535	20000	acidic	1;7
Succinic anhydride*	2103	NA	-	
<i>Alcohols</i>				
1-Butanol	1142	500	fermented	4;7
1-Hexanol	1355	800	herbal	1;2;3;5;7
2,3-Butanediol	1543	4500	creamy	
3-Phenylpropyl alcohol	2039	NA	balsamic	7
4-Vinylguaiacol	2188	3	spicy	4;7
Benzyl alcohol	1870	10000	floral	2;4;7
Chavicol	2334	NA	phenolic	
4-Ethylphenol	2187	NA	smoky	
Phenol	2000	5900	phenolic	
5-methyl-3-hexen-2-ol	1274	NA	green	
3-hexen-1-ol	1398	70	green	2;7
2-Phenylethyl alcohol	1906	4	floral	7
<i>Aldehydes</i>				
Hexanal	1078	NA	-	7
<i>Esters</i>				
3-Methylbutyl acetate	1122	2	fruity	3;7
Butyl acetate	1074	66	ethereal	1;2;3;5;6;7
Ethyl 3-hydroxybutanoate	1515	NA	fruity	7
Ethyl butanoate	1035	1	fruity	1;3;5;6;7
Ethyl (E)-cinnamate	2127	NA	balsamic	7
Ethyl hexanoate	1233	1	fruity	1;3;5;6;7
Ethyl isobutanoate	961	0.1	fruity	7
Isobutyl acetate	1012	66	fruity	7
Methyl 3-hydroxybutanoate	1461	NA	apple	7

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<i>Furans</i>					
2-Hydroxyfuraneol	1518	NA	-		
5-(Hydroxymethyl)-2-furfural	1660	NA	fatty		
Furaneol	2031	0.03	caramellic	1;2;3;4;5;6;7	
Mesifurane	1580	0.03	musty	1;2;3;4;5;6;7	
<i>Ketones</i>					
1,2-Cyclopentanedione*	1742	NA	caramellic		
2-Hexanone	1083	NA	fruity	3;7	
3-Methyl 2-penten-4-one	1187	NA	vegetable		
3-Penten-2-one	1128	1.5	fruity	7	
Acetoin	1284	800	buttery	7	
2-hydroxy-4-pyranone	1990	NA	-		
<i>Lactones</i>					
delta-Caprolactone	1791	NA	herbal	7	
gamma-Caprolactone	1694	1600	tonka	2;5;7	
gamma-Decalactone	2138	11	fruity	1;2;3;4;5;7	
gamma-Dodecalactone	2374	7	fruity	2;7	
Pantolactone	2029	NA	caramellic	4	
<i>Terpenes</i>					
alpha-(E,E)-Farnesene	1746	NA	woody		
beta-Linalool	1547	6	floral	1;2;3;5;6;7	
trans-Linalool oxide (pyranosid)	1721	320	floral	1;2;5;7	
alpha-Muurolene	1726	NA	woody		
1-Terpineol	1576	300	citrus	4;7	
trans-Nerolidol	2042	300	floral	1;2;3;5;7	
<i>Others</i>					
Coumaran	2389	NA	green tea		
Ethyl methyl benzene*	1225	NA	-		
Ethylene glycol diacetate	1535	NA	-		
1,1-diethoxybutane	990	NA	-	7	
unknown	1995	NA	green		

<sup>a</sup> Identified by NIST library (version 2.2)

<sup>b</sup> On polar column (TG Wax)

<sup>c</sup> References where identified volatiles were also found.

<sup>d</sup> 1: Larsen et al. (1992); 2: Menager et al. (2004); 3: Schwieterman et al. (2014); 4: Ubeda et al. (2014); 5: Lambert et al. (1999); 6: Azodanlou et al. (2003); 7: VCF Volatile Compounds in Food database – Strawberry fruit (2007-Feb-08 version 9.1)

\*only identified by MS

**Table 3:** Number of molecules and quantitative repartition for chemical classes in strawberries free-volatiles

	TOTAL ( $\mu\text{g}/\text{kg}^{\text{a}}$ )	<i>Acid</i>		<i>Alcohol</i>		<i>Aldehyde</i>		<i>Ester</i>		<i>Furan</i>		<i>Ketone</i>		<i>Lactone</i>		<i>Terpene</i>		<i>Other</i>	
		Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio
<b>Clery</b>	16608 $\pm$ 1099	6	44%	3	7%	0	0%	4	15%	1	13%	4	6%	4	4%	2	3%	2	8%
<b>Darselect</b>	13187 $\pm$ 2627	2	38%	1	1%	0	0%	2	19%	3	26%	2	5%	3	10%	1	0%	0	0%
<b>FCMO060</b>	9130 $\pm$ 877	3	27%	1	7%	0	0%	1	15%	1	16%	1	4%	2	24%	1	1%	1	7%
<b>Fraise19</b>	9769 $\pm$ 1415	4	33%	1	5%	0	0%	1	13%	2	13%	1	16%	2	14%	1	1%	1	6%
<b>Honeoye</b>	34176 $\pm$ 1785	6	48%	1	2%	0	0%	2	12%	2	8%	4	4%	4	17%	2	2%	2	6%
<b>Honey</b>	30505 $\pm$ 1274	7	28%	5	21%	0	0%	3	10%	2	8%	4	12%	3	14%	2	1%	2	6%
<b>Senga sangana (Bulgaria)</b>	21154 $\pm$ 2672	7	38%	2	5%	0	0%	4	11%	2	19%	3	3%	3	5%	2	2%	1	19%
<b>Senga sangana (Poland)</b>	19822 $\pm$ 2682	5	43%	2	7%	0	0%	1	10%	3	16%	3	4%	3	15%	2	2%	2	2%
<b>Siabel</b>	22984 $\pm$ 2484	8	20%	2	1%	0	0%	2	24%	2	35%	3	5%	3	9%	1	2%	2	4%
<b>Clery PC</b>	41746 $\pm$ 29012	7	3%	0	0%	0	0%	4	90%	1	1%	3	1%	3	0%	2	1%	2	4%
<b>Gariguette</b>	47980 $\pm$ 7387	10	26%	1	0%	1	1%	8	58%	3	4%	2	1%	1	0%	3	5%	3	4%
<b>Clery HS</b>	95633 $\pm$ 9752	8	6%	0	0%	1	1%	5	79%	2	4%	2	1%	2	0%	2	4%	2	4%
<b>CIR121</b>	115250 $\pm$ 22173	6	5%	0	0%	1	1%	4	84%	2	2%	2	1%	2	4%	2	2%	2	1%
<b>Charlotte</b>	58259 $\pm$ 7228	8	6%	0	0%	1	1%	6	79%	2	5%	2	1%	1	2%	3	2%	2	5%

<sup>a</sup> in equivalent 4-nonanol

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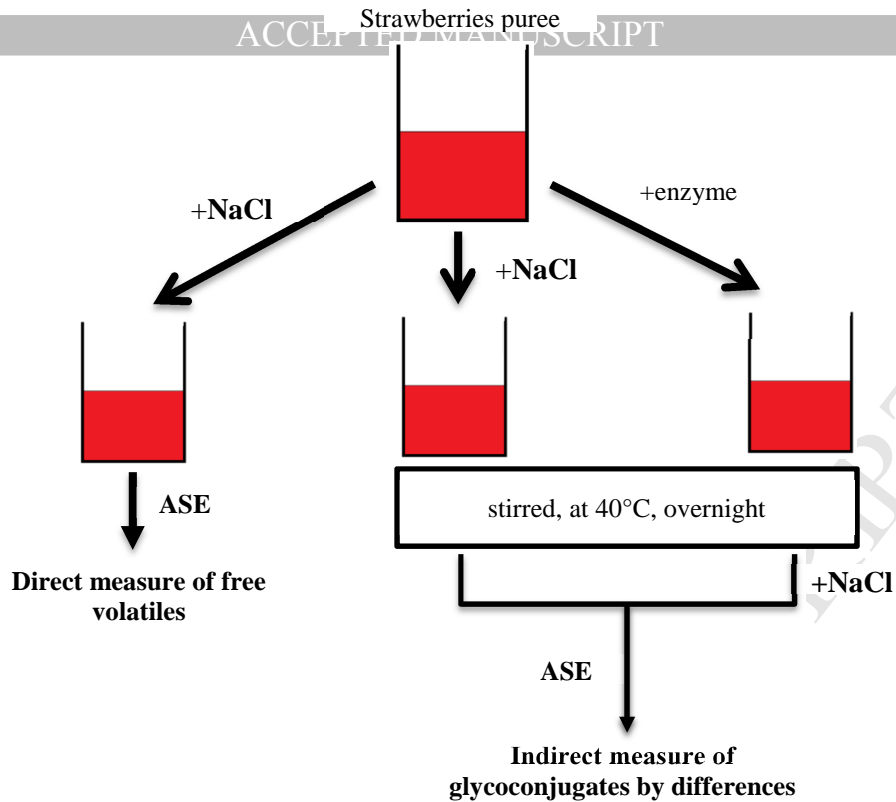
**Table 4:** number of molecules and quantitative repartition of chemical classes in strawberries glycoconjugates

	TOTAL ( $\mu\text{g}/\text{kg}^{\text{a}}$ )	<i>Acid</i>		<i>Alcohol</i>		<i>Aldehyde</i>		<i>Ester</i>		<i>Furan</i>		<i>Ketone</i>		<i>Lactone</i>		<i>Terpene</i>		<i>Other</i>	
		Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio
<b>Clery</b>	10867 $\pm$ 869	6	39%	4	20%	0	0%	1	1%	1	2%	1	3%	2	2%	1	1%	1	33%
<b>Darselect</b>	7705 $\pm$ 2774	3	43%	1	5%	0	0%	0	0%	1	15%	1	2%	2	26%	2	2%	2	7%
<b>FCMO060</b>	4848 $\pm$ 1697	2	13%	2	19%	1	6%	1	3%	0	0%	1	8%	1	13%	0	0%	1	38%
<b>Fraise19</b>	4655 $\pm$ 745	4	30%	2	17%	0	0%	0	0%	0	0%	1	7%	1	10%	0	0%	1	38%
<b>Honeoye</b>	14044 $\pm$ 5056	5	44%	3	11%	0	0%	0	0%	1	5%	1	5%	2	12%	1	1%	1	23%
<b>Honey</b>	9982 $\pm$ 998	6	47%	4	19%	0	0%	0	0%	0	0%	0	0%	1	7%	1	1%	1	27%
<b>Senga sangana (Bulgaria)</b>	20166 $\pm$ 1613	8	51%	5	9%	0	0%	1	1%	1	0%	1	4%	1	0%	0	0%	1	34%
<b>Senga sangana (Poland)</b>	7350 $\pm$ 2352	4	37%	3	23%	0	0%	1	3%	0	0%	1	2%	2	12%	1	1%	1	25%
<b>Siabel</b>	9268 $\pm$ 1112	3	13%	3	9%	0	0%	0	0%	1	54%	0	0%	2	5%	0	0%	1	19%
<b>Clery PC</b>	2524 $\pm$ 606	4	11%	4	22%	1	0%	0	0%	2	0%	1	12%	1	3%	0	0%	2	52%
<b>Gariguette</b>	18074 $\pm$ 9398	10	54%	2	2%	1	2%	0	0%	2	13%	3	5%	1	0%	2	3%	2	23%
<b>Clery HS</b>	5258 $\pm$ 578	6	19%	3	12%	1	0%	0	0%	2	16%	2	11%	0	0%	1	1%	2	40%
<b>CIR121</b>	7052 $\pm$ 3103	8	20%	3	10%	1	1%	0	0%	1	2%	2	8%	2	14%	1	10%	2	43%
<b>Charlotte</b>	3657 $\pm$ 1060	6	10%	2	2%	1	2%	0	0%	1	4%	2	11%	0	0%	0	0%	2	71%

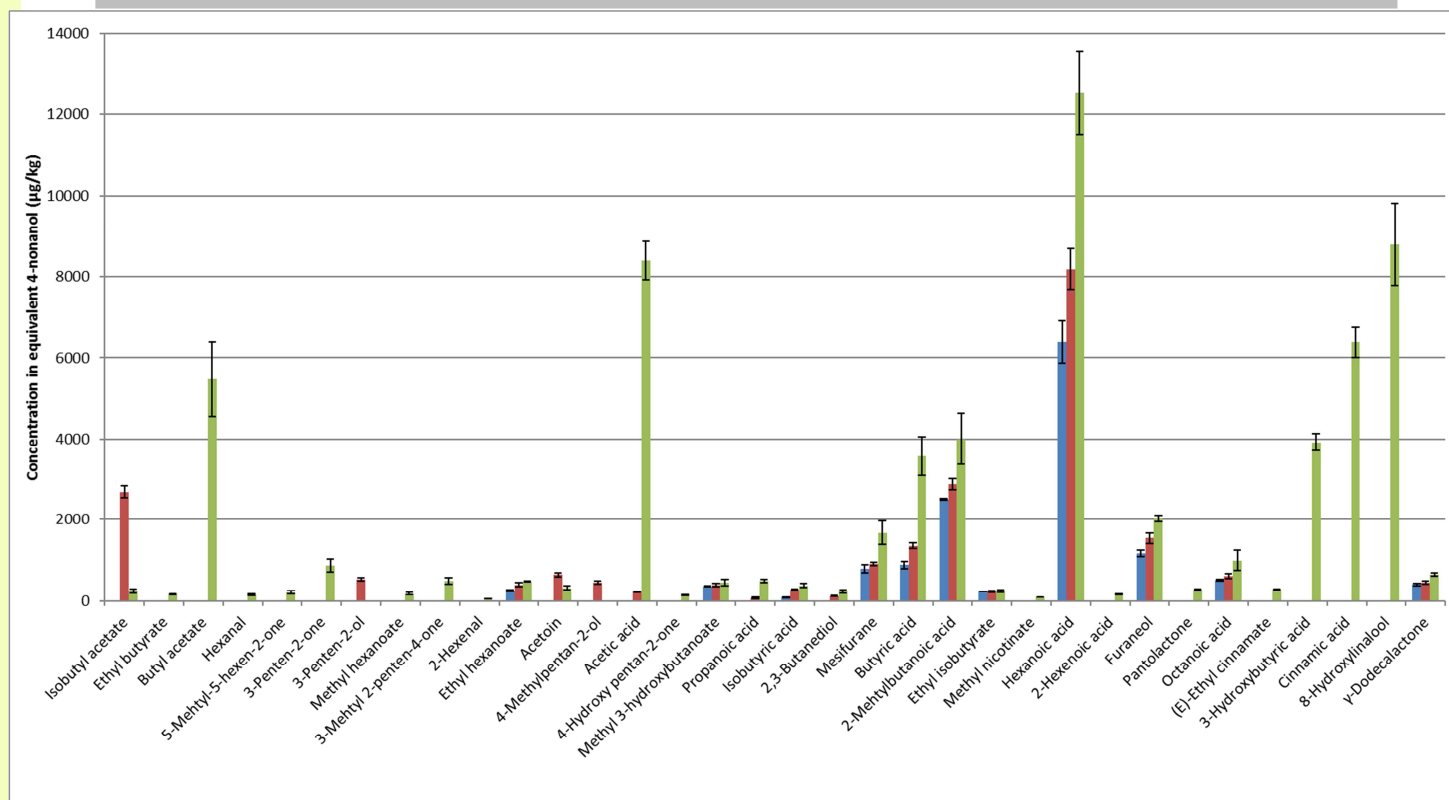
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**Figure 1: Scheme of strawberries preparation for assisted-solvent extraction (ASE) of volatiles and sampling point for analysis of free- and bound-volatiles**



**Figure 2: Concentration of volatiles after extraction by ASE method with: dichloromethane/pentane (1:2) (blue), dichloromethane (red), ethyl acetate/pentane (2:1) (green)**

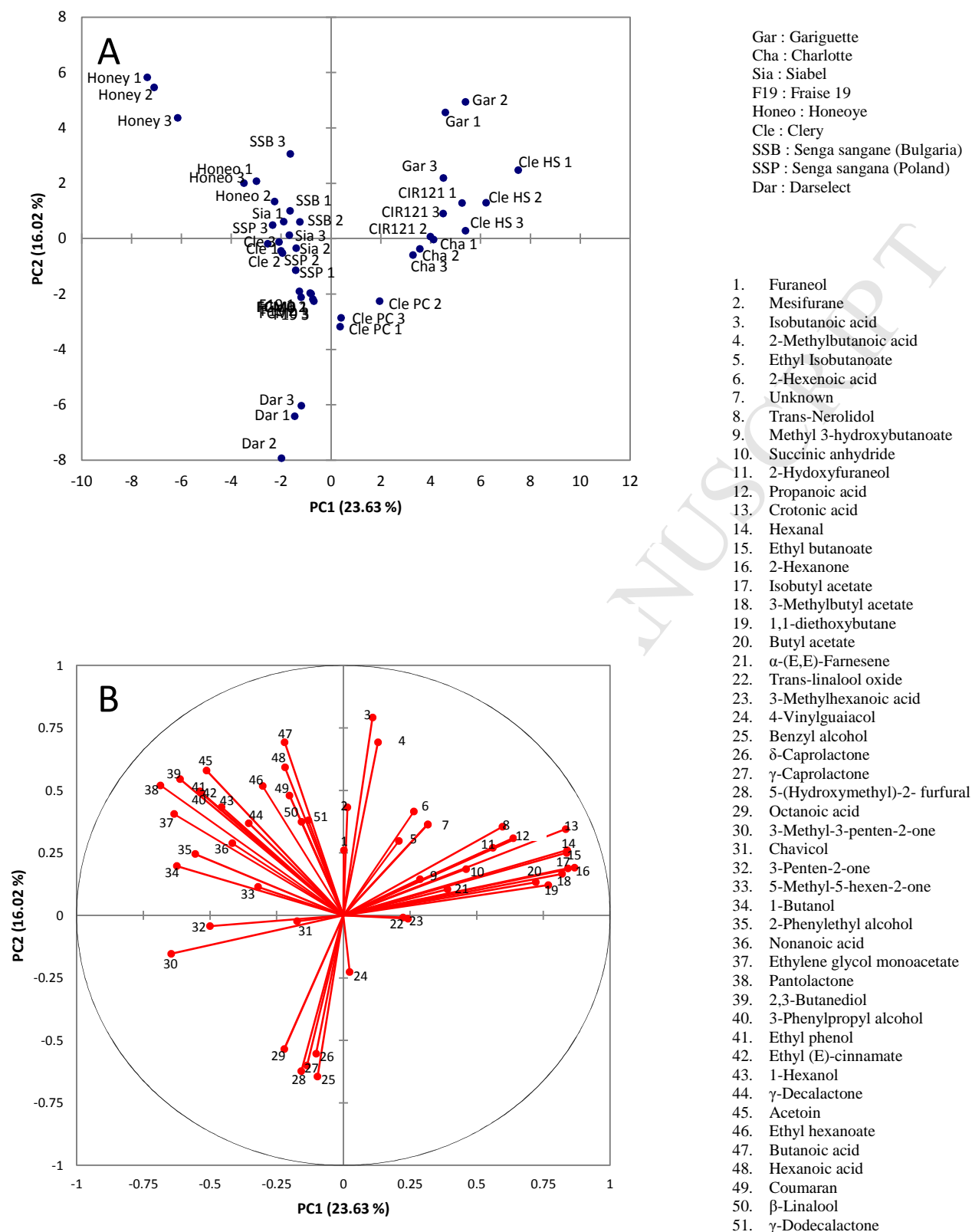
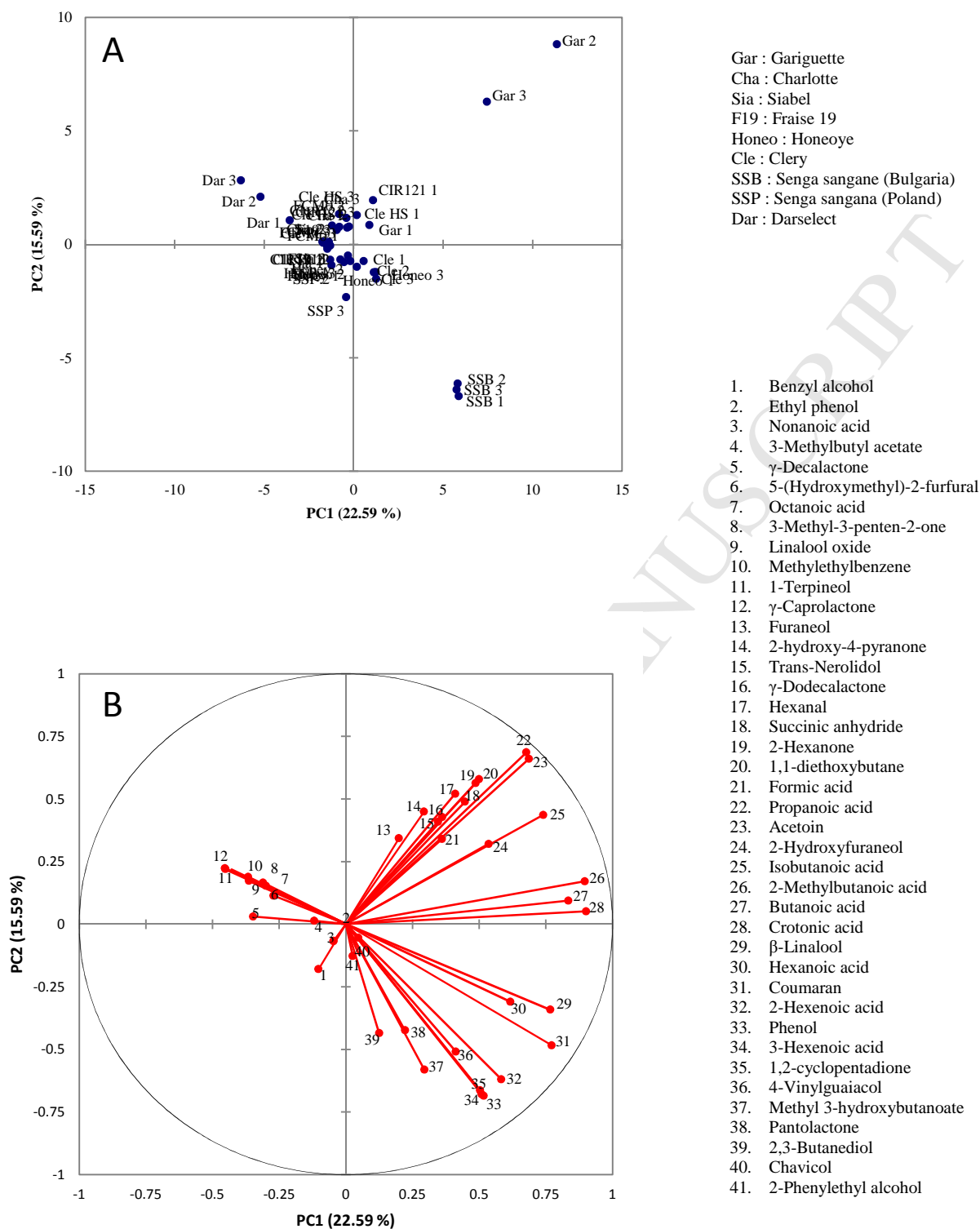


Figure 3: Principal component analysis of free-volatiles in strawberries. A. Sample map; B. Variable correlation map

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**Figure 4: Principal component analysis of glycoconjugates in strawberries. A. Sample map ; B. Variable correlation map**

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**Highlights:**

Glycosylated volatiles can be reliably analyzed by direct enzymatic hydrolysis of the fruit.

Free-volatile profiles split strawberries for fresh market and for processing.

Bound-volatile profiles were similar among the majority of strawberry varieties.

Strawberries for processing were the best candidates for aroma enhancement.

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