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

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

1. Introduction

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

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

Various parameters can influence the aroma of strawberries (Perez, Rios, Sanz, & Olias, 1992; Miszczak, Forney, & Prange, 1995). The first one is genetic. Indeed Aharoni (2004) compared the aroma profile of wild strawberries (*Fragaria vesca*) and cultivated strawberries (*Fragaria x ananassa*). Nerolidol dominates among terpenes in cultivated strawberries, while, in wild strawberries, monoterpenes like β-myrcene and α-pinene, responsible of the characteristic musky aroma of this species, are more prevalent (Bianchi, Lovazzano, Lanubile, & Marocco, 2014). Maturity also impacts the strawberry aroma: Forney, Kalt, & Jordan (2000) showed an increase of methyl butanoate and methyl hexanoate with maturity and Darbellay, Luisier, Villettaz, Amadò, & Azodanlou (2004) a decrease of (Z)-3-hexenyl acetate and an increase of ethyl hexanoate during ripening in ‘Carezza’, ‘Darselect’ and ‘Marmolada’ varieties. The atmosphere during packaging also impacts volatile production: Nielsen & Leufven (2008) showed an increase of ethyl acetate (fermented molecules) with CO_2-enriched atmosphere in the ‘Korona’ variety.

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

These glycosides are traditionally identified and quantified after isolation by solid phase extraction (SPE) (Young & Paterson, 1995). SPE consists in injecting the fruit extract on an XAD-2 or a C18-column, which binds the aglycone moieties of the glycosides. The sugars and free volatiles are first eluted by water and dichloromethane, respectively; the glycosides are thereafter eluted with another solvent (generally methanol) (Ubeda et al., 2012; Roscher, Herderich, Steffen, Schreier, & Schwab, 1996). This method allows to eliminate matrix interactions. However, it is not adapted to compare free volatiles to bound volatiles because of the discrimination that is generated by affinity extraction, and because the natural evolution of volatiles in fruit matrices is not considered. Indeed, Hampel, Robinson, Johnson, & Ebeler (2014) showed that this pre-treatment leads to a loss and a discrimination of many compounds, and can not be used to compare free volatile to glycoconjugated volatiles. They advised direct hydrolysis to compare quantitatively free and bound volatiles.

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

2. Material & method

2.1. Plant material

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

2.2. Reagents and solvents

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

2.3. Fruit characterisation

2.31. Brix and pH

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

2.3.2 Sugars and Acids

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

2.4 Characterization of Volatiles Compounds

2.4.1 Sample preparation:

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

2.4.2 Accelerated solvent extraction (ASE)

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

2.4.3 GC–MS

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

Injection was in splitless mode at 250 °C. The carrier gas was helium with a constant flow of 1.2 $mL.min^{-1}$. Oven temperature program was 35 °C for 2 min ramped at 5°$C.min^{-1}$ to 230°C then held for 5 min. Mass spectra were obtained by electron ionization at 70 eV, with scanning from m/z 35 to 250 at 2 scans.s^{-1}.

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

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

2.5 Statistics

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

3. Results

3.1 General characteristics of the strawberries

Global characteristics of the strawberry are summarized in Table 1. The average pH was 3.5 and the average Brix degree was between 8~9. This is coherent with previous studies (Schwieterman et al., 2014). The main acid was citric acid but with a high proportion (~30%) of malic acid. Acid concentrations were similar among all strawberries (fresh market or for processing). Glucose, fructose and sucrose are present in similar proportions (around 30% for glucose, 45% for fructose and 25% for sucrose) among the strawberries for processing while table strawberries had no sucrose but had higher fructose and glucose concentrations. In strawberries, sucrose does not vary during ripening (Sturm, Koron, & Stampar, 2003) so this difference can not be explained by a simple difference of maturity. Interestingly, the three Clery samples had similar pH but different Brix degree (7.8 for Clery; 6.9 for Clery PC; 8.6 for Clery HS) with an absence of sucrose for Clery PC and Clery HS from CIREF.

3.2 Optimisation of volatile extraction

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

3.3 Identification of volatiles

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

3.4 Free volatiles profile

Free volatiles profiles (Suppl. data) were analyzed by PCA (Figure 3) and their repartition by chemical class is presented in Table 3. The sample map (Figure 3.a.) showed two groups of samples. The first principal component clearly separated strawberries for processing from fresh market strawberries while the second principal component differentiated ‘Darselect’ from all others. Each initial batch was distinct from the others except ‘Fraise19’ and ‘FCM0’, which were co-located. These two “varieties” come from a mix used by food industry, which may explain the similitudes between them. The correlation circle (Figure 3.b.) indicated that strawberries for processing are differentiated by volatiles like 2,3-butanediol, pantolactone, acetoin, 3-phenylpropyl alcohol or butanoic acid. Molecules like benzyl alcohol, caprolactones, octanoic acid and 5-(hydroxymethyl)-2-furfural with, respectively, floral, fruity, acidic and caramel notes, seemed responsible for the ‘Darselect’ differentiation. Fresh market strawberries were differentiated by carbonyls and esters like hexanal, 3-methylbutyl acetate, isobutyl acetate, 2-hexanone. Again the three 'Clery' batches were distinct, indicating that their volatile profile would not depend only on genetics but also on cultivation method and pedoclimatic conditions.

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

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

3.5 Glycoconjugated volatile profile

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

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

The PCA sample map (Figure 4.a) revealed another trend. All strawberries, except 'Darselect', 'Senga Sangana' (Bulgaria) and 'Gariguette' (but this last with very high variability), were clustered in the center of the PCA sample map, indicating that their glycoconjugated pools were qualitatively similar. 'Darselect' differed in having higher concentrations of 1-terpineol, gamma-caprolactone and ethyl 3-hydroxybutanoate than the others. 'Senga Sangana' (Bulgaria) was differentiated by 2- and 3-hexenoic acid, 1,2-cyclopentanedione and phenol. Finally, 2-hexanone, propanoic acid, acetoin, 2-hydroxyfuraneol and isobutanoic acid were glycoconjugates specific to 'Gariguette'.

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

4 Discussion

4.1 Methodology

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

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

4.2 Volatile molecules of strawberries

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

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

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

Glycoconjugated volatiles

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

5. Conclusion

Direct enzymatic hydrolysis allowed to reexamine the free to glycoconjugated volatile ratios in strawberries. Free volatiles were more abundant than glycoconjugated volatiles, especially in fresh market strawberries, and that the glycosylated/free volatiles ratio was higher in strawberries for processing. All tested strawberries had similar glycoconjugates profiles (except for "Darselect", "Senga Sangana" (Bulgaria) and "Gariguette"). Indeed, the release of glycoconjugated volatiles would enhance aroma in a similar manner for all varieties. It can therefore be an interesting way to enhance the aromatic power of strawberry preparations in industry. Besides fresh market strawberries had a lower aromatic reserve, which indicates that strawberries for processing would be better candidates for future experiments on glycoside hydrolysis during process.

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

	Origin	*Harvest year*	*Glucose*[a]	*Fructose*[a]	*Sucrose*[a]	*Citric acid*[a]	*Malic acid*[a]	*Brix*	*pH*
FCMO060	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
Siabel	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
Clery	?	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
Honeoye	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
Honey	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
Senga sangana	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
Darselect	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
Senga sangana	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
Fraise 19	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
CIR121	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
Clery PC	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
Clery HS	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
Charlotte	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
Gariguette	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

[a] in g per 100 g of fresh material ; PC : Full-field ; HS : Hydroponis

Table 2 : Identified[a] volatile compound in GC-MS analyses of strawberries samples

	Calculated RI[b]	Perception threshold in water (µg/L)	Odortype	Ref[c,d]
Acids				
(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	-	
Alcohols				
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
Aldehydes				
Hexanal	1078	NA	-	7
Esters				
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

Furans				
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
Ketones				
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	-	
Lactones				
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
Terpenes				
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
Others				
Coumaran	2389	NA	green tea	
Ethyl methyl benzene*	1225	NA	-	
Ethylene glycol diacetate	1535	NA	-	
1,1-diethyoxybutane	990	NA	-	7
unknown	1995	NA	green	

[a] Identfied by NIST library (version 2.2)
[b] On polar column (TG Wax)
[c] References where identified volatiles were also found.
[d] 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 (µg/kg[a])	*Acid*		*Alcohol*		*Aldehyde*		*Ester*		*Furan*		*Ketone*		*Lactone*		*Terpene*		*Other*	
		Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio	Number	Ratio
Clery	16608±1099	6	44%	3	7%	0	0%	4	15%	1	13%	4	6%	4	4%	2	3%	2	8%
Darselect	13187±2627	2	38%	1	1%	0	0%	2	19%	3	26%	2	5%	3	10%	1	0%	0	0%
FCMO060	9130±877	3	27%	1	7%	0	0%	1	15%	1	16%	1	4%	2	24%	1	1%	1	7%
Fraise19	9769±1415	4	33%	1	5%	0	0%	1	13%	2	13%	1	16%	2	14%	1	1%	1	6%
Honeoye	34176±1785	6	48%	1	2%	0	0%	2	12%	2	8%	4	4%	4	17%	2	2%	2	6%
Honey	30505±1274	7	28%	5	21%	0	0%	3	10%	2	8%	4	12%	3	14%	2	1%	2	6%
Senga sangana (Bulgaria)	21154±2672	7	38%	2	5%	0	0%	4	11%	2	19%	3	3%	3	5%	2	2%	1	19%
Senga sangana (Poland)	19822±2682	5	43%	2	7%	0	0%	1	10%	3	16%	3	4%	3	15%	2	2%	2	2%
Siabel	22984±2484	8	20%	2	1%	0	0%	2	24%	2	35%	3	5%	3	9%	1	2%	2	4%
Clery PC	41746±29012	7	3%	0	0%	0	0%	4	90%	1	1%	3	1%	3	0%	2	1%	2	4%
Gariguette	47980±7387	10	26%	1	0%	1	1%	8	58%	3	4%	2	1%	1	0%	3	5%	3	4%
Clery HS	95633±9752	8	6%	0	0%	1	1%	5	79%	2	4%	2	1%	2	0%	2	4%	2	4%
CIR121	115250±22173	6	5%	0	0%	1	1%	4	84%	2	2%	2	1%	2	4%	2	2%	2	1%
Charlotte	58259±7228	8	6%	0	0%	1	1%	6	79%	2	5%	2	1%	1	2%	3	2%	2	5%

[a] in equivalent 4-nonanol

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

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

[a] in equivalent 4-nonanol

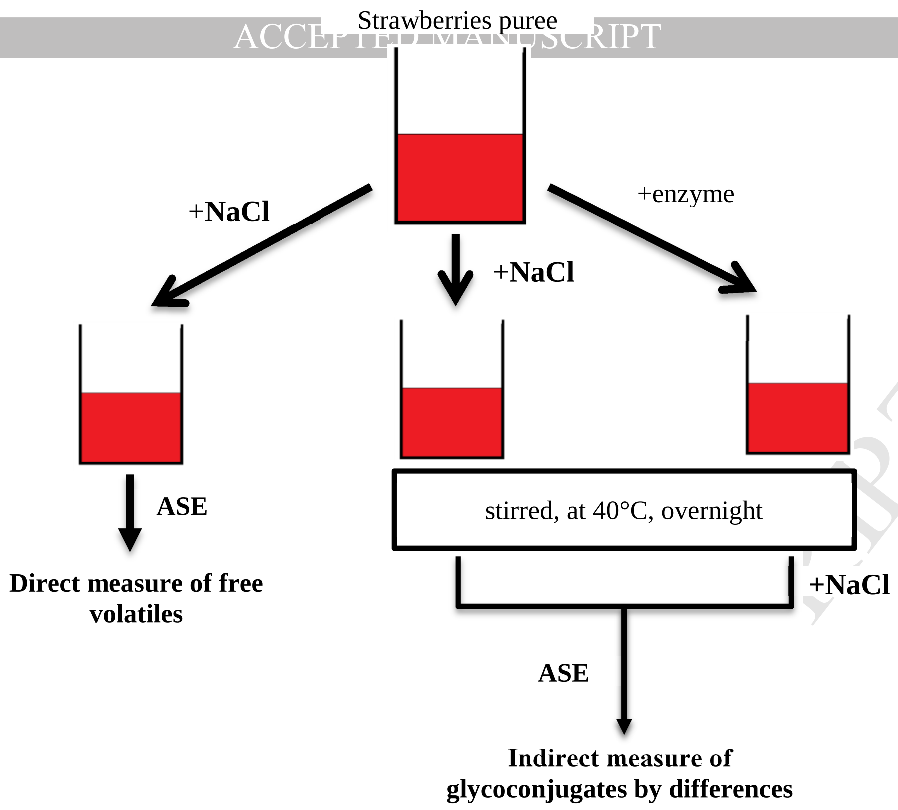


Figure 1: Scheme of strawberries preparation for assisted-solvent extraction (ASE) of volatiles and sampling point for analysis of free- and bound-volatiles

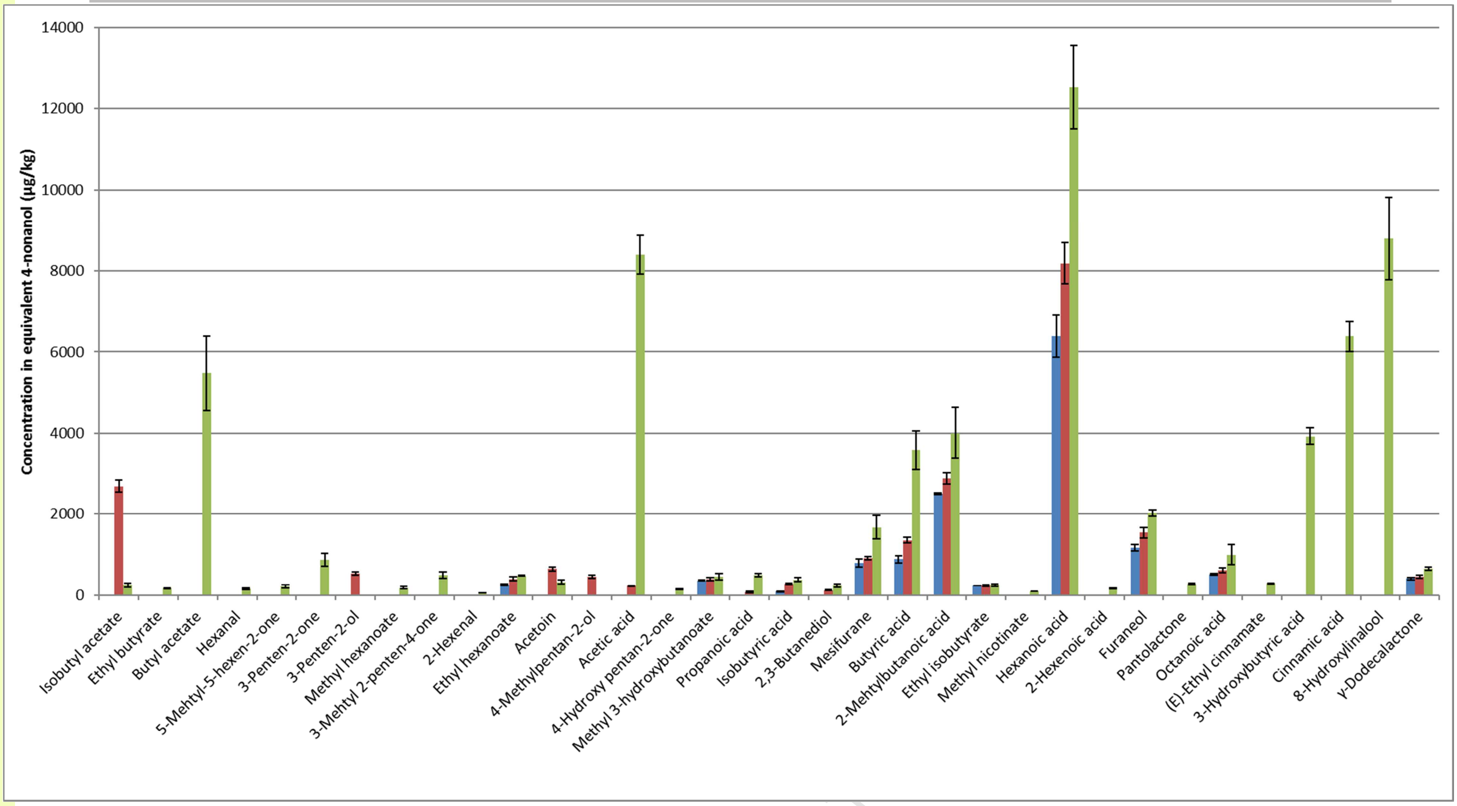
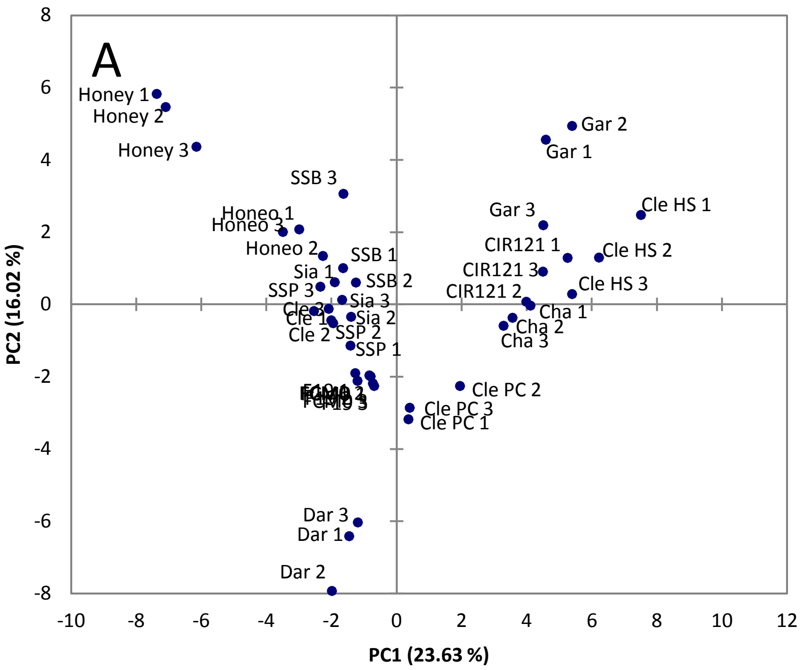
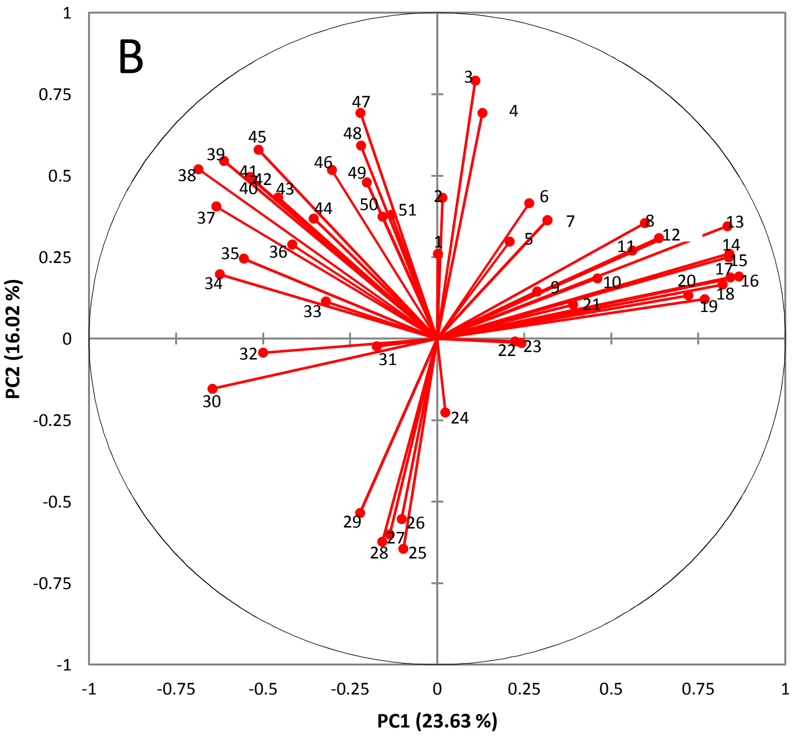


Figure 2: Concentration of volatiles after extraction by ASE method with: dichloromethane/pentane (1:2) (blue), dichloromethane (red), ethyl acetate/pentane (2:1) (green)

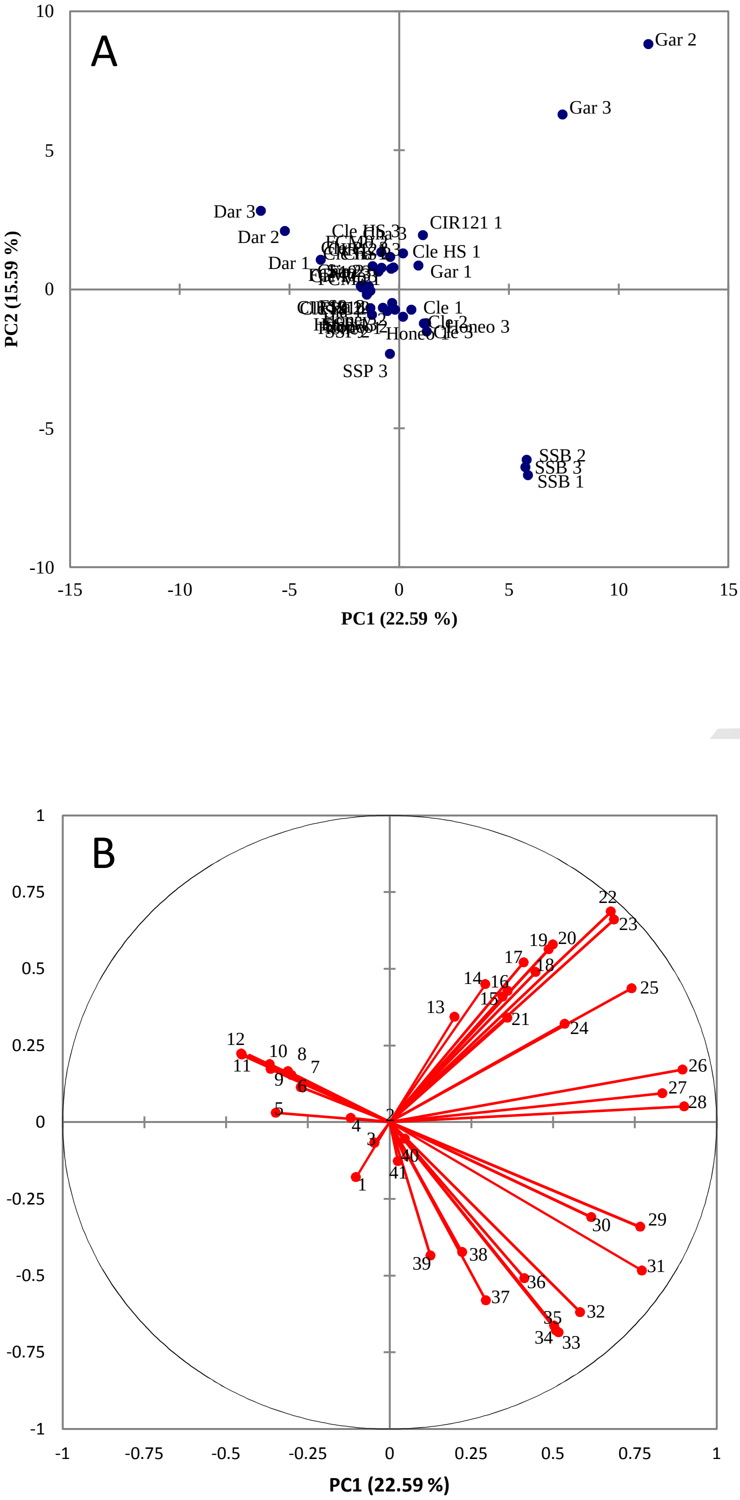


Gar : Gariguette
Cha : Charlotte
Sia : Siabel
F19 : Fraise 19
Honeo : Honeoye
Cle : Clery
SSB : Senga sangane (Bulgaria)
SSP : Senga sangana (Poland)
Dar : Darselect



1. Furaneol
2. Mesifurane
3. Isobutanoic acid
4. 2-Methylbutanoic acid
5. Ethyl Isobutanoate
6. 2-Hexenoic acid
7. Unknown
8. Trans-Nerolidol
9. Methyl 3-hydroxybutanoate
10. Succinic anhydride
11. 2-Hydoxyfuraneol
12. Propanoic acid
13. Crotonic acid
14. Hexanal
15. Ethyl butanoate
16. 2-Hexanone
17. Isobutyl acetate
18. 3-Methylbutyl acetate
19. 1,1-diethoxybutane
20. Butyl acetate
21. α-(E,E)-Farnesene
22. Trans-linalool oxide
23. 3-Methylhexanoic acid
24. 4-Vinylguaiacol
25. Benzyl alcohol
26. δ-Caprolactone
27. γ-Caprolactone
28. 5-(Hydroxymethyl)-2- furfural
29. Octanoic acid
30. 3-Methyl-3-penten-2-one
31. Chavicol
32. 3-Penten-2-one
33. 5-Methyl-5-hexen-2-one
34. 1-Butanol
35. 2-Phenylethyl alcohol
36. Nonanoic acid
37. Ethylene glycol monoacetate
38. Pantolactone
39. 2,3-Butanediol
40. 3-Phenylpropyl alcohol
41. Ethyl phenol
42. Ethyl (E)-cinnamate
43. 1-Hexanol
44. γ-Decalactone
45. Acetoin
46. Ethyl hexanoate
47. Butanoic acid
48. Hexanoic acid
49. Coumaran
50. β-Linalool
51. γ-Dodecalactone

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



Gar : Gariguette
Cha : Charlotte
Sia : Siabel
F19 : Fraise 19
Honeo : Honeoye
Cle : Clery
SSB : Senga sangane (Bulgaria)
SSP : Senga sangana (Poland)
Dar : Darselect

1. Benzyl alcohol
2. Ethyl phenol
3. Nonanoic acid
4. 3-Methylbutyl acetate
5. γ-Decalactone
6. 5-(Hydroxymethyl)-2-furfural
7. Octanoic acid
8. 3-Methyl-3-penten-2-one
9. Linalool oxide
10. Methylethylbenzene
11. 1-Terpineol
12. γ-Caprolactone
13. Furaneol
14. 2-hydroxy-4-pyranone
15. Trans-Nerolidol
16. γ-Dodecalactone
17. Hexanal
18. Succinic anhydride
19. 2-Hexanone
20. 1,1-diethoxybutane
21. Formic acid
22. Propanoic acid
23. Acetoin
24. 2-Hydroxyfuraneol
25. Isobutanoic acid
26. 2-Methylbutanoic acid
27. Butanoic acid
28. Crotonic acid
29. β-Linalool
30. Hexanoic acid
31. Coumaran
32. 2-Hexenoic acid
33. Phenol
34. 3-Hexenoic acid
35. 1,2-cyclopentadione
36. 4-Vinylguaiacol
37. Methyl 3-hydroxybutanoate
38. Pantolactone
39. 2,3-Butanediol
40. Chavicol
41. 2-Phenylethyl alcohol

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

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.