APPLICATION OF GLYCOSIDIC AROMA PRECURSORS TO ENHANCE THE AROMA AND SENSORY PROFILE OF DEALCOHOLISED WINES

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Abstract

The addition of glycosidic aroma precursors isolated from grapes to reinforce the aroma profile of a dealcoholised white wine has been investigated. Moreover, the use of oenological glycosidases and the effect of storage (30 days) on the evolution of the released aglycones were evaluated. Four types of dealcoholised wines: control (CTR), control with enzyme addition (CTR-E), added with aroma precursors (PREC) and with enzyme and aroma precursors (PREC-E) were prepared. The analysis of free volatile compounds by HS-SPME-GC-MS and the application of multivariate statistical analysis confirmed differences in the volatile profile between CTR and PREC wines. By applying aroma dilution and olfactometry analysis (AEDA-GC-O), 20 odour notes were detected. The highest dilution factors eliciting floral *like* odour were found in PREC wines and identified as linalool, geraniol and β-phenylethyl alcohol. Sensory descriptive analysis confirmed higher intensity in *Muscat* and *floral* attributes in these wines, which open the possibility to new technological application based on using aroma precursors to enhance the aroma characteristics of wines with low aroma intensity.

Key words: grape glycosidic aroma precursors; dealcoholised wines, volatile compounds, AEDA-GC-O, sensory descriptive analysis

Highlights

> Glycosidic aroma precursors to aromatise dealcoholised wines. > Volatile profile, odour active compounds and sensory analysis> Great increase in terpenes in treated wines > Enhancement of the *Muscat*, *flowery* and *tropical* aroma attributes.

1. Introduction

In recent years, new consumer demands for light, fruity and low alcohol beverages have pushed the wine industry into a diversification in their production and new types of low-alcohol beverages based on dealcoholised wines are becoming more and more common in the market. Besides the perception of these low alcohol wine-based beverages as healthier, the acceptance of these new products by the consumers greatly depends on their organoleptic characteristics. To reduce the alcohol content, several methods have been patented and/or published to obtain a beverage with very low level of ethanol (below 1% v/v). Some of them are based on vacuum evaporation in a column of rotary cones, extraction with supercritical CO_2, or membrane separation processes (Moro-Gonzalez, Gonzalez-Jimenez, Cortijo-Garcia, Pinto-Solano, & Guadarrama-Rodríguez, 2012; Pérez-Magariño, Ortega-Heras, Rodríguez-Bencomo, Cano-Mozo, González-Huerta, & Herrera, 2008; Ruiz-Rodríguez et al., 2012; Catarino, & Mendes, 2011; Sobota, & Zdarsky, 2011). In some of these methods the aroma compounds are firstly isolated and after the dealcoholisation step (partially or totally) the aroma compounds are added again to the dealcoholised wine (Pérez-Magariño et al., 2008). However, most of the dealcoholisation processes provoke important changes in the sensory characteristics of the dealcoholised wines, many of them associated to a lost in aroma intensity (Gómez-Plaza, Lopez-Nicolas, Lopez-Roca, & Martinez-Cutillas, 1999; Pérez-Magariño et al., 2008; Catarino, & Mendes, 2011).

The characteristic aroma of many wines depends on the varietal compounds of grapes. These varietal compounds can be present in grapes as free volatile compounds and as aroma precursors. Among them, glycosidic precursors could be considered as a pool of aromatic compounds that might be liberated during winemaking or storage by acid or enzymatic hydrolysis. The volatile compounds that could be released from glycosidic

74 aroma precursors are mainly terpenes, C13 nor-isoprenoids, benzenic derivatives,
75 volatile phenols and C6 compounds (Baumes, 2009). These compounds can provide
76 important aromatic characteristics to wine aroma, for example, in the case of terpenes,
77 they could provide flowery notes that are characteristics of some grape varieties such as
78 Muscat (Etievant, 1991).
79 Since aroma precursors are mainly located in the solid parts of the grape (skins), the use
80 of grape pomace produced during winemaking and/or from the juice industry activity,
81 has been proposed as an interesting way to obtain aroma precursors to value this sub-
82 products for different types of industrial applications (Palma, Taylor, Zoecklein, &
83 Douglas, 2000). The effect of the addition of an extract of grape glycosidic precursors
84 on the volatile composition of musts or wines has been evaluated by different authors.
85 For instance, the effect of different yeast strains on the release of the aromatic aglycones
86 from a glycoside extract was studied in real and synthetic musts (Ugliano, Bartowsky,
87 McCarthy, Moio, & Henschke, 2006; Loscos, Hernandez-Orte, Cacho, & Ferreira, 2007
88 and 2009a; Hernandez-Orte, Cersosimo, Loscos, Cacho, Garcia-Moruno, & Ferreira,
89 2008; Gamero, Hernandez-Orte, Querol, & Ferreira, 2011). Moreover, the ability of
90 lactic acid bacteria during malolactic fermentation to liberate the aglycones from the
91 corresponding precursors has been also proven (Ugliano, Genovese, & Moio, 2003;
92 Ugliano & Moio, 2006; Hernandez-Orte, Cersosimo, Loscos, Cacho, Garcia-Moruno, &
93 Ferreira, 2009). The release of varietal compounds from the glycosidic precursors might
94 also occur during the second fermentation of a base wine (Ganss, Kirsch, Winterhalter,
95 Fischer, & Schmarr, 2011). In some of these works a complementary evaluation of the
96 enzymatic activities of the yeasts or lactic acid bacteria were also studied. Other authors
97 have also followed the liberation of the aglycones and their evolution in accelerated

98 wine aging conditions (Loscos, Hernandez-Orte, Cacho, & Ferreira, 2010) and during
99 the aging of wines on lees (Loscos, Hernandez-Orte, Cacho, & Ferreira, 2009b).
100 Since glycosidic aroma precursors can be an interesting source of aroma compounds,
101 this opens the possibility to use them to reinforce the aroma profile of wines from non-
102 aromatic varieties, to improve the organoleptic characteristics of dealcoholised wines, or
103 even, they might be use as flavouring agents in other wine-based beverages.
104 The main objective of this work was to evaluate the possibility of using grape
105 glycosidic aroma precursors to improve the aroma and sensory characteristics of a
106 dealcoholised white wine. Dealcoholisation was done at lab-scale following a gentle
107 vacuum evaporation process. The impact of adding grape aroma precursors was
108 evaluated taking into consideration the effect of the storage in presence or not of
109 glycosidase enzymes. The characterization of the volatile profile of the wines,
110 identification of the odour active compounds and the descriptive sensory analysis of the
111 wines were carried out to achieve this objective.

112 **2. Materials and Methods**

113 *2.1. Reagents and solvents*

114 Solvents (ethanol, dichloromethane, pentane, ethyl acetate and methanol) were obtained
115 from Merck (Darmstadt, Germany) and LabScan (Gliwice, Poland). Pure water was
116 obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). L-(+)-
117 tartaric acid, sodium chloride, sodium hydroxide, sodium phosphate dibasic, sodium
118 phosphate monobasic monohydrate, and citric acid monohydrate came from Panreac
119 (Barcelona, Spain) and Sigma-Aldrich (Steinheim, Germany). Pure volatile compounds
120 were supplied by Aldrich (Gillingham, UK), Fluka (Buchs, Switzerland), Riedel-de
121 Haën (Seelze, Germany) and Firmenich (Geneva, Switzerland).

122 *2.2. Samples.*

123 *2.2.1. Preparation of the aroma precursors extract*

124 To obtain the aroma precursors extract, a methodology based in the methods already
125 published by Loscos et al., 2007 and Ganss et al 2011 were followed. Ten kilograms of
126 Muscat grapes were destemmed, crushed and filtered to separate the musts and the
127 skins. The last ones were macerated with a buffer solution (0.1M Na_2HPO_4/NaH_2PO_4,
128 pH=7 and 13% ethanol v/v) for 36 hours at room temperature and in a nitrogen
129 atmosphere. After that, the buffer was filtered and evaporated (at vacuum) to remove
130 ethanol. Glycosidic aroma precursors were isolated by retention on Amberlite XAD-2
131 resins from Supelco (Bellefonte, PA, USA). The isolation was carried out in glass
132 columns where the resins (20 cm of length ≈ 63 cm^3 of resins) were conditioned with
133 150 mL of dichloromethane, methanol and milli-Q water. After passing the musts or the
134 buffers from the extracted skins, resins were washed with water (300 mL) to remove
135 high-polar compounds, then with pentane/dichloromethane (300 mL) (2:1 v/v) to
136 remove free volatile compounds, and finally aroma precursors were eluted with 300 mL
137 of ethyl acetate/methanol (9:1 v/v). The eluate was evaporated to dryness, dissolved in
138 milli Q water and extracted twice with pentane and dichloromethane to remove traces of
139 free volatile compounds. The extract was stored at -20ºC.

140 *2.2.2. Preparation of the dealcoholised wines with the precursors extracts*

141 Four litres of a commercial white wine from "Airen" grape variety produced in Castilla
142 la Mancha (Spain) (11.5 % ethanol v/v) were purchased in a local grocery and
143 dealcoholised in our laboratory by applying a gentle vacuum evaporation at 35ºC and 40
144 mbar. The wine was evaporated until leave 60% of the initial volume. Dealcoholised
145 wine was reconstituted to the initial volume (4 L) with milli-Q water obtaining a wine
146 of ≈0.8 % ethanol v/v. The ethanol content was confirmed by direct injection (1µL) of
147 the wine in a gas chromatograph provided with a flame ionisation detector (GC-FID)

system (Split 1:20) (Hewlett-Packard 5890). The chomatographic column was a SGL-20 (30 m x 0.25mm x 0.25 μm) from Sugelabor (Barcelona, Spain) and the column oven program was 60ºC (6 min) , from 60ºC to 200ºC at 12ºC/min and hold 200ºC for 5 min.

Dealcoholised wine was separated in twelve 200-mL amber bottles with screwcap. Four sets of three bottles were prepared as following: two sets without addition of the precursor extract: Control wines (CTR), from which one set was prepared with addition of a commercial oenological enzyme preparation with glycosidase activity (Enovin, Agrovin, Ciudad Real, Spain) (CTR-E); the others two sets of wines were prepared with the aroma precursor extract: Precursors wines (PREC), from which one of them, was prepared with addition of the oenological enzyme preparation (PREC-E). The amount of aroma precursors extract added to each of the 200 mL wines was the equivalent to 300 g of Muscat grapes. On the other hand, the oenological enzyme was added to obtain a final concentration of 20 mg/L. All samples were stored at room temperature in the darkness during 30 days, taking samples for analysis at 15 and 30 days.

2.3. Analysis of free volatile compounds

The analysis of wine volatile compounds was carried out by head space solid phase microextraction coupled to gas chromatography spectrometry (HS-SPME-GC-MS). Wine samples (8 mL), 2.3 g of NaCl and 40 μL of an internal standards solution (400 mg/L 3,4-dimethylphenol, 10 mg/L 3-octanol and 2.5 mg/L methyl nonanoate) were added to a 20 mL SPME vial. The SPME procedure and chromatographic conditions were detailed in Rodríguez-Bencomo, Muñoz-González, Andujar-Ortiz, Martin-Alvarez, Moreno-Arribas, & Pozo-Bayon, 2011. The extraction procedure was automatically performed using a CombiPal system (CTC Analytics AG, Zwingen, Switzerland) with a 50/30 μm DVB/CAR/PDMS fibre of 2 cm length from Supelco.

Samples were pre-incubated for 10 min at 50 ºC and extraction was performed in the headspace of each vial for 30 min at 50 ºC. Desorption was performed in the injector of the GC system in splitless mode for 1.5 min at 270 ºC. After each injection the fibre was cleaned for 20 min to avoid any memory effect. The chromatographic separation was performed in a GC-MS (Agilent 6890GC, Agilent 5973N MS) equipped with a Supra-Wax fused silica capillary column (60 m × 0.25mm i.d. × 0.50 μm film thickness) from Konik (Barcelona, Spain). Helium was the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 40 ºC for 5 min, then, it increased at 4 ºC/min to 240 ºC and was held for 20 min. The acquisitions were performed in Scan (from 35 to 350 amu) and Sim modes for some specific compounds (electronic impact mode, 70 eV). The MS conditions were 270, 150 and 230 ºC for the transfer line, quadrupole and ion source respectively. The signal corresponding to a specific ion of quantification was calculated by the data system. The identification of compounds was carried out by comparison of retention times and mass spectra of the references compounds with those reported in the mass spectrum library NIST 2.0. Quantitative data were obtained by calculating the relative peak area in relation to that of the corresponding internal standard. To calculate the concentration of each aroma compound, calibration curves of each reference compound at different concentrations covering the concentration ranges expected in the samples were prepared.

2.4. Analysis of glycosidic aroma precursors

The glycosides aroma precursors present in wine were indirectly analysed by enzymatic hydrolysis according with the methods proposed by Loscos et al., 2007 and 2009a with some modifications. Thirty mL of dealcoholised wine were percolated through a 100 mg Lichrolut SPE cartridge (Merck, KGaA, Darmstadt, Germany) previously conditioned with 5 mL of dichloromethane, 5 mL of methanol and 10 mL of milli-Q

198 water. After that, the cartridge was washed with 4 mL of water and 4 mL of pentane/
199 dichloromethane (2:1 v/v). The glycosides were eluted with 7 mL of ethyl
200 acetate/methanol (9:1 v/v). After evaporation to dryness, the extract was dissolved in 4
201 mL of citrate/phosphate buffer (pH=5). The hydrolysis was carried out by addition of 20
202 mg of the oenological enzyme and incubated 16 hours at 40ºC.
203 The liberated aglycones, added with 20 µL of β-damascone solution (0.25 mg/mL) as
204 internal standard, were retained on 50 mg of a Lichrolut SPE cartridge previously
205 conditioned with 2 ml of dichloromethane and methanol and 5 mL of a water-ethanol
206 solution (12% in ethanol v/v). After passing 5 mL of methanol, cartridges were dried
207 with air (0.6 bars, 10 min) and the volatile compounds were eluted with 1 mL of
208 dichloromethane. The eluate, added with 20 µL of an internal standard solution of 2-
209 octanol, (65 mg/L) was evaporated with a gentle nitrogen flow until ≈100 µL. Two µL
210 of this extract were injected (splitless mode) in the GC-MS system (Agilent 6890GC,
211 Agilent 5973N MS). Column oven program was the same described in section 2.3.
212 *2.5. Aroma Extract Dilution Analysis-Gas Chromatography-Olfactometry (AEDA-GC-*
213 *O)*
214 The dealcoholised wine (CTR) and the same wine supplemented with the glycosidic
215 aroma precursor extract and the enzymatic preparation (PREC-E) were analysed by GC-
216 MS-O following the AEDA methodology (Schieberle & Grosch, 1987, Ullrich &
217 Grosch, 1987), using three experienced sniffers. For AEDA, the concentrated aromatic
218 extract (200 µL) of the wine samples (obtained in the conditions described in 2.4 for the
219 analysis of free volatiles but using 200 mg of Lichrolut SPE cartridge), was stepwise
220 diluted 1:1 using dichloromethane as the solvent to obtain dilutions of 1:1, 1:2, 1:4, 1:8,
221 1:16 and samples up to 1:512 of the original extracts. Sniffing of dilutions was
222 continued until no odorant could be detected by GC-O. Each odorant was thus assigned

to a flavor dilution factor (FD factor) representing the last dilution in which the odorants was still detectable. The gas chromatography system consisted of an Agilent 6890 chromatograph equipped with a flame ionisation detector (FID) (Wilmington, DE, USA), an Agilent 5973 mass selective detector (MSD) (Wilmington, DE, USA), and a Gerstel ODP-2 (Baltimore, MD, USA) sniffing port using a deactivated capillary column (30 cm x 0.3 mm) heated at 240 °C and supplied with humidified air at 40°C. This system allowed us to simultaneously obtain a FID signal for the quantification, an MS signal for the identification, and the odour characteristics of each compound detected in the sniffing port. GC effluent was split 1:1:1 among the FID, MSD, and the sniffing port. Aroma compounds were separated on DB-Wax (30 m length x 0.25 mm i.d. x 0.5µm thickness, J&W Scientific Folsom, CA , USA) column. A total of 3 µL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. Injector and FID detectors were set at 270°C and 280°C, respectively. The flow rate of carrier gas (helium) was 1.5 mL/min. The oven temperature of the DB-Wax column was first increased from 50° to 200 °C at a rate of 5 °C/min and then to 260°C at 8 °C/min, with a final hold at 260 °C for 5 min. The same oven temperature programs were used for the mass-selective detector. The MS (electronic impact ionisation) conditions were: ionisation energy of 70 eV, mass range m/z of 30-300 amu, transfer line temperature of 250 °C, and source temperature of 180°C (Cayhan & Selli, 2011).

2.6. Sensory descriptive analysis

Sensory descriptive analysis was performed by a panel previously trained in the odour of typical descriptive attributes of Muscat wines (Campo, Ferreira, Escudero, & Cacho, 2005; Sanchez-Palomo, Pérez-Coello, Díaz-Maroto, González-Viñas, & Cabezudo, 2006). The panel was composed by 17 panellists (6 males and 11 females) previously selected from 28 people recruited from the CIAL staff on the basis of their performance

and reproducibility in the training sessions. The aroma reference standards employed to define each of the term evaluated are listed in Table 1. All the references employed in the training were prepared in model wine solutions (3.5 g/L tartaric acid; 0.5% ethanol v/v; pH=3.5) and were evaluated at two levels of intensity. The three types of samples chosen for the sensory analysis (CTR, PREC and PREC-E samples at 30 days of storage time) were presented in code wine glasses in random order. All the wines were orthonasally evaluated in duplicate by the 17 panellists in two separate sessions using a 4 point-scale (0=not detected; 1=weak detected-hardly recognizable; 2=clear-but not intense; 3=intense). The data were processed as a mixture of intensity and frequency of detection called modified frequency (MF) and defined as MF (%)=$[F(\%)\cdot I(\%)]^{1/2}$, where F(%) is the detection frequency of an attribute in percentage and I(%) in the average intensity expressed as percentage of the maximum intensity (Campo et al., 2005).

2.7. Statistical Analysis

The statistical methods used for the data analysis were: three-way analysis of variance (ANOVA) to examine together the main effects of the three estudied factors (addition of aroma precursors, storage time, and addition of enzyme); one way-ANOVA and Least Significance Difference (LSD) test for means comparison of the aroma precursors data at 30 days of storage time; and principal component analysis (PCA), from correlation matrix, to examine the relationship between the analyzed variables and between the wine samples. For the sensory analysis, the modified frequencies (MF) were statistical analysed by the non-parametric Krustal-Wallis rank test. A value of P = 0.05 was fixed for the level of significance of the tests. The STATISTICA program for Windows version 7.1 was used for data processing (StatSoft, Inc., 2005, www.statsoft.com).

3. Results and Discussion

3.1. Effect of the addition of the glycosidic precursors with and without enzymes on the free volatile composition of dealcoholised wines

Dealcoholised wines obtained in the conditions described in the Material and Methods section were prepared by adding the aroma precursor extract and using or not the glycosidase enzyme preparation. The analysis of the volatile profile was performed in the initial dealcoholised wine and in the treated wines after 15 and 30 days of storage. These results together with those from a three-way ANOVA (only considering the main effects), therefore, taking into consideration the storage time, addition of precursors and addition of enzymes like factors, are presented in Table 2. From the three studied factors, the addition of aroma precursors and the storage time seemed to be the most significant for the majority of aroma compounds. The addition of aroma precursors clearly affected the group of terpenes and C13 norisoprenoids, while other compounds such as C6 alcohols, lactones and furanic compounds did not seem affected for any of them. In general, in the wines added with the aroma precursor extract (PREC and PREC-E), an important increase in their concentration compared to the initial dealcoholised wine was observed. As it was said, this was especially remarkable for most terpenic compounds. For instance, the concentration of linalool increased 230 times at the end of the storage time compared to its initial concentration in the INI-0 sample. Other terpenes, such as limonene, α-terpineol, terpinen-4-ol and β-citronellol were also found between 7 and 47 times more concentrated in the treated wines after 30 days of storage. For some of them such as linalool, α-terpineol and limonene a strong rise in their concentration was observed in the first 15 days of storage, while for others,

298 such as β-citronellol, the main increase in the concentration was observed after 15 days,
299 especially in wines treated with enzymes (PREC-E). It is noteworthy that the
300 concentration values calculated for linalool in the treated wines were in the same order
301 of magnitude than those determined by Ugliano et al., 2006 in synthetic musts added
302 with Muscat aroma precursors and fermented with different yeasts strains. In addition,
303 the calculated range (393-402 µg/L) for this compound, was in agreement with that
304 reported by other authors in Muscat wines (Ribereau-Gayon, Boidron, & Terrier, 1975;
305 Sanchez-Palomo et al., 2006) and it was much higher than that determined in Airen
306 wines (lower than 50 µg/L) (Cejudo-Bastante, Castro-Vázquez, Hermosín-Gutiérrez, &
307 Pérez-Coello, 2011; Peinado, Moreno, Bueno, Moreno & Mauricio, 2004; Bueno,
308 Peinado, Medina, & Moreno, 2006).
309
310 Control wines (CTR and CTR-E) exhibited lower concentration of these terpenes
311 compared to PREC wines, however in the case of control wines treated with enzymes
312 (CTR-E) a slight increase in the concentration of most terpenes was observed after 15
313 days of storage likely due the liberation of aglycones from aroma precursors originally
314 present in the initial dealcoholised wine. The progressive decrease in the concentration
315 of terpenes observed during storage was likely due to the involvement of these
316 compounds in oxidative reactions or acid catalyzed rearrangements, as it has been
317 already described (Loscos et al., 2010)
318 The results for β-damascenone, the only C13 nor-isoprenoid, quantified in the wines
319 followed the same trend observed for the terpenic compounds. In PREC and PREC-E
320 wines an important increase in its concentration (16 to 17 times), was observed in the 15
321 first days of storage. The range of concentration of β-damascenone (3.83-4.02 µg/L) in
322 the wines treated with the precursors extract was in agreement with the values reported

by other authors in Muscat wines (Selli, Canbas, Cabaroglu, Erten, & Gunata, 2006) and similar to those obtained by Loscos et al., 2010 in synthetic wines aged in accelerated conditions and supplemented with extracts of aroma precursors from different grapes varieties (Chardonnay, Muscat). In addition, the concentration of this compound in the wines added with the precursor extract was only slightly higher than that reported by other authors in synthetic musts added with an aroma precursor extract from non-aromatic grapes and fermented with different yeasts or when synthetic wines were subjected to malolactic fermentation (Hernández-Orte et al., 2008 and 2009). Therefore, this seemed to indicate, that the grape variety from which the precursor came from did not have a critical influence on the levels of this compound in the wines.

Some other volatiles compounds identified in the wines such as phenols and benzoic compounds, were also significantly influenced by the addition or the precursor extract or the storage time, however the addition of enzyme was not as important to explain their changes in concentration. Among them, the ethylphenols, 4-ethylguaiacol and 4-ethylphenol, eugenol and β-phenylethyl alcohol showed higher concentrations in the wines supplemented with the aroma precursors. The average content of these compounds are shown in Table 2, and it was between 17% and 63% higher for ethylguaicol and β-phenylethyl alcohol respectively, in the wines added with precursors (PREC) than in the control wine aged 30 days. Only, 2-methoxy-4 vinylphenol, 4-vinylphenol and benzyl alcohol showed a significant effect due to the storage time. The two first compounds experienced strong increases in their concentration mainly at the end of the storage time (30 days), and although they showed a large variation in the three wines replicates, it is not possible to discard that both factors (addition of

348 precursors and enzyme) and/or other reasons related to the presence of residual
349 enzymatic activities (cinnamate decarboxylase) in the oenological preparation might
350 induce their formation.
351 Some other groups of volatile compounds, such as acids and esters also showed
352 differences between wines added or not with the aroma precursors (Table 2), although
353 “a priori”, these compounds are not directly related to the glycosidic aroma precursors.
354 In this sense, a significant higher content of low molecular weight esters (ethyl acetate,
355 butyrate and propanoate and butyl acetate) was observed in the wines supplemented
356 with the aroma precursor extract (Table 2). On the other hand, other esters such ethyl
357 octanoate, decanoate and hexanoate showed a significant effect because of the enzyme
358 addition; while the storage time affected the behaviour of ethyl octanoate, ethyl lactate
359 and β-phenylethyl acetate, which showing increases and decreases in their content
360 during the storage. It is likely that the variation observed in their concentration, which
361 means formation and hydrolysis, could be better explained by a re-equilibration in their
362 concentration as a consequence of the variation on the levels of ethanol, higher alcohols,
363 acids and esters due to the dealcoholisation process. In addition, possible residual
364 esterase activities in these types of enzymatic preparation (Sumby, Grbin, & Jiranek,
365 2010) widely used in winemaking to enhance wine aroma, might be also an influent
366 factor. Anyway, the global effect of all of these variations in the content of esters might
367 have an effect at sensory level on the fruity aromatic notes (Etievant, 1991).
368 Regarding the group of volatile fatty acids, some of them such as hexanoic and
369 octanoic, also showed a significant effect due to the addition of the aroma precursors
370 (Table 2). Their content was in general, higher in the wines added with precursors
371 (between 34% and 50%) than in the control wines at the end of storage. In addition,
372 hexanoic and decanoic acids were significantly influenced by the storage time and their

373 concentration fluctuated during aging. These acids could contribute to freshness and to
374 equilibrate the fruity aromas of wines (Etievant, 1991).
375 Conversely, as it was indicated before, the C6 alcohols, lactones and furanic compounds
376 identified in the wines were not affected by any of the studied factors, in spite that some
377 of them, such as C6 alcohols, might be originated from glycosidic precursors.
378 In order to obtain more information on the causes of variability in the concentration of
379 volatile compounds due to the different studied factors, Principal Component Analysis
380 have been carried out considering as variables all free volatile compounds quantified in
381 the samples (Table 2). It was observed that more than 60 % of the variation in the data
382 could be explained by the two first principal components (PC1 and PC2). Figure 1
383 shows all the wine samples in the plane defined by these components. As can be seen,
384 all samples treated with aroma precursors showed negative values for PC1, while initial
385 and control wines exhibited positive values for this component. Therefore, PC1 seemed
386 to be related with the liberated aglycones from the aroma precursors. On the other hand,
387 the initial wine (INI-0) had positive and low values for PC2, whereas the values were, in
388 general, positive and higher in the control wines after 15 and 30 days. These results
389 seem to indicate that PC2 could be related with the evolution of the volatile compounds
390 in the control wines during the storage time. The weight of each variable on each
391 principal component showed that PC1 (explained the 41.49% of the total variance) was
392 highly correlated with the free volatiles: ethyl propanoate (-0.901), ethyl butyrate (-
393 0.846), butyl acetate (-0.856), limonene (-0.878), linalool (-0.897), terpinen-4-ol (-
394 0.891), β-damascenone (-0.859), eugenol (-0.816), 4-ethylphenol (-0.911) and ethyl
395 acetate (-0.909). All these compounds presented a negative correlation, so their levels in
396 the wines treated with the aroma precursor extract were higher than those found in the
397 control wines. On the other hand, PC2 (explained the 18.78% of the total variance) was

398 also correlated with the free volatiles, furfural (0.724), β-phenyl ethyl acetate (-0.808),
399 hexanoic acid (0.613), β-phenylethyl alcohol (0.651), octanoic acid (0.643) and
400 decanoic acid (-0.611). Although, a clear separation among control samples was not
401 observed, the compounds positively correlated with PC2 showed, in general, lower
402 contents at the end of the storage time in the control wines compared to the initial
403 wines. On the contrary, the compounds negatively correlated with PC2, in general,
404 showed an increase in their concentration during the storage time.
405
406 *3.2. Quantification of the remaining glycosidic aroma precursors in the dealcoholised*
407 *wines stored for 30 days*
408 In order to evaluate the presence of remaining glycosidic aroma precursors in the wines
409 after 30 days of storage, which still might be a potential source for volatile compounds
410 in the wines, the concentration of glycosidic terpenes was determined in the initial wine
411 and in the wines submitted to 30 days of storage. This group of compounds was chosen
412 to quantify the remaining glycosidic aroma precursors in the samples because they are
413 the most representative aroma compounds of Muscat wines. As can be seen in Table 3,
414 in the wines aged 30 days, terpenic compounds had higher values of concentration in
415 those wines treated with the aroma precursors extract than in the control wines. This
416 finding seemed to indicate that, although an important part of aroma precursors were
417 hydrolyzed and released as free volatiles (as it was already commented in the previous
418 section), a very little part of them remained in the wine, so during wine storage these
419 precursors might still release the corresponding aglycones contributing to the terpenic
420 aroma of wine. In addition, it was not observed a significant effect of the addition of
421 enzymes, so probably the main hydrolytic mechanism involved in the release of terpenic
422 compounds from the corresponding glycosidic precursors might have been acid-

423 catalysed hydrolysis. These results are in accordance with the results obtained in the
424 free terpenes analysis, in which in general, there was not a significant effect due to the
425 enzyme addition in most of the identified terpenes. However, geraniol followed a
426 different trend compared to the rest of terpenic compounds. Taking into consideration
427 the increase observed in the levels of this compound (Table 3). The explanation could
428 be due to the great reactivity of terpene compounds and therefore, the subsequent
429 transformations or rearrangements of the released aglycones during the different steps
430 involved in the analysis of aroma precursors. This fact has been already described for
431 the transformation of linalool into geraniol (Ebeler, 2001).
432
433 *3.3. Identification of the odour active compounds in the dealcoholised wines added with*
434 *glycosidic aroma precursors*
435 In order to determine the odour active compounds, therefore, the most relevant
436 compounds for the aroma characteristics of the dealcoholised wines added with the
437 aroma precursor extract, the wines were submitted to an olfatometric analysis by GC-O.
438 To do that, the two wines with "a priori" more different sensory characteristics, the
439 control wine (CTR) and the wine added with precursors and enzymes (PREC-E) were
440 chosen for this study. An aroma extract of each wine was obtained and the AEDA
441 technique was used for the GC-O study. The results are presented in Table 4. As can be
442 seen, twenty odour notes were detected in the AEDA in the FD range 4-512. Except
443 three of them, all the perceived odours were assigned to different volatile compounds by
444 comparison of their retention index with those tabulated in the bibliography, mass
445 spectra data of reference compounds and/or MS spectra libraries, as well as based on
446 their odorant quality. Some of the identified compounds, such as peaks 5, 7, 8, 11, 12,
447 13, 16 and 19, were not showed in Table 2, since in this table only the groups of

448 compounds more related with their release from the glycosidic precursors or more
449 representative of the volatile profile of wines were analysed. In Table 4, the highest FD
450 factors in the PREC-E wines were determined for peak 9 (FD: 512), peak 17 (FD: 64)
451 and peak 18 (FD: 64), all of them eliciting a *floral-like* odour that were identified as
452 linalool, geraniol and 2-phenylethanol respectively. Linalool is one of the most
453 important compounds related to the aroma of Muscat wines (Etievant, 1991). This
454 compound was only detected by GC-O in the PREC-E wine but not in the control wine.
455 In addition, other GC-O peaks identified as geraniol (FD: 64) α-terpineol (FD: 6) and
456 hotrienol (FD: 8) also associated with flowery notes, presented higher FD in the PREC-
457 E wine than in the control wine. Other two odour active compounds, identified as ethyl
458 octanoate and ethyl 4-hydroxybutanoate (peak 6 and peak 16) associated to *fruity-like*
459 notes, also showed dilution factors higher in the PREC-E wine compared to the control
460 wine. In fact, peak 16, was not detected in the control wine. On the other hand, several
461 peaks eliciting unpleasant sensory notes were detected in the wines. These peaks,
462 exhibited higher FD in the control than in the PREC-E wine. For instance, two unknown
463 compounds (peak numbers 3 and 10) presented an odour described as *plastic, burnt* and
464 *chemical*. Moreover, peak 13, identified as butanoic acid was associated to *chemical*
465 and *mouldy odour-like* aroma and its FD was higher in the control wine (FD: 32) than in
466 PREC-E wine (FD: 16). All of these compounds (except peak 10) were detected in both
467 wines (although at different intensity), therefore, they were already present in the
468 dealcoholised wine and they might have originated during the dealcoholisation process
469 and/or they could have been initially present in the original wine before
470 dealcoholisation. The differences found by GC-O in the aroma active compounds
471 between both types of wines, might be related to sensory differences in the
472 dealcoholised wines, as was checked as following.

3.4. Sensory Descriptive Analysis

To determine whether the addition of aroma precursors might change the sensory profile of dealcoholised wines, a descriptive sensory analysis was performed with the control wine (CTR) and with the wines treated with the aroma precursor extract, with and without enzyme addition (PREC and PREC-E respectively) at the end of the storage time (30 days). The results of the sensory analysis are shown in Figure 2. As can be seen, the "spider web" represents the ten sensory attributes characteristic of Muscat wines selected for this study and the modified frequency calculated for each of them in the three wines. The results clearly showed a different sensory profile between control and treated wines. PREC and PREC-E wines showed significant differences in the *Muscat* and *floral* aromatic notes, more intense in these wines compared to the Control wine. These results were in agreement with the analytical results and the olfactometric study that showed levels of linalool and some other monoterpenes (α-terpineol) and β-phenylethyl alcohol in much greater concentration in the wines treated with the glycosidic precursor extract. However, the *tropical fruity* note was significantly more intense only in the wine treated with precursors (PREC), while panelists did not show significant differences in the intensity of this attribute between PREC-E and control wines. The perception of the *tropical fruity aroma* could be more related with the concentrations of esters and the relative proportions of ethyl esters and acetates (Etievant, 1991, Ferreira, Fernández, Peña, Escudero, & Cacho, 1995), which as it was previously shown was higher in the PREC wine. In addition, *yeast* and *oxidized* notes, which could be considered as off-flavours in wines, were significantly higher in the control wine than in those wines treated with the aroma precursors (PREC and PREC-E). The oxidized character could be due to the dealcoholisation process which, in spite

498 of the mild conditions applied to the wine samples, might have produced the oxidation
499 of some volatile compounds. The presence of very intense *floral/Muscat* notes in the
500 PREC and PREC-E wines might have masked the perception of the unpleasant notes in
501 these wines. However, during the GC-O analysis, as it was shown before, in where
502 isolated peaks were detected, some odour active compounds associated to these types of
503 off-flavours were also detected in the PREC-E wine.
504
505 **4. Conclusions**
506 The results of this study have shown the aroma enhancement of dealcoholised wines
507 due to the addition of glycosidic aroma precursors. The main effect of this addition is an
508 increase in terpenes and some C13 norisoprenoids, which seems to be more related to
509 acid-catalysed reactions than to the enzymatic release using commercial glycosidase
510 enzymes. The aromatic aglycones released from the precursors in the wines, have been
511 shown to present high dilution factors and mainly a *floral* odour quality, which could be
512 responsible for the greater aroma intensity in typical *Muscat* and *floral* attributes and
513 lower intensity in some off-flavours notes originated during the dealcoholisation
514 process. Although in this work, dealcoholisation was performed at lab scale, these
515 results show the interesting technological potential of glycosidic aroma precursor to
516 enhance the aroma of dealcoholised wines. This application could be even more
517 interesting considering the potential of industrial winemaking by-products (such as
518 grape pomace) as a source of these types of compounds. Ongoing research trying to
519 control the aromatization process and the stability of these compounds during wine
520 aging will be carried out in order to better know the potential of using these compounds
521 as flavoring agents.
522

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639

640 **Figure captions**

641

642 **Figure 1.** Representation of the wine samples in the plane defined by the two first
643 principal components (PC1, PC2) obtained from the PCA of the data corresponding to
644 the free volatile composition. Legends refer to the Initial wine with 0 days of storage
645 (INI-0) and Control wines with and without enzyme addition (CTR, CTR-E) and Wines
646 added with the aroma precursor extract with and without enzyme (PREC and PREC-E)
647 during storage (15 and 30 days).

648

649 **Figure 2**. Graph of the mean sensory modified frequency MF(%) ratings of the three
650 types of wines: Control wine (CTR) and wines added with aroma precursors with and
651 without enzyme (PREC and PREC-E), obtained by sensory descriptive analysis (17
652 judges, two repetitions). * denotes significance at $p < 0.05$ in the ANOVA analysis.

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Table 1. Aroma references used in the training of the sensory panel.

Attributes	**Reference Standard** [a]		**Definition**
	High intensity	Low intensity	
Muscat	133 µL of 1900 mg/L Linalool solution	10 µL of 1900 mg/L Linalool solution	Floral-Linalool-Muscat
Tropical-Fruit	2.5 mL of Multifruit juice [b]	0.5 mL of Multifruit juice [b]	Passion Fruit-Pineapple
Tree-Fruit	2.5 mL of peach juice + 2.5 mL of apple juice [b]	0.5 mL of peach juice + 0.5 mL of apple juice [b]	Peach-Apple
Citric	0.5 cm^2 of grapefruit peel + 6 drops of lemon juice	0.25 cm^2 of grapefruit peel + 2 drops of lemon juice	Lemon-Orange-Grapefruit
Froral	300 µL of 2 g/L β-Phenylethanol solution	100 µL of 2 g/L β-Phenylethanol solution	Floral-Rose
Anise	5 mL infusion of Chamomile/Anise	1 mL infusion of Chamomile/Anise	Anise-Licorice
Dry-Fruit	A prune crushed	1/4 prune crushed	Sweet-Caramel-Dry Fruit
Oxidize	5 mL of sherry wine	1.5 mL of sherry wine	Acetaldehyde-Oxidized
Yeast	0.1 g of baker yeast	100 µL of high intensity solution	Yeasty
Herbaceous	6 pieces of grass	3 pieces of grass	Vegetative-Fresh-Green

657
658 [a] All the references were prepared in 25mL of a model wine (3.5 g/L tartaric acid, 0.5%
659 ethanol v/v, pH=3.5)
660 [b] Commercial fruit juices were used.
661

662

663

Table 2. Free volatile compounds determined in the wines (µg/L except indicated).

	0 Days	15 days				30 Days				ANOVA [a]		
	Initial	CTR	CTR-E	PREC	PREC-E	CTR	CTR-E	PREC	PREC-E	Prec.	Time	Enz.
ESTERS												
Ethyl propanoate	0.404 ± 0.04	0.977 ± 0.315	1.05 ± 0.13	9.7 ± 2.96	9.26 ± 0.5	1.44 ± 0.33	1.16 ± 0.38	8.34 ± 2.79	10.4 ± 2.1	*		
Ethyl butyrate	1.91 ± 0.13	3.13 ± 0.28	3.49 ± 0.45	4.5 ± 0.37	4.1 ± 0.1	3.79 ± 0.78	3.44 ± 0.89	4.61 ± 1.12	5.33 ± 0.74	*		
Ethyl hexanoate	12.2 ± 1.2	16.3 ± 0.6	24.9 ± 1.2	18.7 ± 0.2	35 ± 4.8	25 ± 12.7	25.8 ± 7.4	22.5 ± 12.7	37.4 ± 2.6			*
Ethyl octanoate	27.1 ± 3.3	20.2 ± 1	324.8 ± 128.8	26.5 ± 2.2	538.7 ± 92	19 ± 7.4	158.3 ± 5	17.5 ± 7.9	194.6 ± 68.6		*	*
Ethyl decanoate	13.6 ± 1.3	5.43 ± 0.29	19.5 ± 7.7	6.82 ± 0.32	18.4 ± 3.1	5.32 ± 2.25	18.3 ± 2.8	3.73 ± 1.05	13.9 ± 2.9			*
Ethyl lactate (mg/L)	26.0 ± 0.4	27.6 ± 0.4	25.9 ± 1.9	28.6 ± 3.1	25.7 ± 0.4	21.8 ± 2.9	20.4 ± 0.1	23.4 ± 1.3	24.4 ± 1.2		*	
Ethyl acetate (mg/L)	nd	0.367 ± 0.219	0.307 ± 0.055	4.22 ± 1.12	4.43 ± 0.23	0.413 ± 0.194	0.274 ± 0.148	4.22 ± 0.72	4.74 ± 0.14	*		
Butyl acetate	nd	nd	nd	8.40 ± 2.31	6.93 ± 0.18	nd	nd	6.54 ± 1.78	6.66 ± 0.36	*		
Isoamyl acetate	8.59 ± 0.71	11.3 ± 1.8	14.6 ± 0.8	14.4 ± 1.2	10.8 ± 0.5	13.0 ± 3.8	12.4 ± 3.3	13.3 ± 3.1	13 ± 1.3			
Hexyl acetate	0.316 ± 0.034	0.484 ± 0.02	0.626 ± 0.106	0.587 ± 0.04	0.58 ± 0.072	0.562 ± 0.260	0.502 ± 0.27	0.463 ± 0.027	0.628 ± 0.128			
β-Phenylethyl acetate	4.86 ± 0.62	5.3 ± 0.26	5.61 ± 0.45	5.19 ± 0.31	7.44 ± 3.2	11.3 ± 6.7	9.23 ± 6.01	6.79 ± 3.77	12.6 ± 1.4		*	
C6 ALCOHOLS												
1-Hexanol	15.2 ± 2.1	23.4 ± 2.5	19.7 ± 3.3	22.2 ± 1.3	20.8 ± 3.8	23.2 ± 7.7	21.7 ± 6.3	22.6 ± 7	33.2 ± 2.7			
Trans-3-Hexen-1-ol	1.94 ± 0.13	2.45 ± 0.28	2.24 ± 0.31	2.3 ± 0.61	2.41 ± 0.15	1.98 ± 0.30	2 ± 0.04	2.4 ± 0.11	2.54 ± 0.11			
Cis-3-Hexen-1-ol	32.4 ± 1.2	39.1 ± 1	36.2 ± 4.9	52.1 ± 29.2	34.1 ± 1.8	32.6 ± 3.3	33.3 ± 0.1	44.1 ± 20.8	34.4 ± 2.6			
TERPENES AND C13 NOR-ISOPRENOIDS												
Limonene	0.264 ± 0.018	0.148 ± 0.027	0.146 ± 0.014	12.9 ± 2.4	12.2 ± 0.4	0.137 ± 0.012	0.171 ± 0.014	11.9 ± 0.7	12.4 ± 0.8	*		
Linalool	1.71 ± 0.17	0.813 ± 0.036	1.26 ± 0.14	331 ± 9	364 ± 6	0.981 ± 0.259	1.42 ± 0.03	393 ± 20	402 ± 33	*	*	
Terpinen-4-ol	0.180 ± 0.004	0.151 ± 0.014	0.135 ± 0.016	1.16 ± 0.01	1.22 ± 0.04	0.157 ± 0.004	0.156 ± 0.012	1.37 ± 0.11	1.38 ± 0.05	*	*	
α-Terpineol	2.80 ± 0.27	2.10 ± 0.04	1.94 ± 0.08	58.6 ± 5	60.4 ± 2.7	1.87 ± 0.15	1.88 ± 0.22	62.2 ± 6.1	64.1 ± 3.1	*		
β-Citronellol	0.239 ± 0.054	0.197 ± 0.03	0.197 ± 0.073	0.396 ± 0.121	1.17 ± 0.8	0.164 ± 0.041	0.235 ± 0.033	2.16 ± 2.69	7.54 ± 1.55	*	*	*
β-Damascenone	0.236 ± 0.01	0.315 ± 0.088	0.318 ± 0.019	4.15 ± 0.99	4.11 ± 0.15	0.336 ± 0.068	0.401 ± 0.051	4.02 ± 0.35	3.83 ± 0.45	*		

VOLATILE PHENOLS AND BENZENIC DERIVARIVES												
4-Ethylguaiacol	2.56 ± 0.31	2.71 ± 0.09	2.77 ± 0.19	2.72 ± 0.06	2.8 ± 0.05	2.27 ± 0.33	2.47 ± 0.19	2.66 ± 0.22	2.89 ± 0.14	*		
4-Ethylphenol	3.03 ± 0.28	3.7 ± 0.01	3.67 ± 0.11	3.81 ± 0.09	3.99 ± 0.1	3.2 ± 0.46	3.38 ± 0.18	3.85 ± 0.43	4.56 ± 0.22	*		
2-Methoxy-4-vinylphenol	76.8 ± 9.1	77 ± 1.3	87.6 ± 4.4	90.3 ± 5.6	128 ± 57	250 ± 166	86.6 ± 1.2	329 ± 409	819 ± 442		*	
4-vinylphenol	48.4 ± 7.2	58.6 ± 4	65.8 ± 6.6	62.3 ± 4.2	175 ± 191	431 ± 331	68.9 ± 5.7	312 ± 431	1878 ± 1155		*	
Eugenol	0.430 ± 0.051	0.433 ± 0.028	0.524 ± 0.037	0.486 ± 0.031	0.578 ± 0.031	0.43 ± 0.059	0.49 ± 0.03	0.511 ± 0.028	0.667 ± 0.044	*		*
β-Phenylethyl alcohol (mg/L)	12.8 ± 0.4	11.2 ± 3.1	10.1 ± 0.8	12.9 ± 2.9	12.9 ± 0.6	7.66 ± 1.43	8.78 ± 1.14	12.5 ± 1.2	12.5 ± 1	*		
Benzyl alcohol (mg/L)	1.5 ± 0.19	1.99 ± 0.07	1.9 ± 0.1	1.78 ± 0.11	1.82 ± 0.02	1.5 ± 0.3	1.68 ± 0.11	1.65 ± 0.16	1.82 ± 0.17		*	
ACIDS												
Hexanoic acid (mg/L)	3.11 ± 0.2	2.89 ± 0.44	2.6 ± 0.31	3.18 ± 0.29	3.21 ± 0.05	2.06 ± 0.41	2.09 ± 0.2	3.1 ± 0.23	3.08 ± 0.26	*	*	
Octanoic acid (mg/L)	2.67 ± 0.06	2.28 ± 0.62	1.73 ± 0.53	2.61 ± 0.65	2.29 ± 0.05	1.73 ± 0.28	1.70 ± 0.12	2.45 ± 0.39b	2.28 ± 0.26	*		
Decanoic acid	105 ± 20	88.5 ± 6.3	78.8 ± 31.7	95.9 ± 16	80.1 ± 5.7	125 ± 27	130 ± 39	100 ± 15	111 ± 9		*	
LACTONES AND FURANIC COMPOUNDS												
γ-Nonalactone	264 ± 33	314 ± 16	254 ± 83	279 ± 17	252 ± 6	283 ± 36	264 ± 2	269 ± 23	268 ± 23			
Fulfural	48.8 ± 5.2	82.7 ± 8.2	73.2 ± 11.6	81.2 ± 17.5	56.7 ± 43.8	39.2 ± 46.4	67.0 ± 81.5	78.5 ± 58.4	10.9 ± 4.5			

nd: not detected. [a] Three way-ANOVA results: Prec: addition of precursor aroma extract; Enz: Addition of enzymes. * indicates a significant effect.

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Table 3. Terpenes released from glycosidic aroma precursors (μg/L)

	0 Days	30 Days			
	Initial	**CTR**	**CTR-E**	**PREC**	**PREC-E**
α-Pinene	0.177 ± 0.004	0.177 ± 0.086	0.259 ± 0.069	0.261 ± 0.112	0.282 ± 0.031
Limonene	0.581 ± 0.051	0.661 ± 0.202 a	0.636 ± 0.23 a	1.66 ± 0.51 b	1.44 ± 0.62 ab
Linalool	nd	nd a	nd a	9.92 ± 5.91 b	12.1 ± 1.2 b
α-Terpineol	nd	nd a	nd a	2.73 ± 0.83 b	2.39 ± 0.18 b
β-Citronellol	5.07 ± 2.12	4.14 ± 0.39 a	4.2 ± 0.17 a	8.16 ± 1.97 b	8.74 ± 0.54 b
Nerol	2.93 ± 0.15	1.59 ± 0.26 a	2.35 ± 0.25 a	61.7 ± 29.3 b	72.7 ± 8.6 b
Geraniol	5.52 ± 0.13	194 ± 1 b	193 ± 2 b	78.5 ± 34.2 a	85.5 ± 10.6 a

nd: not detected. Different letters indicate significant differences among values within the same line ($p<0.05$).

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Table 4. Aroma-active compounds selected by AEDA-GCO in the Control and in the wines added with the aroma precursor extract.

Peak	Compound	Odour Description [a]	FD Factor[b]	
			CTR	PREC-E
1	Isoamyl alcohol	alcoholic	16	32
2	Ethyl hexanoate	fruity	4	8
3	Unknown	burnt, plastic	8	4
4	Cis-3-hexen-1-ol	green plant	4	4
5	Acetic acid	vinegar	32	32
6	Ethyl octanoate	fruity	nd	32
7	Benzaldehyde	almond, spicy	4	8
8	Ethyl-3-hydroxy-butanoate	fruity, grape	16	16
9	Linalool	floral, rose	nd	512
10	Unknown	chemical, plastic	2	nd
11	γ-Butyrolactone	sweet, caramel	4	4
12	Hotrienol	floral	nd	8
13	Butanoic acid	chemical, mouldy	32	16
14	Unknown	floral	nd	4
15	α-Terpineol	floral	4	16
16	Ethyl-4-hydroxy-butanoate	fruity	8	16
17	Geraniol	floral	nd	64
18	2-Phenylethanol	floral	64	64
19	Pantolactone	burnt	8	8
20	4-Vinylguaiacol	smoky	8	8

[a] Odour description as perceived by panelists during olfactometry. [b] FD factor is the highest dilution of the extract at which an odorant is determined by aroma extract dilution analysis. nd: not detected

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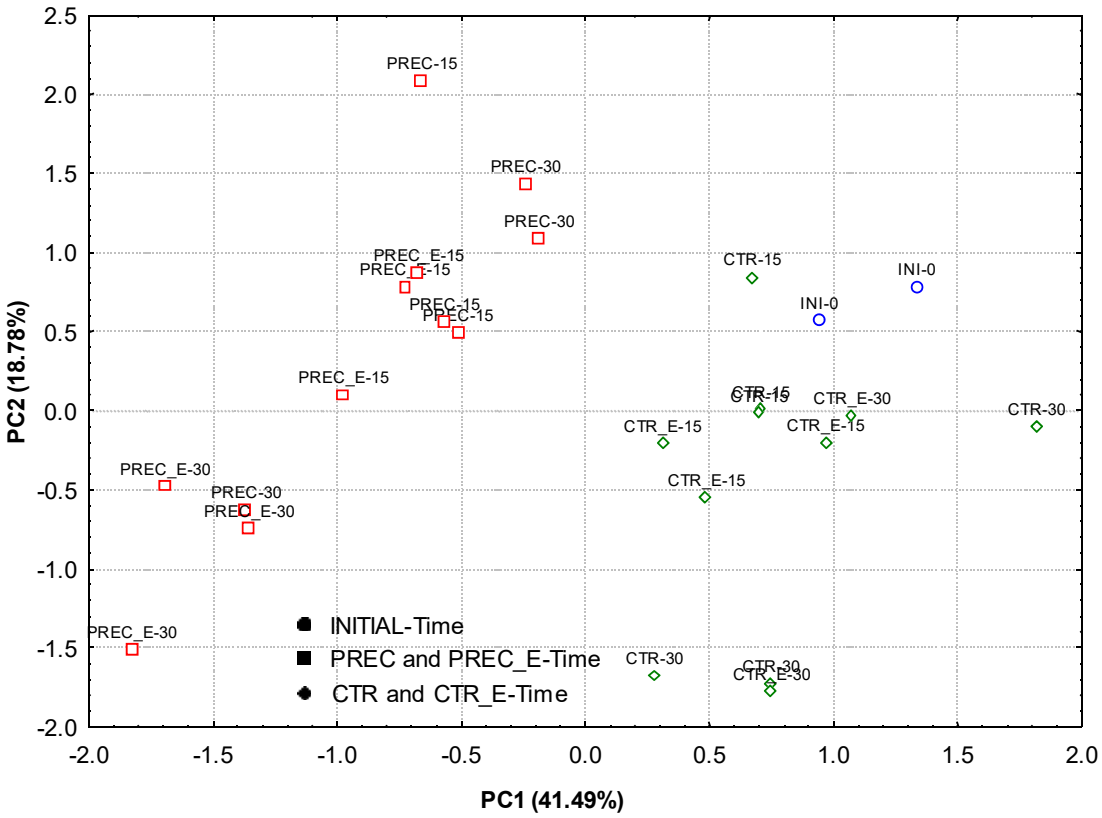
PC2 (18.78%)
PC1 (41.49%)
PREC-15
PREC-30
PREC-30
PREC_E-15
PREC_E-15
PREC-15
PREC-15
PREC_E-15
PREC_E-30
PREC-30
PREC_E-30
PREC_E-30
CTR-15
INI-0
INI-0
CTR-15
CTR_E-30
CTR-30
CTR_E-15
CTR_E-15
CTR_E-15
CTR-30
CTR-30
CTR-30
INITIAL-Time
PREC and PREC_E-Time
CTR and CTR_E-Time

Figure 1

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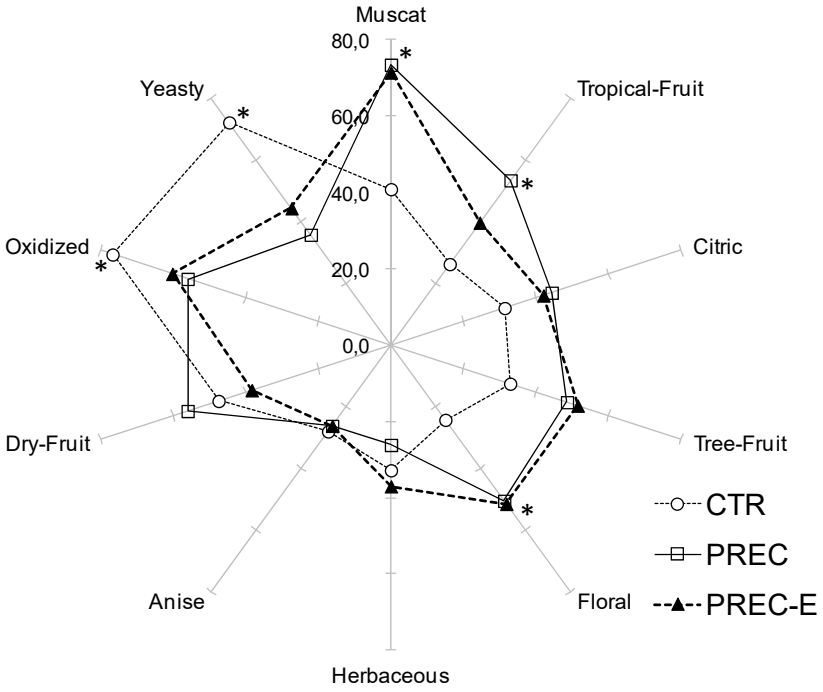

680
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682 Figure 2
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