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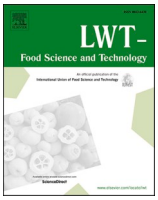

Aroma enhancement of dealcoholized wines using enzyme treatment and glycosidic aroma precursors

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ABSTRACT

This research has studied the treatment of dealcoholized wines with glycosidic enzymatic preparation and glycosidic aroma precursors extract isolated from aromatic grapes in order to enhance the aroma profile of non-alcoholic white wines. The effect of the addition of the precursors extract and the enzymatic treatment was studied after 15 and 30 d of storage, using dealcoholized wine as control. The analysis of free and bound volatile compounds was carried out by gas chromatography-mass spectrometry (GC-MS) after isolation using solid-phase extraction (SPE). The application of multivariate statistical analysis confirmed differences in the free and bound volatile profile of the studied samples when applicated the different treatments after 15 d and 30 d of storage. The combination of both treatments increased in a significant way the aromatic profile of Verdejo dealcoholized wine, showing high concentrations of terpenic and bencenic compounds due to the addition of precursors extract from an aromatic grape variety. This brings to light that this technique could be considered as a possible way of enhancing the aroma profile of dealcoholized wines in La Mancha region, as well as a way of using by-products from the wine industry, thus contributing to the concept of circular economy.

1. Introduction

The reduction in alcohol consumption is becoming increasingly common due to the detrimental effects alcohol has on health (Catarino & Mendes, 2011; Osorio, Sánchez-Palomo, & González-Viñas, 2023), leading to an increased demand for beverages with low alcohol content by consumers (Deroover, Siegrist, Brain, McIntyre, & Bucher, 2021; Margallo et al., 2015; Raineau et al., 2023; Rodríguez-Bencomo, Selli, Muñoz-González, Martín-Álvarez, & Pozo-Bayón, 2013). As a result, despite original wine remaining the most consumed, these demands have driven the wine industry to adopt various strategies to adjust the alcohol content of wines (Osorio et al., 2023; Pham, Stockdale, Jeffery, Tuke, & Wilkinson, 2019), a task that has not been easy due to the current climatic situation (Ubeda, Hornedo-Ortega, Cerezo, Garcia-Parrilla, & Troncoso, 2020).

Based on this, the CAP reform (Common Agricultural Policy, EU regulation 2117/2021), based on OIV resolutions OIV-ECO 523–2016, OIV-ECO 433–2012 and OIV-ECO 432–2012 includes dealcoholized wines, fully or partially under the new EU rules. The reduction of wine alcohol content through various techniques has been studied by different authors (Catarino & Mendes, 2011; Labanda, Vichi, Llorens, & López, 2009; Taran, Stoleicova, Soldatenco, & Morari, 2014), however, all dealcoholizing techniques result in a loss of aromatic compounds that negatively affect sensory properties, such as esters, acids, alcohols, C_6 compounds, terpene compounds … and consequently impacting consumer acceptance and overall liking (Catarino & Mendes, 2011; Longo, Blackman, Torley, Rogiers, & Schmidtke, 2017; Mangindaan, Khoiruddin, & Wenten, 2018; Osorio et al., 2023; Sam et al., 2021). Therefore, the enhancement of wine aroma following dealcoholization is necessary and can assist winemakers in reducing undesirable effects in dealcoholized wines, potentially leading to greater consumer acceptance (Ma et al., 2022). Various studies have proposed different techniques to improve the aromatic profile of wines that have undergone dealcoholization, such as the addition of flowers (Ma et al., 2022; Sam et al., 2021) or the introduction of grape glycosidic aroma precursors from grape varieties classified as aromatic (Rodríguez-Bencomo et al., 2013). However, their use is not approved as an oenological practice by the European Union (EU) and the International Organization of Vine and Wine (OIV), which is an important limitation, although they could be added in the case of special and aromatized wines.

The development of new products from dealcoholized wines reconstituted with fruit-based additives, herbs or new aromas represents a

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new potential market for the wine industry. Therefore, the development of techniques to improve the aroma of dealcoholized wines will benefit the wine industry as it will not only allow market diversification but will also be able to meet the growing consumer demand for new dealcoholized wines with unique and pleasant aromatic profiles.

According to this, the aim of this study was to research the use of grape glycosidic aroma precursors of Muscat a Petit Grains and the glycosidic enzyme treatment as techniques to enhance the aroma of dealcoholized white wines. It is important to note that in addition to improving the aroma of dealcoholized wines, the use of grape skins could be considered a process that would make the winemaking process more sustainable, since by-products of the process would be reused or utilized, thus reducing its environmental impact.

2. Material & methods

2.1. Reagents and standards

The dichloromethane, ethanol and methanol originated from Merck (Darmstadt, Germany), while the anhydrous sodium sulfate was sourced from Panreac (Barcelona, Spain). L-(+)-tartaric acid was obtained from Panreac (Barcelona, Spain). Ultrapure water, with a conductivity of 0.000006 S/m, was generated through the Milli-Q purification system by Millipore (U.S.). LiChrolut EN resins were provided by Merck (Darmstadt, Germany). An alkane solution (C_7-C_{24}, Supelco, Bellefonte, PA, USA) dissolved in dichloromethane was utilized to ascertain the linear retention index (RI) of each volatile compound. Pure standards were obtained from Sigma-Aldrich (Madrid, Spain), Merck (Darmstadt, Germany), Fluka (Madrid, Spain), Lancaster (Strasbourg, France), and Firmenich (Geneva, Switzerland). This information is shown in Table S1.

2.2. Samples

2.2.1. Aroma precursors extract

The precursors were extracted from the Muscat "a petit grains" grape variety cultivated in La Mancha region according to the method proposed by Rodríguez-Bencomo et al., 2013. Three kilograms of grapes, harvested at their optimal maturity point (21° Brix), were manually destemmed, homogenized with a mixer Ultra Turrax T25 (Ika, Labortechnik) and filtered to separate the musts and the skins.

The skins were macerated with an ethanolic solution (400 g skins/L solution) that simulates a wine matrix (10 % ethanol in water, 5 g/L tartaric acid, pH 3.5) during 48 h at room temperature in darkness, in order to extract the precursors. This solution was centrifuged at 4500 rpm for 15 min at 5 °C, and the supernatant was filtered through filter paper. The extraction of glycosidically bound volatile compounds of ethanolic solution was carried out using the method proposed by Sánchez-Palomo, Pérez-Coello, Díaz-Maroto, González-Viñas, & Cabezudo, 2006. Once all the ethyl acetate extract was obtained, it was evaporated under vacuum to dryness thus to eliminate the solvent and the dry extracts were reconstituted with 1 mL of Milli-Q water (precursor extract) being available for its addition to the dealcoholized wine samples and stored at −20 °C.

2.2.2. Preparation of the dealcoholized wines with the precursors extract

Dealcoholized Verdejo white wine supplied by a regional cellar of Castilla-La Mancha was divided into eight amber bottles (200 mL) in order to prepare the four sets under study, all samples was prepared in duplicate. The first two sets were prepared without precursor extract, being the first set called control set as it only contained 200 mL of dealcoholized wine (Set 1). The second set (Set 2) was produced with the addition of a commercial oenological enzyme preparation with glycosidase activity (Lallzyme Beta, Lallemand). The other two sets (Set 3 and Set 4) were prepared each adding 3 mL of precursor extract (Rodriguez Bencomo et al., 2013). In the set 3 no enzyme was added, and the set 4 consisted of the addition of enzyme and precursor extract to the wine. The oenological enzyme preparation was added to obtain a final concentration of 20 mg/L. All samples were stored in darkness at room temperature, taking samples for analysis at 15 d and 30 d.

2.3. Analysis of free and glycosidically bound minor volatile compounds of wines

2.3.1. Isolation of free glycosidically bound minor volatile compounds of wines

Analysis of free and bound volatile compounds were carried out at 0 d (control), 15 d and 30 d. Samples (100 ml) of dealcoholized white wine with and without aroma precursors addition and enzyme treatment, containing 40 μL of the internal standard (4-nonanol 1.04 g/L in absolute ethanol), were passed through SPE cartridges (500 mg) supplied by Merck at 1 mL/min (Sánchez-Palomo, Alonso-Villegas, & González-Viñas, 2015). Ten milliliters of dichloromethane were used to elute the minor free volatile compounds, and 25 mL of ethyl acetate were used to elute the glycosidically bound fraction of the aroma. The dichloromethane extract was concentrated under a nitrogen stream to a final volume of 200 μL. The ethyl acetate extract was evaporated to dryness under vacuum, and then re-dissolved with 1 mL of methanol.

2.3.2. Enzymatic hydrolysis of bound fraction

Five hundred μL of the methanol extract were concentrated to dryness under nitrogen stream, and the resulting dried glycosidic extract was reconstituted in 100 μL citrate–phosphate buffer (0.1 mol/L-0.2 mol/L, pH 5). Enzymatic preparation (Lallzyme Beta, Lallemand) was added to the reconstituted extracts in order to obtain a concentration of 20 mg/L of enzyme. This treatment was carried out at 40 °C for 16 h, following the method established by Sánchez-Palomo et al., 2006. Subsequently, the reaction mixture underwent five extractions with 2 mL of dichloromethane using discontinuous liquid-liquid extraction. Following the addition of 10 μL of 4-nonanol (1.04 g/L) as an internal standard, the resulting extract was concentrated to a final volume of 200 μL under a stream of nitrogen.

2.3.3. Identification and quantification of volatile compounds by gas chromatography coupled to mass spectrometry (GC-MS)

Gas chromatography coupled to mass spectrometry (GC-MS) was used to determine the minor volatile compounds in the studied wines. An Agilent 6890N GC system connected to a Mass Selective Detector (model 5973 inert) via a DB-WAX column (60 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Inc. Santa Clara, CA, USA) was used for sample analysis. The extract (1 μL) was injected at 250 °C in splitless mode, and the carrier gas was helium at a flow rate of 1 mL/min. The oven temperature program was as follows: an initial temperature of 70 °C was maintained for 5 min, increased at a rate of 1 °C/min to 95 °C, maintained for 10 min, followed by an increase to 200 °C at a rate of 2 °C/min, and maintained for 40 min. The MS operated in the electron impact mode with an electron energy of 70 eV. The global run time was recorded in full scan mode (40–450 m/z mass range), with an ion source temperature of 230 °C.

The identification of volatile compounds was carried out through the comparison of the mass spectra with those of authentic compounds and with those in the data system libraries (NBS75K). In order to confirm the identity of each volatile compound, the linear retention index (LRI) was calculated for each volatile compound with the retention times of a mixture of straight-chain alkanes (C_7-C_{24}), injected under the same chromatographic conditions.

The response factor for each volatile compound was determined by injecting commercially available standards into the analysis system at an intermediate concentration typically found in wines. An equal amount of internal standard was added to both the standards and the samples. In the case of compounds not commercially available the response factor of compounds with similar chemical structures were used. Then, the different response factors were used to calculate the concentration of

each compound. Determinations were performed in duplicate.

2.4. Odor impact compounds

The impact of every volatile aroma compound to the aroma of wine was quantified through OAVs that were determined as the relation of c/t, being c the total concentration of each volatile compound in the wine samples and t the odor threshold of the compound available in the literature (Étievant, 1991; Güth, 1997; Ferreira, López, & Cacho, 2000) (Table 3). The volatile compounds of wine were grouped based on their aroma descriptors into aromatic series in order to establish the sensory profile of a wine based on the chemical composition (Moyano, Zea, Moreno, & Medina, 2002; Franco, Peinado, Medina, & Moreno, 2004; Sánchez-Palomo, Gómez García-Carpintero, Alonso-Villegas, & González-Viñas, 2010).

2.5. Sensory descriptive analysis

Sensory descriptive analysis was performed by a panel previously trained in the odor of typical descriptive attributes of Verdejo wine (Delgado, Osorio, Alonso-Villegas, Sánchez-Palomo, & González-Viñas, 2021). Dealcoholized Verdejo wines and wines from different innovations for aroma enhanced were evaluated, in duplicate, by a trained panel of 12 wine tasters (5 female and 5 male) aged between 28 and 57 years. They were informed of the nature and risks of the present study and were asked to give their consent to participate in the sensory analyses.

The wine samples were stored at 5 °C and presented at 10 °C (30 ml) for odor and aroma detection. The assessment took place in a standard sensory analysis chamber equipped with separate booths, uniform lighting, absence of noise and distracting stimuli and air-conditioned at 20 °C (UNE-EN ISO 8589:2010). Normalized-tasting glass wine was used (UNE 87022:1992), labeled with three-digit random numbers and covered with a watch glass to minimize the escape of volatile components were use.

The five types of samples chosen for the sensory analysis wine control, storage control wine, enzyme treatment wine, wine added of precursors and wine added of precursors and with enzyme treatment after 30 d of storage time. All the wines were orthonasally evaluated in duplicate by the 11 assessors in two separate sessions using a 10 cm unstructured scale to rate the intensity of each attribute. The left-hand end of the scale indicated respectively “attribute not perceptible,” and “attribute strongly perceptible.

2.6. Statistical analysis

The significance of the treatment (enzyme, precursors and enzyme + precursors) and storage time was determined via 3-way ANOVA. Principal Component Analysis (PCA), from correlation matrix, to examine the relationship between the studied factors and the concentration of different groups of aroma compounds. One way-ANOVA test for mean intensity comparison of the aroma sensory data at 30 days of storage time and control wine. The SPSS 28.0 for Windows statistical package was used for data processing.

3. Results and discussion

3.1. Influence of the treatment on the free aroma compounds of dealcoholized wine

Free volatile compounds were analyzed in the initial dealcoholized wine and in the dealcoholized wines treated with glycosidic aroma precursors with and without enzyme treatment after 15 d and 30 d of storage in controlled conditions. Table 1 shows the results of the mean concentrations, relative standard deviation of volatile compounds and the result of three-way ANOVA, taking into account the addition of glycosidic aroma precursors, enzyme treatment and storage time.

The enzyme treatment with and without glycosidic aroma precursors addition, increased the total concentration of volatile compounds compared to dealcoholized control wine, coinciding with other research studied by Rodríguez-Bencomo et al., 2013, in which the aroma of dealcoholized wines was enhanced with the addition of Muscat precursors.

Terpenes were the group of volatile compounds that mainly increased, especially after 30 d of storage in wines from all treatment tested. The initial increase of terpenic compounds could be attributed to the chemical hydrolysis of glycosidically bound terpenes. Linalool, α-terpineol, *trans* linalool oxide pyranic and 2,6-dimethyl-3,7-octadiene-2,6-diol were the terpenes that showed a major increase, coinciding with the studied by Rodríguez-Bencomo et al., 2013. Moreover, the total concentrations of these group of compounds were in concordance with other authors for this grape aromatic variety, Muscat “a petit grains” in La Mancha region (Sánchez-Palomo et al., 2006).

Control wine (Set 1) presented the lowest concentrations of terpenic compounds as it can be seen in Table 2, thus when it is treated with the enzymatic preparation (Set 2), a slight increase in their concentration of most terpenes was observed after 15 d of storage, coinciding with Rodríguez-Bencomo et al., 2013. This increase is related to the release of aromatic compounds, which were in their bounded form, after the application of a glycosidic enzymatic treatment, whose effect is higher after 15 d of storage. This increase can also be observed in Set 4 over Set 3, as the enzymatic treatment is applied.

Bencenic compounds were another group of volatile compounds that were of major importance in qualitative and quantitative terms. Coinciding previous studies (Loscos, Hernández-Orte, Cacho, & Ferreira, 2009), the total concentration of bencenic compounds mainly raises with the application of enzymatic treatment (Set 2), though, the addition of aroma precursors extract, with or without enzymatic treatment (Sets 3 and 4), was not as significant in their increase, bringing to light the importance of the bound fraction for this group of volatile compounds. Benzaldehyde, benzyl alcohol and 2-phenylethanol presented the highest concentrations, although a general decrease has been observed in every case after 30 d of storage. The release of homovanillic acid after enzymatic treatment must be highlighted in Set 2, as well as in Set 4, coinciding with the studied by Loscos et al., 2009 in Muscat grapes cultivated in other regions of Spain.

Some other identified volatile compounds were C_{13} norisoprenoids and C_6 compounds, and they were also significantly influenced by the three studied factors but to a lesser extent, as there was not observed a difference neither with the addition of aroma precursors extract nor with the enzymatic treatment, though after 30 d of storage it was observed a decrease in their total concentration. It is remarkable the presence of β-damascenone due to its contribution to the aromatic profile because of its low olfactory detection threshold (Guth, 1997), in line with the studied by Selli, Canbas, Cabaroglu, Erten, & Günata, 2006, in wines of other *cv*. Muscat grape varieties. The range concentration of β-damascenone in the wines was similar to those studied by Loscos, Hernandez-Orte, Cacho, & Ferreira, 2007; Rodríguez-Bencomo et al., 2013. C_6 compounds were a minor quantitative and qualitative group (Selli et al., 2006), and their total concentration decreased when aroma precursors extract was added to samples, with or without enzyme treatment.

Acids and esters were groups of volatile compounds that seem not to be directly related to the glycosidic aroma precursors. In this way, hexanoic and octanoic acids experimented an increase when the aroma precursor extract was added (Sets 3 and 4) compared to control and enzyme-treated wine (Rodríguez-Bencomo et al., 2013). The concentration of acids suffered a decrease when wines were stored 30 d. Hexanoic and decanoic acids may contribute to freshness in wines (Lan et al., 2019; Ma et al., 2022), and, together with octanoic acid, their increased concentration might favor the synthesis of their corresponding esters due to the application of enzymatic treatment (Ma et al., 2022).

Table 1
Mean values (μg/L) and relative standard deviations (n = 2) of free volatile compounds determined in the studied samples after 15 and 30 days of storage.

COMPOUND	CONTROL		SET 1				SET 2				SET 3				SET 4				ANOVA[a]		
	INITIAL		15 DAYS		30 DAYS		15 DAYS		30 DAYS		15 DAYS		30 DAYS		15 DAYS		30 DAYS		Precursor	Enzyme	Time
Hexanoic acid	1898.98	(3.76)	1895.24	(2.46)	1906.96	(2.54)	1830.72	(3.51)	1871.31	(4.21)	2107.87	(1.40)	1759.43	(5.78)	2647.77	(0.25)	1967.49	(5.08)	b	b	b
Octanoic acid	2131.59	(3.35)	1883.82	(4.39)	1923.05	(3.97)	1881.15	(4.15)	1983.91	(4.02)	2329.59	(4.69)	1902.07	(2.02)	2809.51	(3.40)	2039.21	(1.67)	b	b	b
Decanoic acid	102.07	(1.00)	98.12	(1.92)	101.68	(6.86)	106.49	(4.08)	108.57	(4.87)	114.97	(0.40)	99.67	(7.51)	100.29	(2.53)	106.46	(4.58)	n.s.	n.s.	n.s.
ACIDS	**4132.64**		**3877.18**		**3931.69**		**3818.36**		**3963.78**		**4552.43**		**3761.17**		**5557.56**		**4113.17**				
2-methyl-2-buten-1-ol	3.38	(2.11)	8.42	(2.56)	5.95	(0.32)	8.05	(7.60)	6.23	(1.88)	5.25	(2.34)	3.76	(2.50)	4.49	(2.01)	4.00	(0.11)	b	n.s.	b
2,3-Butanediol	4.82	(1.48)	8.57	(3.39)	10.25	(5.35)	7.70	(2.95)	10.15	(7.02)	3.94	(4.54)	5.79	(2.05)	4.83	(2.78)	2.89	(2.97	b	b	b
3-methylthio-1-propanol	139.27	(5.12)	132.70	(1.15)	138.92	(3.76)	147.60	(1.26)	131.22	(5.63)	158.54	(0.81)	144.30	(0.91)	157.53	(0.83)	149.83	(0.33)	b	n.s.	b
ALCOHOLS	**147.47**		**149.69**		**155.12**		**163.35**		**147.61**		**167.74**		**153.85**		**166.85**		**156.72**				
Benzaldehyde	35.10	(2.03)	120.36	(4.33)	103.50	(5.29)	127.13	(0.72)	130.28	(5.67)	99.80	(1.51)	103.98	(7.03)	117.17	(1.87)	141.94	(3.06)	n.s.	b	n.s.
Phenethyl acetate	4.20	(1.70)	4.29	(1.00)	4.15	(0.85)	4.52	(0.63)	4.40	(0.29)	3.47	(3.30)	4.82	(1.65)	4.59	(4.12)	4.90	(3.48)	b	b	b
Guaiacol	73.79	(0.97)	36.17	(1.49)	10.02	(5.49)	43.06	(4.35)	44.96	(0.51)	30.80	(1.93)	38.17	(1.08)	33.64	(1.70)	31.05	(2.74)		b	b
Benzyl alcohol	139.59	(5.11)	160.71	(3.95)	160.30	(3.16)	177.22	(0.95)	177.45	(0.88)	103.59	(0.94)	98.17	(7.69)	98.63	(2.10)	93.49	(1.01)	b	b	
2-Phenylethanol	5518.20	(1.29)	5602.30	(2.13)	5270.54	(2.05)	6111.63	(2.97)	5374.81	(1.27)	5913.93	(1.11)	5513.93	(1.20)	5963.93	(0.08)	5558.93	(0.21)	b	b	b
Eugenol	2.60	(1.28)	2.65	(0.08)	2.73	(3.11)	4.30	(3.62)	3.07	(0.88)	4.24	(2.78)	2.71	(0.21)	4.69	(1.10)	3.54	(0.75)	b	b	b
Syringol	45.67	(1.56)	36.28	(0.62)	24.49	(1.82)	29.30	(0.56)	34.26	(2.70)	38.28	(5.85	36.79	(0.61)	40.61	(4.74)	34.82	(0.78)	b	b	b
Benzoic acid	84.10	(0.85)	91.29	(2.49)	112.02	(4.83)	89.80	(1.27)	108.41	(4.88)	97.44	(0.36)	84.08	(2.81)	83.15	(2.11)	83.76	(0.74)	b	b	b
Benzeneacetic acid	33.78	(2.11)	36.29	(0.30)	30.80	(1.30)	37.03	(1.15)	34.68	(0.67)	44.37	(0.02)	36.22	(2.44)	45.76	(2.13)	38.88	(0.15)	b	b	b
Vanillin	17.61	(4.05)	8.67	(2.53)	13.20	(3.99)	9.76	(1.93)	16.44	(4.67)	11.70	(0.54)	13.50	(4.49)	10.72	(0.97)	12.03	(3.88)	n.s.	n.s.	b
Acetovanillone	67.73	(1.05)	69.12	(1.63)	59.12	(1.90)	71.10	(0.00)	66.99	(0.44)	70.10	(1.87)	60.89	(2.14)	76.23	(1.07)	60.64	(0.45)	n.s.	b	b
Vanillic acid	10.96	(6.51)	10.77	(1.08)	10.12	(1.35)	26.03	(2.01)	18.42	(2.51)	16.94	(6.28)	13.70	(0.17)	29.47	(1.91)	21.82	(1.46)	b	b	b
Zingerone	49.27	(1.45)	40.46	(5.28)	27.57	(2.99)	35.79	(0.81)	40.74	(0.66)	40.60	(2.89)	43.80	(2.72)	34.84	(1.74)	42.39	(0.01)	b	n.s.	n.s.
Homovanillic acid	54.38	(1.31)	54.13	(0.98)	43.31	(0.71)	61.09	(2.97)	44.40	(2.08)	83.69	(0.81)	61.72	(0.21)	93.69	(0.72)	71.72	(0.18)	b	b	b
BENCENIC COMPOUNDS	**6136.97**		**6273.52**		**5871.87**		**6827.74**		**6099.30**		**6558.95**		**6112.47**		**6637.11**		**6199.90**				
α-ionone	7.79	(2.75)	7.77	(1.92)	6.54	(4.54)	7.44	(5.24)	5.76	(1.96)	7.42	(2.20)	6.58	(4.91)	6.27	6.98	6.19	0.58	n.s.	b	b
Naphthalene, 1,2-dihydro-1,1,6-trimethyl	4.42	(1.61)	4.41	(1.47)	4.18	(1.17)	3.40	(3.14)	1.70	(4.55)	2.28	(5.20)	1.41	(4.11)	2.93	(1.52)	2.53	(1.05)	b	b	b
4-oxoisophorone	5.36	(1.33)	5.40	(4.38)	6.93	(0.17)	5.03	(3.90)	7.56	(1.70)	4.50	(1.56)	6.98	(3.21)	5.35	(2.16)	5.04	(0.23)	b	b	b
β-Damascenone	4.67	(1.53)	5.86	(2.93)	6.06	(1.76)	4.43	(2.36)	6.91	(2.68)	7.29	(1.81)	6.14	(3.10)	6.29	(0.94)	5.82	(0.71)	b	b	b
3-oxo-α-ionol	55.98	(1.27)	56.36	(1.83)	42.30	(0.02)	59.67	(0.17)	53.06	(2.38)	53.13	(1.87)	53.65	(4.14)	52.94	(0.29)	45.35	(1.09)	b	b	b
C_{13} NORISOPRENOIDS	**78.22**		**79.80**		**66.01**		**79.97**		**74.99**		**74.61**		**74.77**		**73.77**		**64.93**				
2-Hexanol	21.32	(0.33)	23.00	(8.00)	12.09	(4.42)	25.23	(2.64)	12.68	(5.80)	26.85	(4.13)	15.15	(0.24)	22.87	(1.11)	12.85	(3.54)	b	n.s.	b
1-Hexanol	8.35	(0.34)	10.16	(8.84)	7.30	(0.47)	8.79	(0.99)	4.90	(2.15)	9.09	(0.62)	6.85	(3.19)	6.81	(3.23)	6.65	(0.54)	b	b	b
***trans* 3-Hexen-1-ol**	2.13	(3.35)	3.51	(1.08)	2.31	(1.97)	3.37	(3.12)	3.60	(2.61)	n.d.		n.d.		1.83	(1.66)	2.00	(1.74)	b	b	b
2-ethyl-1-hexanol	3.98	(1.79)	5.10	(0.27)	4.48	(0.66)	4.72	(3.54)	4.50	(0.51)	4.02	(1.99)	4.81	(1.64)	4.82	(1.20)	4.81	(1.18)	n.s.	b	n.s.
C_6 COMPOUNDS	**35.78**		**41.77**		**26.17**		**42.12**		**25.67**		**39.96**		**26.82**		**36.33**		**26.30**				
Isoamyl acetate	1.89	(3.77)	2.82	(2.21)	3.49	(2.84)	2.59	(2.69)	2.82	(1.34)	3.92	(2.97)	1.40	(4.21)	2.51	(4.63)	2.96	(0.68)	b	b	b
Ethyl lactate	97.12	(0.15)	107.79	(5.04)	92.58	(0.62)	109.07	(0.09)	106.80	(0.14)	91.12	(1.03)	89.36	(0.36)	100.89	(0.92)	100.26	(0.16)	b	b	b
Ethyl octanoate	6.32	(1.13)	6.45	(3.82)	3.76	(0.93)	34.35	(0.43)	34.86	(3.78)	5.08	(3.42)	3.49	(0.91)	55.26	(1.31)	33.42	(0.23)	b	b	b
3-hydroxy ethylbutyrate	132.57	(5.38)	163.73	(6.13)	154.82	(3.30)	178.51	(1.83)	155.87	(4.88)	226.44	(7.00)	129.37	(0.03)	223.09	(2.22)	128.06	(0.80)	b	n.s.	b
Diethyl succinate	19.97	(3.57)	19.82	(0.96)	16.80	(2.98)	21.09	(0.19)	20.86	(3.87)	17.50	(2.36)	17.34	(0.72)	16.90	(0.46)	17.28	(2.24)	b	b	b
Diethyl malate	6.19	(0.10)	7.76	(2.35)	7.69	(1.54)	4.60	(1.41)	6.13	(1.89)	7.46	(0.86)	5.17	(1.81)	4.87	(2.69)	5.89	(0.42)	b	b	n.s.
ESTERS	**264.07**		**308.38**		**279.15**		**350.21**		**327.34**		**351.53**		**246.12**		**403.52**		**287.87**				
γ-Butyrolactone	8.87	(0.80)	10.20	(0.98)	9.69	(5.70)	8.40	(3.99)	7.44	(1.71)	4.98	(0.86)	6.10	(0.68)	3.39	(0.46)	4.23	(1.55)	b	b	n.s.
γ-Heptalactone	10.89	(6.55)	8.46	(1.87)	8.00	(0.05)	10.04	(3.96)	9.83	(5.47)	6.06	(5.34)	4.35	(1.39)	9.04	(0.75)	8.33	(2.03)	b	b	b
LACTONES	**19.76**		**18.66**		**17.68**		**18.44**		**17.28**		**11.04**		**10.45**		**12.42**		**12.56**				
***cis* linalool oxide furanic**	14.26	(5.00)	16.14	(0.55)	14.81	(3.48)	17.86	(7.32)	14.74	(5.11)	14.66	(0.88)	15.95	(3.18)	15.37	(5.27)	17.77	(2.14)	n.s.	b	n.s.
***trans* linalool oxide furanic**	5.63	(1.27)	5.00	(2.28)	5.74	(1.44)	5.34	(2.74)	5.81	(1.95)	5.77	(1.14)	5.83	(2.06)	6.06	(0.87)	6.44	(0.51)	b	b	b
Linalool	0.15	(0.97)	3.33	(3.31)	6.53	(6.46)	3.01	(2.01)	3.80	(2.52)	125.19	(1.25)	171.59	(0.68)	121.61	(0.51)	172.64	(2.24)	b	n.s.	b
α-terpineol	6.85	(1.04)	5.51	(3.69)	5.46	(2.02)	9.14	(1.54)	10.69	(5.10)	66.62	(0.90)	89.22	(2.42)	71.80	(1.54)	85.93	(0.76)	b	b	b
***trans* linalool oxide pyranic**	2.11	(0.64)	1.55	(1.23)	2.19	(4.34)	50.88	(2.65)	78.31	(1.67)	18.04	(5.00)	19.21	(1.04)	54.84	(0.03)	88.99	(0.41)	b	b	b
***cis* linalool oxide pyranic**	2.65	(2.69)	0.55	(3.02)	2.84	(1.47)	2.97	(1.28)	2.74	(3.15)	4.36	(1.80)	3.15	(0.38)	4.18	(2.65)	3.21	(0.51)	b	b	n.s.
Nerol	18.01	(3.96)	6.07	(8.07)	3.97	(0.84)	4.46	(2.38)	5.24	(2.07)	5.71	(1.27)	6.05	(2.13)	6.35	(3.68)	6.58	(0.46)	b	n.s.	n.s.

(*continued on next page*)

Table 1 (*continued*)

COMPOUND	CONTROL		SET 1		SET 2		SET 3		SET 4		ANOVA[a]		
	INITIAL		15 DAYS	30 DAYS	15 DAYS	30 DAYS	15 DAYS	30 DAYS	15 DAYS	30 DAYS	Precursor	Enzyme	Time
Geraniol	16.59	(0.11)	16.52 (2.28)	15.60 (2.49)	26.25 (0.57)	22.95 (0.67)	28.31 (1.16)	25.40 (0.50)	35.95 (1.10)	32.54 (4.47)	b	b	b
2,6-dimethyl-3,7-octadiene-2,6-diol	10.74	(4.33)	11.74 (3.97)	10.39 (3.87)	27.10 (2.28)	13.36 (0.27)	18.50 (1.19)	16.75 (4.60)	43.04 (0.99)	37.70 (0.21)	b	b	b
Hydroxycitronellol	12.72	(5.61)	14.02 (3.76)	12.14 (5.86)	14.05 (4.03)	14.99 (5.04)	15.44 (6.84)	12.73 (0.49)	18.72 (1.17)	14.57 (2.94)	b	b	b
***trans* 8-hydroxylinalool**	8.73	(0.82)	7.20 (1.90)	6.27 (2.06)	8.16 (3.66)	6.79 (0.04)	7.43 (7.51)	6.62 (0.39)	8.28 (1.93)	14.73 (0.64)	b	b	b
TERPENIC COMPOUNDS	**98.43**		**87.64**	**85.93**	**169.21**	**179.44**	**310.03**	**372.51**	**386.19**	**481.08**			

n.d.: non-detected.
n.s., non-significant differences among samples.
SET 1: wine storage.
SET 2: wine with enzyme treatment.
SET 3: wine with precursors extract.
SET 4: wine with precursors extract and enzyme treatment.
[a] Three way-ANOVA results: Precursor: addition of precursor aroma extract; Enzyme: Addition of enzymes; Time: time of storage.
[b] Different superscripts in the same row indicate statistical differences at the 0.05 level according to the ANOVA.

This can be explained because the glycosidic enzymatic preparation may contain residual esterase activity and might release esters from fatty acids (Sumby, Grbin & Jiranek, 2010; Ma et al., 2022), therefore, the factor that mainly affected the concentration of the identified esters was the treatment with the enzymatic preparation. 3-hydroxy-ethylbutyrate was the only ester where the addition of aroma precursor extract increased its concentration. Esters like ethyl lactate, ethyl octanoate and isoamyl acetate showed increases and decreases in their content during storage (Rodríguez-Bencomo et al., 2013). These esters seem to play an important role in the glycosylated aroma of wines, as it was studied by other authors in research of dealcoholized wine aroma enhancement through different techniques (Ma et al., 2022).

3.2. *Influence of the treatment on the enzymatically released aroma compounds of dealcoholized wine*

The glycosidically bound fraction of the aroma can be considered as a potential source for volatile compounds in wines. The volatile compounds released from the bound fraction by enzyme hydrolysis in studied wines after 15 d and 30 d of storage are shown in Table 2.

The principal groups of volatile compounds identified and quantified in this fraction were bencenic and terpenic compounds, regardless of the storage time and treatment applied. As it can be observed, the concentration of these compounds decreases after 30 d of storage of wine in the bottle, being the wines treated only with glycosidic aroma precursors (Set 3) the ones which present a major concentration of terpenes and bencenic compounds. These results bring to light that, even though most of the glycosidically bound compounds had been released with the enzymatic treatment (Lallzyme Beta), there is a remaining part in the wine that could be liberated during the storage of these wines in the bottle by acid hydrolysis.

Nerol, geraniol and linalool, and benzaldehyde, benzyl alcohol and 2-phenylethanol, appeared to be the terpenic and bencenic compounds, respectively, with the highest concentrations, independently of the treatment applied. This coincides with the studied for the glycosidically bound fraction of wines elaborated with Muscat “a petit grains” grape variety cultivated in La Mancha region (Sánchez-Palomo et al., in 2006), and with the research of the volatile free and bound fractions of La Mancha Verdejo wines (Sánchez-Palomo et al., 2015).

As can be observed the concentration of C_6 compounds bound is higher than free fraction in all studied wines probably because during the wine dealcoholization process there is a great loss of volatile alcohols such as C_6 compounds (Osorio Alises et al., 2023).

3.3. *Influence of treatment in odor activity values*

Table 3 shows the odor descriptors, odor threshold obtained by the bibliographic references and OAVs values for the 19 aroma compounds with OAV >0.1 in wines samples after 30 d of storage. Among all the free volatile compounds identified in the samples after 30 d of storage, seven had an OAV >1 and twelve had an OAV ≥0.1 in at least one sample, which indicates that they would contribute to the aroma of the wines in an individual or synergically way, respectively. β-damascenone appeared to be the volatile compound with the highest OAV value regardless of the treatment applied, contributing to the aroma of wines with fruity and floral odors (Guth, 1997).

In control wine, guaiacol was the second highest volatile compound with an OAV >1, providing smoky and sweet notes to the wine. The concentration of this compound decreased with storage time, that is why its odor activity value was lower in Set 1. Moreover, this value increased in Sets 2 and 4 with respect to set 1, which can be explained because of the release of bencenic compounds from the bound fraction with the use of glycosidic enzyme. Hexanoic and octanoic acids and ethyl octanoate need to be mentioned in the control sample due to their high odor activity values. The calculated OAV of hexanoic and octanoic acids appeared to be similar in all the studied samples, revealing that neither

Table 2
Mean values (μg/L) and relative standard deviations (n = 2) of bound volatile compounds released by enzymatic hydrolysis determined in the studied samples after 15 and 30 days of storage.

COMPOUND	CONTROL		SET 1				SET 2				SET 3				SET 4						
	INITIAL		15 DAYS		30 DAYS		15 DAYS		30 DAYS		15 DAYS		30 DAYS		15 DAYS		30 DAYS		Precursor	Enzyme	Time
Hexanoic acid	235.22	(1.26)	271.27	(0.76)	300.93	(0.32)	256.53	(0.66)	266.47	(0.40)	362.17	(0.12)	366.54	(0.47)	248.95	(0.57)	250.80	(0.76)	a	a	a
Octanoic acid	392.47	(4.00)	317.66	(1.60)	325.01	(0.17)	381.23	(0.23)	377.22	(0.89)	447.60	(0.95)	499.03	(0.80)	405.07	(0.02)	339.77	(4.07)	a	a	n.s.
Decanoic acid	279.83	(1.43)	240.80	(0.42)	225.56	(0.08)	19.32	(0.36)	30.41	(0.61)	111.96	(0.53)	131.87	(1.77)	80.56	(1.22)	144.51	(1.94)	a	a	a
ACIDS	**907.53**		**829.72**		**851.49**		**657.08**		**674.10**		**921.74**		**997.44**		**734.58**		**735.09**				
1-Butanol	18.64	(0.03)	17.22	(2.00)	14.55	(2.49)	4.00	(2.46)	4.31	(0.92)	17.55	(2.06)	16.53	(0.12)	9.69	(0.46)	9.89	(0.16)	a	a	a
3-Octanol	10.65	(0.01)	6.30	(4.37)	6.76	(0.81)	6.38	(2.27)	5.27	(1.12)	7.20	(1.10)	7.13	(0.07)	7.26	(0.89)	7.21	(3.18)	a	a	a
2,3-Butanediol	4.71	(0.09	5.54	(3.23)	5.66	(4.24)	5.87	(2.76)	5.57	(1.86)	6.38	(1.38)	5.93	(1.10)	7.09	(1.89)	5.58	(5.65)	a	n.s.	a
1-Octanol	n.d.		n.d.		n.d.		6.52	(2.15)	15.60	(0.76)	15.52	(2.14)	22.71	(3.75)	n.d.		n.d.		a	a	a
ALCOHOLS	**34.00**		**29.06**		**26.97**		**22.77**		**15.14**		**31.13**		**29.58**		**24.04**		**22.67**				
Benzaldehyde	5291.67	(1.49)	4734.23	(2.89)	3837.50	(0.12)	2559.40	(1.95)	2710.97	(4.10)	6925.00	(0.56)	6176.68	(0.14)	4334.13	(0.78)	4079.10	(1.90)	a	a	a
Guaiacol	11.41	(1.31)	8.21	(2.05)	6.16	(5.43)	7.83	(1.23)	7.42	(1.10)	14.36	(2.44)	39.81	(2.88)	8.59	(0.91)	31.93	(0.37)	a	a	a
Benzyl alcohol	6144.27	(2.64)	5990.95	(3.39)	4156.69	(0.24)	2828.88	(2.08)	2129.54	(2.87)	7459.22	(0.01)	7287.63	(0.62)	6230.85	(0.69)	5117.76	(3.47)	a	a	a
2-phenylethanol	1594.16	(4.23)	1445.70	(4.10)	1308.50	(1.65)	851.37	(0.86)	954.64	(0.46)	2394.66	(1.47)	2133.41	(0.69)	2060.14	(3.85)	2326.40	(0.64)	a	a	n.s.
Benzoic acid	115.60	(0.17)	86.36	(1.26)	58.82	(0.67)	43.62	(2.53)	39.40	(1.33)	53.89	(0.56)	84.37	(7.18)	59.36	(2.99)	85.28	(1.56)	a	a	a
Vanillin	47.37	(2.91)	48.98	(2.97)	49.15	(2.96)	19.45	(0.34)	26.50	(2.71)	40.28	(0.19)	154.27	(1.26)	74.02	(1.66)	41.88	(0.82)	a	a	a
Acetovanillone	93.16	(0.34)	89.66	(1.02)	63.76	(0.75)	20.11	(1.57)	20.97	(6.34)	39.78	(0.17)	160.66	(1.15)	35.86	(2.61)	141.91	(2.02)	a	a	*
Zingerone	107.59	(0.01)	109.29	(0.11)	109.55	(3.87)	69.28	(1.82)	63.40	(4.22)	92.08	(4.73)	172.39	(0.95)	84.05	(1.02)	143.73	(1.96)	a	a	a
Homovanillic acid	280.01	(0.60)	237.41	(2.89)	303.00	(0.30)	57.33	(2.90)	53.83	(2.29)	112.06	(3.59)	525.68	(2.60)	122.13	(2.80)	376.16	(0.66)	a	a	a
Acetosyringone	202.64	(1.09)	236.79	(2.89)	222.75	(1.80)	84.20	(0.91)	80.92	(0.98)	136.45	(2.66)	138.84	(0.85)	148.19	(1.03)	121.13	(2.75)	a	a	a
BENCENIC COMPOUNDS	**13887.88**		**12987.58**		**10115.88**		**6541.48**		**6087.57**		**17267.78**		**16873.72**		**13157.32**		**12465.29**				
4-oxoisophorone	17.32	(0.88)	13.46	(3.11)	15.30	(6.28)	6.87	(1.87)	11.41	(1.79)	20.90	(1.16)	29.40	(5.05)	17.59	(0.81)	19.50	(8.04)	a	a	a
2,3-dehydro-4-oxo-β-ionol	31.12	(0.61)	28.17	(1.27)	28.41	(0.44)	13.90	(0.77)	27.19	(0.44)	23.49	(0.85)	44.75	(2.75)	1.73	(3.45)	0.69	(2.75)	a	a	a
6-Hydroxy-3-oxo-α-ionol (Vomifoliol)	n.d.		n.d.		n.d.		n.d.		n.d.		1558.70	(1.17)	1575.84	(7.08)	n.d.		n.d.		a	a	n.s.
3-oxo-α-ionol	317.31	(1.53)	270.58	(3.18)	230.53	(4.27)	115.20	(0.24)	104.88	(0.99)	233.75	(0.87)	220.96	(2.75)	339.29	(1.45)	361.99	(0.59)	a	a	a
6,7-dehydro-7,8-dihydro-3-oxo-α-ionol	196.79	(0.33)	174.21	(2.03)	165.08	(0.51)	79.09	(1.18)	80.29	(0.75)	94.06	(4.60)	102.50	(0.76)	224.15	(0.28)	182.81	(1.06)	a	a	a
3-hydroxy-7,8-dihydro-β-ionol	100.85	(0.86)	95.19	(1.58)	83.50	(0.98)	57.83	(2.85)	51.70	(3.88)	125.70	(3.03)	104.20	(1.05)	210.52	(0.06)	226.64	(0.41)	a	a	a
C_{13} NORISOPRENOIDS	**663.39**		**581.60**		**522.82**		**272.90**		**275.47**		**2056.60**		**2077.65**		**793.28**		**791.63**				
1-Hexanol	192.67	(2.23)	140.99	(4.47)	143.12	(2.08)	81.18	(1.07)	76.24	(1.82)	240.55	(0.26)	254.08	(0.63)	192.67	(0.66)	182.36	(2.33)	a	a	
***Trans* 3-Hexen-1-ol**	14.57	(0.61)	10.66	(3.15)	7.65	(0.91)	6.63	(2.06)	8.97	(2.84)	12.19	(2.72)	10.88	(2.51)	7.21	(1.11)	7.25	(2.39)	a	a	a
***Cis* 3-Hexen-1-ol**	59.99	(0.27)	56.62	(5.17)	54.34	(2.52)	31.37	(2.21)	35.10	(1.97)	85.59	(1.57)	48.85	(0.78)	64.07	(2.02)	63.26	(2.47)	a	a	a
***Cis* 2-Hexen-1-ol**	7.67	(0.91)	6.40	(3.51)	6.00	(2.73)	5.39	(3.31)	5.77	(1.29)	32.99	(3.71)	35.04	(0.41)	6.41	(1.76)	5.42	(1.42)	a	a	n.s.
***Trans* 2-Hexen-1-ol**	50.02	(2.64)	33.41	(1.89)	24.65	(4.19)	18.42	(1.40)	23.07	(1.04)	48.47	(1.02)	46.68	(1.35)	36.24	(2.77)	33.07	(4.41)	a	a	a
C_6 ALCOHOLS	**324.92**		**248.08**		**235.75**		**142.98**		**149.14**		**419.80**		**395.53**		**306.60**		**291.36**				
Isoamyl acetate	3.06	(1.78)	3.07	(2.39)	3.39	(5.85)	n.d.		n.d.		6.68	(6.14)	7.03	(0.04)	n.d.		n.d.		a	a	
Ethyl lactate	9.86	(8.47)	9.77	(2.38)	16.01	(2.10)	n.d.		n.d.		15.36	(0.05)	16.39	(1.34)	n.d.		n.d.		a	a	a
Methyl hexanoate	14.47	(2.20)	11.10	(1.72)	13.31	(0.59)	5.29	(2.06)	5.87	(0.51)	21.68	(4.20)	25.29	(0.10)	n.d.		n.d.		a	a	a
Methyl salicylate	17.34	(1.01)	19.56	(0.50)	18.91	(1.52)	10.64	(3.39)	9.12	(9.10)	24.32	(1.45)	20.26	(0.47)	31.16	(0.05)	29.86	(0.24)	a	a	a
ESTERS	**41.67**		**40.43**		**48.24**		**15.93**		**14.99**		**61.36**		**61.94**		**31.16**		**29.86**				
***Cis* linalool oxide furanic**	128.32	(0.38)	131.73	(0.27)	124.21	(0.26)	44.61	(1.07)	33.82	(2.29)	249.21	(0.13)	191.73	(0.90)	101.85	(4.12)	67.46	(2.18)	a	a	a
***Trans* linalool oxide furanic**	224.23	(1.21)	134.35	(3.12)	129.15	(1.22)	58.62	(1.30)	75.61	(6.13)	344.88	(0.39)	217.11	(0.53)	129.97	(2.87)	94.68	(0.48)	a	a	a
Linalool	10.24	(0.33)	15.26	(1.03)	13.41	(2.84)	9.47	(2.58)	9.25	(1.60)	383.12	(0.52)	421.64	(0.83)	308.46	(0.26)	236.09	(2.03)	a	a	a
α-terpineol	64.89	(1.50)	74.54	(0.27)	68.59	(0.82)	14.68	(0.94)	0.70	(1.74)	315.94	(3.18)	336.11	(0.47)	75.74	(0.87)	73.58	(1.69)	a	a	n.s.
***Trans* linalool oxide pyranic**	72.54	(1.10)	39.77	(2.43)	39.13	(0.15)	31.34	(0.65)	25.78	(0.57)	183.96	(0.78)	179.75	(1.11)	82.39	(0.93)	80.42	(1.23)	a	a	a
***Cis* linalool oxide pyranic**	93.98	(0.32)	85.79	(2.77)	85.40	(0.40)	29.87	(0.58)	33.57	(2.28)	113.51	(2.34)	95.54	(4.10)	48.89	(2.85)	48.67	(2.90)	a	a	a
β-citronellol	32.36	(1.49)	37.82	(0.61)	35.73	(2.01)	27.75	(1.94)	29.70	(2.12)	62.68	(0.99)	45.32	(0.93)	33.91	(0.09)	34.18	(2.56)	a	a	a
Nerol	22.24	(0.59)	24.12	(0.67)	21.44	(1.14)	19.34	(4.31)	1.53	(1.00)	1325.76	(1.75)	986.83	(3.90)	734.29	(0.85)	677.36	(2.16)	a	a	a
Geraniol	14.60	(0.62)	14.16	(1.29)	13.58	(2.79)	10.94	(1.96)	5.04	(1.93)	704.49	(4.82)	523.09	(3.44)	488.06	(0.44)	461.69	(2.86)	a	a	a

(*continued on next page*)

Table 2 (*continued*)

COMPOUND	CONTROL	SET 1		SET 2		SET 3		SET 4				
	INITIAL	15 DAYS	30 DAYS	15 DAYS	30 DAYS	15 DAYS	30 DAYS	15 DAYS	30 DAYS	Precursor	Enzyme	Time
Hydroxycitronellol	15.89 (0.76)	12.87 (0.89)	10.42 (2.31)	12.78 (1.38)	16.31 (1.05)	106.18 (6.06)	79.21 (1.51)	32.21 (0.18)	40.15 (2.73)	a	a	a
***Trans* 8-hydroxylinalool**	9.03 (0.43)	88.93 (7.41)	66.95 (0.80)	59.25 (5.35)	30.98 (2.19)	365.90 (0.46)	358.92 (0.58)	200.60 (1.28)	217.38 (1.89)	a	a	a
TERPENIC COMPOUNDS	**688.32**	**659.34**	**608.00**	**318.65**	**262.29**	**4155.63**	**3435.24**	**2236.39**	**2031.66**			

n.d.: non-detected.
n.s., non-significant differences among samples.
SET 1: wine storage.
SET 2: wine with enzyme treatment.
SET 3: wine with precursors extract.
SET 4: wine with precursors extract and enzyme treatment.
aThree way-ANOVA results: Precursor: addition of precursor aroma extract; Enzyme: Addition of enzymes; Time: time of storage.
[a] Different superscripts in the same row indicate statistical differences at the 0.05 level according to the ANOVA.

the addition of aroma precursors extracts, nor the enzymatic treatment affected these compounds. Fatty acids are generally associated with cheesy, rancid and fatty aromas (Sánchez-Palomo, González-Viñas, Díaz-Maroto, Soriano-Pérez, & Pérez-Coello, 2007), and if they are found in high concentrations they could contribute to negative odors to wine, though in the studied samples this is not expected to happen.

On the other side, ethyl octanoate must be highlighted in sets 2 and 4, bringing to light that its contribution to wine aroma would be higher when an enzymatic treatment is applied, contributing to fruity notes in the wines (Flanzy, 2003).

The addition of aroma precursors extract increased the concentration of terpenic compounds, and regarding odor active compounds, especially linalool needs to be mentioned, giving floral notes to the wines (Guth, 1997). This highlights the importance of improving the aroma of dealcoholized wines with the extract of aromatic precursors of Muscat grape variety in terms of terpenic compounds.

Other compounds need to be highlighted due to their contribution to the aroma of wines in a synergetic way, especially geraniol, eugenol, syringol, 2-phenylethanol, benzaldehyde and vanillin. This is in concordance with other studies for wines elaborated with La Mancha Verdejo grape varieties (Sánchez-Palomo, Alonso-Villegas, Delgado, & González-Viñas, 2017).

3.4. Principal component analysis

With the aim of researching the causes of variability in the concentration of aroma compounds due to the different studied factors, principal component analysis has been carried out considering as variables the total concentration of each group of free and bound volatile compounds in the analyzed samples (Tables 1 and 2). The first three principal components accounted for 88.48% of the total explained variance between the samples. Table 4 shows the rotated principal component loadings for group of free and bound volatile compounds. The results of the principal component analysis (PCA) of the total concentration of free and bound volatile compounds from control wines and treated with enzyme and precursors extract are represented in Fig. 1, which illustrates the simultaneous projection of the eighteen wines and the eleven group of free and bound volatile compounds with absolute correlation coefficient values greater than 0.7.

As can be seen, control wine, control wines after 15 d of storage and samples treated with aroma precursors extract, regardless of the storage time, showed positive values for Principal Component 1, while the rest of studied wines exhibited negative values for this component. Therefore, principal component 1 is related to the higher concentrations of the main groups of aroma precursors (Table 2). On the other hand, Principal Component 2 could be related with the evolution of the main groups of free and bound volatile compounds in wine during storage in bottle. Although there is no clear separation between samples, the group of volatile compounds correlated positively with Principal Component 2 showed a general higher concentration of free volatile compounds after 30 d of storage. On the other hand, the samples situated in the negative part of this component, showed a slight decrease in their concentration during storage.

3.5. Sensory descriptive analysis

To determine whether the addition of aroma precursors and enzyme treatment might change the sensory profile of Verdejo dealcoholized wines, a descriptive sensory analysis was performed with the control wine, control wine at the moment of analyzed, control wines after 30 d of storage (Set 1) and with the wines treated with the aroma precursor extract, with and without enzyme addition (Set 2, Set 3 and Set 4) at the end of the storage time (30 d). The results of the sensory analysis are shown in Fig. 2. As can be observed, the "spider web" represents the ten sensory attributes characteristic of Verdejo wines selected for this study. The aroma of control wines was characterized by moderately intense

Table 3
Odor descriptor, aromatic series, odor threshold (μg/L) and odor activity values (OAV) of free volatile compounds of the control wine and the studied samples after 30 days of storage.

Free compounds	Odor descriptores	Aromatic series [d]	Odor threshold (ug/L)	CONTROL	SET 1	SET 2	SET 3	SET 4
β-damascenone	Sweet, fruity	1,4	0.05[a]	93.45	121.23	138.23	122.82	116.43
Guaiacol	Medicine, sweet, smoke	4,6	10[c]	7.38	1.00	4.50	3.82	3.11
Hexanoic acid	Sweat	6	420[b]	4.52	4.54	4.46	4.19	4.68
Octanoic acid	Sweat, cheese	6	500[c]	4.26	3.85	3.97	3.80	4.08
Ethyl octanoate	Caramel, fruity	1,4	5[c]	1.26	0.75	6.97	0.70	6.68
Syringol	Phenolic, medicine	6	57[b]	0.80	0.43	0.60	0.65	0.61
2-phenylethanol	Floral, rose	2	10000[a]	0.55	0.53	0.54	0.55	0.56
Geraniol	Rose, geranium	2	30[a]	0.55	0.52	0.77	0.85	1.08
Eugenol	Spices, clove, honey	4,5	6[c]	0.43	0.46	0.51	0.45	0.59
Vanillin	Vanillin	5,7	60[b]	0.29	0.22	0.27	0.22	0.20
3-methylthio-1-propanol	Cooked vegetable	6	1000[a]	0.14	0.14	0.13	0.14	0.15
Benzaldehyde	Sweet, fruity	1.4	350[c]	0.10	0.30	0.37	0.30	0.41
Decanoic acid	Rancid fat	6	1000[b]	0.10	0.10	0.11	0.10	0.11
Benzoic acid	Chemical	6	1000[b]	0.08	0.11	0.11	0.08	0.08
Acetovanillone	Sweet spices	5	1000[b]	0.07	0.06	0.07	0.06	0.06
Isoamyl acetate	Banana	1	30[c]	0.06	0.12	0.09	0.05	0.10
α-terpineol	Sweet, floral	2,4	250[c]	0.03	0.02	0.04	0.36	0.34
2-phenylethyl acetate	Floral	2	250[a]	0.02	0.02	0.02	0.02	0.02
Linalool	Floral	2	15[a]	0.01	0.44	0.25	11.44	11.51

[a] Guth, 1997.
[b] Etiévant, 1991.
[c] Ferreira et al., 2000.
[d] 1: fruity; 2: floral; 4: sweet; 5: spicy; 6: fatty; 7: oak.

Table 4
Correlation coefficients of total concentrations of free and bound groups of volatile components in control wine and treated with enzymatic preparation and glycosidic aroma precursors extract against principal components 1. 2 and 3.

Group of volatile compounds	Component		
	1	2	3
Acids free	0.099	0.757	−0.100
Alcohols free	0.176	0.885	−0.056
Bencenic compounds free	−0.104	0.863	0.400
C13 norisoprenoids free	−0.010	0.111	0.846
C6 alcohols free	0.086	0.475	0.848
Esters free	−0.333	0.876	0.162
Lactones free	−0.570	−0.504	0.609
Terpenic compounds free	0.281	0.543	−0.690
Acids bound	0.928	−0.340	0.062
Alcohols bound	0.816	−0.175	0.436
Bencenic compounds bound	0.963	0.117	−0.045
C13 norisoprenoids bound	0.905	0.196	−0.208
C6 alcohols bound	0.957	0.156	−0.151
Esters bound	0.949	−0.158	−0.037
Terpenic compounds bound	0.805	0.448	−0.337
% Variance explained by PC	42.08	27.36	19.05
Cumulative% of variance	42.08	69.44	88.49

Loading values > 0.700 are marked throughout in boldface type.

fresh, green apple, fruity, floral, tropical fruit and citric with notes of one attribute described by tasters as "Muscat aroma" and sweet. After 30 d of storage control wines (Set 1) exhibit a similar profile without significant differences in the intensity of sensory aroma attributes. Nevertheless, the results clearly showed a different sensory profile between control wines, control wine after 30 d of storage and treated wines (Sets 2, 3 and 4). Wines with precursors, with and without enzyme treatment, showed significant differences in the fruity, floral, citric, banana, topical fruit, sweet and muscat aroma attributes, more intense in these wines compared to the other studied wines. These results were in agreement with the analytical results that showed higher concentration of terpenic compounds related to the muscat, floral and citric notes, and higher concentration of isoamyl acetate, ethyl octanoate and β-damascenone related with fruity, banana and tropical fruit attributes in in the wines added of precursors, with and without enzyme treatment. The higher of sweet aroma can be due to the higher concentration of some benzenic compounds and to the liberation of sugar from glycosidic precursors by enzymatic hydrolysis (Cabaroglu, Selli, Canbas, Lepoutre, & Günata, 2003; Sánchez-Palomo, Díaz-Maroto, González-Viñas, & Pérez-Coello, 2005).

4. Conclusions

The result of this research has shown that the aroma enhancement of dealcoholized wines can be carried out by enzymatic treatment, the addition of aroma precursors extracts or the combination of both techniques. Regarding free volatile compounds, differences were observed in the total concentration of the groups of free volatile compounds depending on the treatment applied and the storage time, although in all cases the highest concentrations were obtained in the group of acids and bencenic compounds.

The aglycones released from the glycosidic fraction by enzymatic activity increase the concentration of terpenic and benzenic compounds in the wines in which this treatment was applied and which could be eliminated by the dealcoholizing process. Storage of the samples for 15 d increased the concentrations of these compounds, although after 30 d a decrease in these compounds was observed due to possible interactions of the compounds with each other and with other components of the wine.

The highest concentration of free volatile compounds in the treated wines was found in the samples in which the treatments of addition of aromatic precursor extract and enzymatic treatment were combined, showing that these techniques constitute a good possibility to enhance the aromatic profile of dealcoholized wines.

The use of grape skins, which are a by-product of the grape, is not only a strategy to improve the aromatic profile of dealcoholized wines, but it is also beneficial to revalorize the use of winemaking by-products in a circular economy context.

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Institutional review board statement

Not applicable.

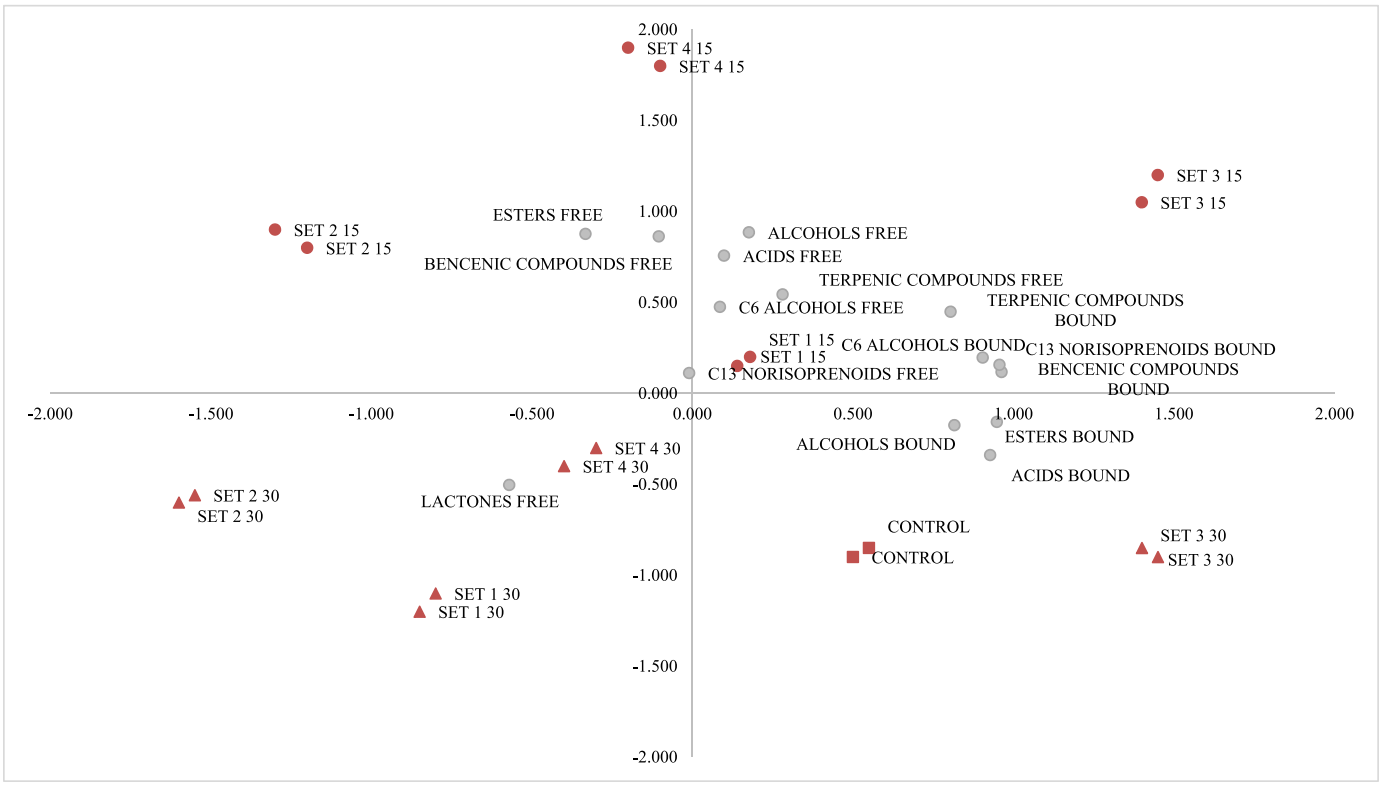

Fig. 1. Principal component bi-plot illustrating the simultaneous projection of the 18 wines and the total concentration of free and bound groups of volatile compounds. Control samples are represented by a red square, samples after 15 days of storage by a red dot and samples after 30 days of storage by a red triangle, and groups of compounds by a gray dot. The axis x represents Principal Component 1 (42.75% variance) and the axis y represents Principal Component 2 (27.35% variance).

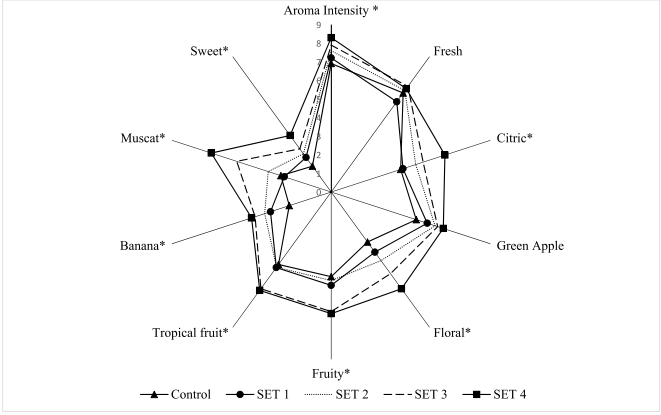

Fig. 2. Graph of the mean sensory attribute intensity of the five types of wines: Control wine (CTR) and control wines after 30 d of storage (SET 1) and wines with enzyme treatment with and without precursors addition (SET 2, SET 3, SET 4), obtained by sensory descriptive analysis (11 judges, two repetitions). * denotes significance at $p < 0.05$ in the ANOVA.

Informed consent statement

Not applicable.

CRediT authorship contribution statement

M. Osorio Alises: Writing – original draft, Methodology, Formal analysis, Data curation. **E. Sánchez-Palomo:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Conceptualization. **M.A. González Viñas:** Writing – review & editing, Visualization, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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