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# Impact of glucose oxidase treatment in high sugar and pH musts on volatile composition of white wines

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ABSTRACT

Climate change is modifying the composition of the grapes, increasing sugar and pH levels, which produces unbalanced wines with high alcohol degree, low acidity and poor aromatic notes. In this study, glucose oxidase (GOX) and catalase (CAT) were applied in white must with high sugar content and pH (>3.8) to simultaneously decrease glucose concentration and pH. The effect of enzyme treatment on volatile composition of wine was investigated. A concentration of 0.17 mkat/L for both GOX and CAT was sufficient to produce a remarkable reduction of glucose concentration in the must (61.5 g/L), achieving similar results within the pH range of 3–4. The musts subjected to enzymatic treatment yielded more balanced wines, lowering their alcohol content by 10–27 mL/L and pH by 0.3–0.5, while leaving their chromatic characteristics unchanged compared to the control wines. As positive effects, enzyme treatment reduced the concentrations of C6-alcohols with green-herbaceous notes and high-chain ethyl esters with soapy notes in wines, and did not modify the concentrations of short-chain ethyl esters, acids and higher alcohols. However, the concentrations of heptyl acetate and 2-phenylethanol with floral notes, and some ketones with floral and fruit notes, were lower in wines from treated musts.

## 1. Introduction

The effects of climate change are of increasing concern in viticulture, as the change in climatic variables (increase in temperature, decrease in rainfall, etc.) directly and indirectly affect grape quality (Droulia & Charalampopoulos, 2022), generating wines with altered physico-chemical and sensory characteristics. One of the problems that most concerns the wine sector due to climate change, most critical in warm climate regions, is the increase of sugar concentration and pH in the musts, and the unbalanced phenolic and aromatic quality of grapes. As a result, wines produced from these grapes will probably be out of balance with high ethanol concentration, low acidity and reduced aromatic quality (Jones et al., 2022).

To mitigate this problem, some strategies to reduce sugar levels of must have been developed (Sam et al., 2021; Schmidtke, Blackman, & Agboola, 2012). One of them is based on the use of glucose oxidase (GOX), which allows a simultaneous reduction of glucose concentration and an acidification of the must. GOX catalyzes the oxidation of β-D-glucose in the presence of oxygen to gluconic acid and hydrogen peroxide ($H_2O_2$) (Khatami et al., 2022). Due to $H_20_2$ can cause oxidation of the pigments of must, GOX deactivation and even problems during alcoholic fermentation, GOX is applied together with catalase (CAT) to break down $H_2O_2$ (Bankar, Bule, Singhal, & Ananthanarayan, 2009; Röcker, Schmitt, Pasch, Ebert, & Grossmann, 2016; Wong, Wong, & Chen, 2008). The oxygen released in the reaction of CAT can be used by GOX to carry out glucose oxidation (Bauer, Zámocká, Majtán, & Bauerová-Hlinková, 2022).

The first studies about the use of the GOX to reduce the alcohol degree of wines date back to 1998, where Pickering, Heatherbell, and Barnes (1998) carried out several trials with white grapes to produce low alcohol wines. They used calcium carbonate to increase the pH of the must to 6.0 to improve the GOX activity, obtaining a large decrease in ethanol of about 42%, although the organoleptic characteristics of the wines were altered.

It was not until 2009 that Biyela, du Toit, Divol, Malherbe, and van Rensburg (2009) re-evaluated the use of GOX to obtain wines with a moderate reduction in alcohol degree to mitigate the effects of climate change. These authors achieved a limited reduction in alcohol content by 5 mL/L and a consequent low decrease in wine pH (<0.2), using red grapes at pH 3.5. In white grapes, Röcker et al. (2016) reported a higher reduction in alcohol content by about 20 mL/L in musts at pH 3.4–3.5, resulting in wines with a very low pH (2.7–2.8). A too low pH level in

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must can generate, among other potential shortcomings, a delay or stuck fermentations, decrease in the efficacy of the potassium metabisulfite used as a wine preserving agent, and undesirable wine sensory characteristics (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006). Therefore, it is hypothesized that the use of GOX to mitigate the effects of climate change and to achieve a suitable alcohol content decrease by 10–20 mL/L should focus on high pH musts (>3.8).

These high values of pH are becoming more frequent in grapes from warm climate regions (Botezatu, Elizondo, Bajec, & Miller, 2021; Essary & Bajec, 2022), where the acidification caused by GOX could be balanced due to buffering capacity of must (Torija et al., 2003). Recently, Botezatu et al. (2021) proposed the use of GOX as acidifying agent to increase the acidity of white musts with a high pH (3.9–4.0), obtaining wines with values of pH of 3.2–3.5 and with a positive impact on their sensory characteristics.

Regarding the volatile composition of wines from musts treated with GOX to mitigate the effects of climate change, a thorough literature review has shown that this issue has been addressed with only five volatile organic compounds (VOCs) (Biyela et al., 2009) or analyzed in over-acidified wines that needed a deacidification step (Röcker et al., 2016).

In this sense, this work shows the results obtained with GOX and CAT in white grapes rich in sugars and with a high pH (3.8–4.0). The main goal of this work was to study the volatile composition of wines from GOX-treated musts. Other interesting parameters, such as alcohol content, pH, total and volatile acidities, as well as chromatic characteristics and phenolic composition of the wines were also evaluated. As a preliminary to this aim, the main factors affecting GOX activity under oenological conditions (enzyme dose, pH and aeration system) were studied to establish the best conditions to reduce the concentration of glucose in must.

## 2. Materials and methods

### 2.1. Grapes and musts

The different experiments of this paper were carried out with Verdejo grapes from two consecutive vintages (2020 and 2021). Grapes were harvested each season in a commercial vineyard located in La Seca (Valladolid, Spain), crushed, destemmed, pressed and sulfited at 30 mg/L of free $SO_2$ to obtain the respective must. The must from 2020 vintage had the following composition: 21.9 ± 0.1 °Brix; pH 3.59 ± 0.01; 4.3 ± 0.1 g/L of total acidity expressed as tartaric acid; 30 ± 2 mg/L of free $SO_2$; 62 ± 5 mg/L of total $SO_2$. The basic composition of the musts from 2021 vintage are summarized in Table 1.

### 2.2. Enzymes

Gluzyme Mono 10.000 (0.17 mkat/g) and Catazyme 25 L (0.42 mkat/g) was used as sources of GOX and CAT. Both commercial preparations were kindly provided by Novozymes® (Bagsvaerd, Denmark). Gluzyme Mono 10.000 is a formulation for baking industry containing wheat flour as matrix and it was not used directly. The enzyme extract was prepared by dissolving 28 g of Gluzyme in 100 mL of water. Then, the mixture was stirred for 30 min and centrifuged at 2320×*g* for 10 min (Sorvall ST 8R Centrifuge, Osterode am Harz, Germany) (Valencia, Espinoza, Ramirez, Franco, & Urtubia, 2017). The supernatant was used as source of GOX. Catazyme 25 L was used without modification.

### 2.3. Preliminary studies

Must from 2020 season was used to investigate the effect of GOX and CAT concentration and must pH, as well as the aeration regime on the reduction of glucose. The must was pasteurized for 40 s at 90 °C (Röcker et al., 2016) and stored at 6 °C until used. The assays, in triplicate, were performed in 100 mL Erlenmeyer flasks with 25 mL of must. All samples were shaken on an orbital agitator at a speed of 150 rpm (Orbital Shaker SO1, Stuart Scientific, Stone, UK) at 25 °C. All experiments were tested with the same concentration of GOX and CAT (0.17 mkat/L of must), except for the runs that studied the GOX and CAT doses (section 2.3.1). After the addition of GOX and CAT, the concentration of glucose of the must was monitored during 24 h.

#### 2.3.1. Effect of GOX and CAT concentration

Four concentrations of GOX (0.08, 0.17, 0.50 and 0.83 mkat/L of must) were evaluated using a GOX:CAT ratio of 1:1. Further trials were performed varying the concentrations of CAT (0.00, 0.17 and 0.34 mkat/L of must) with a concentration of GOX of 0.17 mkat/L of must. Control samples without GOX and CAT were also tested.

#### 2.3.2. Effect of must pH

The effect of must pH was investigated at four different pH (3.0, 3.4, 3.6 and 4.0) modifying the pH of the must by adding 5 mol/L NaOH or 1 mol/L HCl.

#### 2.3.3. System of aeration

The study of the system of aeration was carried out in 1 L Erlenmeyer flaks with 300 mL of must. A first trial was performed shaking the samples at 150 rpm on an orbital agitator. A second trial was carried out with pressured air (0.021 MPa) at 0.5 L/h during 24 h through an air compressor (AS186, Ariston Thermo, Fabriano, Italy) without agitation. The air was filtered in-line using a 0.20 μm PTFE filter (Whatman, GE Healthcare, Little Chalfont, United Kingdom). An airflow meter (LZB-4, Acrylic flowmeter, Zhejiang Yuyao Instrument, Yuyao, China) and pressure regulators were used to control air flow rate. A control sample without agitation was also included.

### 2.4. Enzyme treatment and fermentation of musts

Enzyme treatments were performed using two different batches of Verdejo must from 2021 vintage (must A and B) without thermal treatment and in triplicate. The first assay was carried out with the must A having a pH of 3.8. The same concentration of GOX and CAT (0.17 mkat/L) was applied to 1-L Erlenmeyer flasks containing 300 mL of must A. The enzyme-treated must was maintained at 20 °C during 48 h in an orbital shaker at 150 rpm. Musts were not aerated with pressurized air. Enzyme untreated musts A were refrigerated at 6 °C until fermentation. Enzyme treated must (EA) was blended, in two adequate proportions,

**Table 1**
Physicochemical properties of enzyme untreated and treated musts (GOX must)†.

| Samples (n = 3) | Glucose (g/L) | pH | Total acidity (g/L) | Browning index (AU) | TPI (AU) | Flavonoids (AU) | Hydroxycinnamic acids (AU) |
|---|---|---|---|---|---|---|---|
| **A** | **175$^{b}$** | **3.80$^{b}$** | **3.51$^{a}$** | 0.575 | **6.5$^{b}$** | **1.41$^{b}$** | **4.19$^{b}$** |
| **EA** | **101$^{a}$** | **3.10$^{a}$** | **9.61$^{b}$** | 0.525 | **6.1$^{a}$** | **1.02$^{a}$** | **2.48$^{a}$** |
| **B** | **176$^{B}$** | **4.00$^{B}$** | **2.43$^{A}$** | 0.411 | **6.9$^{B}$** | **1.35$^{B}$** | **4.16$^{B}$** |
| **EB** | **105$^{A}$** | **2.81$^{A}$** | **10.8$^{B}$** | 0.447 | **5.6$^{A}$** | **0.77$^{A}$** | **2.77$^{A}$** |
| ***Sp*** | 8 | 0.03 | 0.1 | 0.08 | 0.1 | 0.03 | 0.3 |

†Values within the same column with different lower case letters indicate significant differences between A and EA musts and with different capital case letters between B and EB musts ($P < 0.05$). GOX: glucose oxidase; AU: arbitrary units; A and B: control musts; EA: enzyme treated must A; EB: enzyme treated must B; *Sp*: pooled standard deviation; TPI: total polyphenol index; n: number of replicates.

with untreated must A to produce wines with a moderate reduction in their alcohol content by around 10–20 mL/L. The mixtures were labeled as MA1 and MA2.

The second assay was performed with the must B having a pH of 4.0 under the experimental conditions described above. Enzyme treated must (EB) was mixed, in two appropriate ratios, with the untreated must B (mixtures MB1 and MB2), with the goal of reducing the wine alcohol content by about 20–30 mL/L. As the pH of the must B was higher than the pH of must A, a bigger reduction of the alcohol degree could be possible without observing an extreme lowering of the pH due to the production of gluconic acid.

Initial A and B musts, as well as MA1, MA2, MB1 and MB2 blending musts were fermented to obtain their respective wines, which were labeled similarly to the originals. Musts were inoculated with 0.3 g/L of activated *Saccharomyces cerevisae* (N96, Anchor Yeast Biotechnologies, Johannesburg, South Africa). All fermentations were carried out in duplicate at 20 °C and monitored by weight loss. Once fermentations were completed, the wines were racked, sulfited at 30 mg/L of free $SO_2$ and left to settle a day at 8 °C until analysis.

### 2.5. Composition of musts and wines

Oenological analysis of musts and wines, such as total acidity (OIV-MA-AS313-01), volatile acidity (OIV-MA-AS313-02), pH (OIV-MA-AS313-15) and alcohol content (OIV-MA-AS312-01) were analyzed according to OIV methods (OIV, 2020). Glucose and fructose (OIV--MA-AS311-02), and gluconic acid (OIV-MA-AS313-28) were determined using enzyme kits (Megazyme, Sidney, Australia) (OIV, 2020). Polyphenolic index, hydroxycinnamic acids and flavonols were measured using the absorbances at 280, 320 and 365 nm, respectively, using a quartz cell of 1 cm path length after sample dilution 1:10 with distilled water (Somers & Ziemelis, 1985). Browning index of musts was directly measured at 420 nm using a 1 cm path length glass cell (Glories, 1984). Glories' method was used to determine the chromatic characteristics of wines (Glories, 1984). This analysis was conducted based on the absorbance measurements at three specific wavelengths (420, 520 and 620 nm) using a glass cell with 1 cm path length. The method involved calculating the total absorbance sum to determine the color intensity. Additionally, the tonality was established by comparing the absorbance at 420 nm to that at 520 nm. The percentages of yellow, red, and blue colors were derived by comparing the absorbance at 420, 520 or 620 nm, respectively, to the combined absorbance of all three wavelengths. Spectrophotometric measurements were performed using a UV–Vis spectrophotometer (Genesys™ 150 Vis/UV–Vis, Thermo Fisher, Madrid, Spain). Analysis of musts and wines were carried out in triplicate.

### 2.6. Determination of VOCs

Headspace-solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC-MS) was carried out to determine the concentration of VOCs in wines (Sánchez et al., 2022). A CombiPal RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland) connected with a 7890A gas chromatograph (Agilent Technologies, Santa Clara, USA) and a 5977 mass selective detector (Agilent Technologies) in the were used for the quantification of wine VOCs. The extraction of wine VOCs were carried out by the methodology described in a previous published work (Massera et al., 2012) with slight modifications. A 20-mL vial was filled with 5 mL of wine saturated with 3 g of NaCl and 50 μL of methyl nonanoate (0.059 mg/L) as internal standard. The vial was sealed with a magnetic screw cap provided with a PTFE/silicone septa and heated at 40 °C for 15 min with agitation (250 rpm). Extraction of VOCs was performed in the headspace vial at 40 °C for 30 min with agitation (250 rpm) using the fiber 50/30 μm DVB/CAR/PDMS (Supelco, Inc., Bellefonte, USA), previously preconditioned at 270 °C for 15 min. After extraction, the fiber was introduced into the injector of GC (250 °C) to desorb the volatiles for 15 min.

The GC conditions were as follows: injector temperature, 250 °C; injection mode, splitless (1 min); capillary column, HP-Innowax column (60 m, 0.250 mm, 0.5 μm) (J &W Scientific, Folsom, CA, USA); oven temperature, 40 °C held for 5 min, then increased to 230 °C at a rate of 2.5 °C/min and then maintained at this temperature for 20 min; carrier gas, helium at a constant pressure of 0.154 MPa and a flow of 1.2 mL/min. MS was operated under the SCAN mode and the mass range studied was from 30 to 200 m/z. The identification was performed by comparing GC mass spectra with pure standards and with spectra from the NIST08 y Wiley7 libraries (Table S1). Quantification was carried out using the internal standard quantification method with standards as was described by Sánchez et al. (2022). Samples were analyzed in triplicated.

### 2.7. Statistical analysis

The data was analyzed using Kruskal-Wallis 1-way ANOVA ($P < 0.05$), followed by post-hoc tests. The statistical analysis was realized using IBM SPSS Statistics (v25, IBM Corp, Chicago, IL, USA).

## 3. Results and discussions

### 3.1. GOX and CAT dose

Fig. 1A shows the effect of the concentration of GOX on the glucose conversion during 24 h of enzyme treatment. An increase in the glucose conversion was observed in enzyme treated musts, as opposed to the control, being more pronounced as the GOX concentration was higher. These results are consistent with those reported by several authors (Biyela et al., 2009; Pickering et al., 1998), who observed improved glucose conversion with increasing doses of GOX. However, a linear correlation between the GOX concentration and the glucose conversion was not obtained. The experiment with the lowest doses of GOX (0.08 mkat/L) resulted in a glucose conversion of 42.5 g/L, recording only 81.8 g/L in the assay with a concentration of GOX ten times higher (0.83 mkat/L). Based on the results obtained, a dose of 0.17 mkat/L was used in subsequent experiments, as it provides a notable glucose conversion (61.5 g/L) which reduces considerably the monetary cost of the enzyme preparation.

The effect of CAT concentration on glucose conversion can be observed in Fig. 1B. The presence of CAT improved the glucose conversion, showing the best results with 0.17 mkat/L. However, the use of the highest concentration of CAT did not improve the GOX performance, showing a reduction of 23% in glucose oxidation as CAT concentration was increased from 0.17 to 0.34 mkat/Liu and Cui (2007) also observed a reduction in glucose conversion in a membrane enzyme reactor when a high CAT/GOX ratio was used. They attributed this phenomenon to the denaturation and aggregation of CAT during the process. Based on these results, a CAT concentration of 0.17 mkat/L was used in the further experiments.

### 3.2. Must pH

As shown in Fig. 1C, no statistically significant differences in glucose conversion by GOX were observed in the must pH range from 3.0 to 4.0. Like these results, Biyela et al. (2009) report no differences for ethanol concentration in wines elaborated with enzyme treated synthetic musts at pH 3.0 and 4.0, although glucose conversion data are not provided by the authors. In the trials of Röcker et al. (2016), similar glucose conversion is achieved in Rhein Riesling must at pH 3.1 and 3.5 (25.0 and 27.5 g/L, respectively).

A probable explanation could be that the enzyme dose used was high enough to compensate for the differences in GOX activity due to must pH. In addition, the studied pH range is very narrow (pH 3.0–4.0) compared to the pH range where the GOX enzyme shows activity (2.5–8.0), so that the enzyme activity under these test conditions would

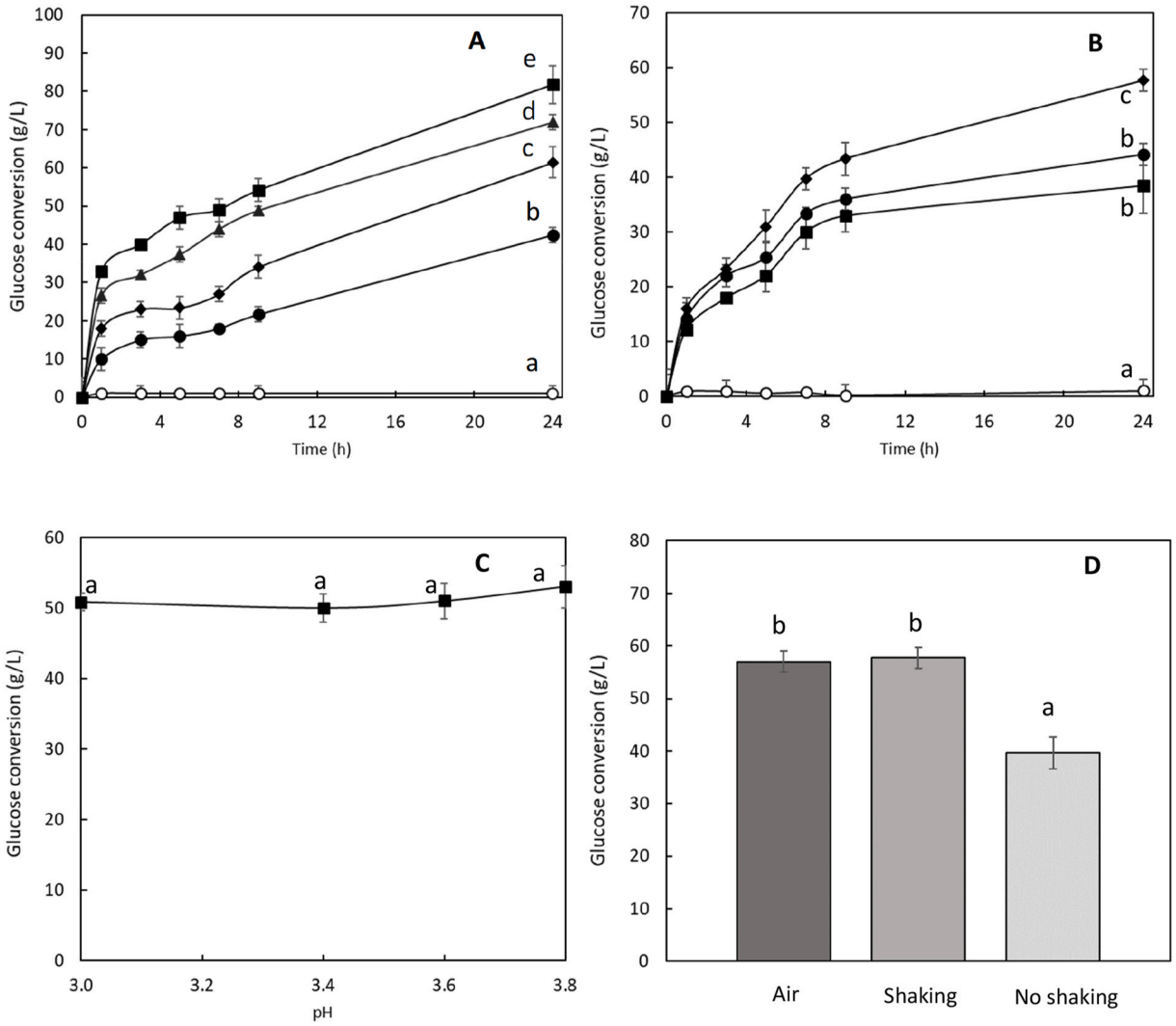


**Fig. 1.** Effects of (A) the concentration of glucose oxidase (GOX) (Control: sample without enzyme (○); GOX: 0.08 (●), 0.17 (◆), 0.50 (▲) and 0.83 (■) mkat/L of must), (B) the concentration of catalase (Control: sample without enzymes (○); CAT: 0 (■), 0.17 (◆) and 0.34 (●) mkat/L of must), (C) the initial must pH, and (D) the conditions of aeration (air: pressured air during 24 h; shaking: orbital shaking at 150 rpm; no shaking) on the enzyme glucose conversion (g/L) of must. Different letters indicate significant differences between samples ($P < 0.05$).

be similar. It has been described that an increase in pH from 3.0 to 4.0 causes a slight increase in pure GOX activity (12%), rising notably (75%) when the pH changes from 4.0 to 6.0 (Godfrey & Reichelt, 1996).

### 3.3. Aeration system

As Fig. 1D shows, similar glucose conversions were found using pressured air during 24 h and agitation at 150 rpm on an orbital shaker, and higher values than the non-stirred experiment. Röcker et al. (2016) reported an increase on the production of gluconic acid employing an aeration by pressured air together with an agitation of must at 53 rpm on an orbital shaker. Pickering et al. (1998) suggest optimal aeration regime for GOX-treated must at 5.7 L of air/min per L of must, significantly higher than 1.6 L of air/min per L of must utilized in this work. The reduction of the aeration rates may be very advantageous in preventing oxidation of the must colour and loss of its aroma (Pickering et al., 1998). Foam formation was observed in the musts with an aeration based on pressured air during 24 h, therefore the further experiments were carried out with agitation at 150 rpm.

### 3.4. Basic composition of enzyme treated musts

Two batches of must from 2021 vintage at pH 3.8 and 4.0 were treated with GOX and CAT during 48 h. The oenological composition of enzyme untreated and treated musts are listed in Table 1. A significant reduction of glucose content was observed in enzyme treated musts (EA and EB) compared to their respective controls (A and B). There were not significant differences for this parameter according to the initial pH of the EA and EB musts. These results agree with those observed in section 3.2. The depletion of glucose concentration was of 74 and 71 g/L in EA and EB musts, respectively, which corresponded to 43 and 42 mL/L less potential alcohol content. The pH of enzyme treated musts dropped drastically as the acidity increased due to the formation of gluconic acid by GOX.

Regarding the browning index, no significant differences in absorbance at 420 nm were detected between the controls and their respective enzyme treated musts (Table 1). Both the concentration of CAT and aeration regime used were probably adequate to avoid the oxidation of the musts, preventing a harmful accumulation of $H_20_2$ and an excessive oxygenation, respectively. Contrary to these results, Pickering, Heatherbell, & Barnes (1999b) reported a lower absorbance at 420 nm in the control must than in enzyme treated must, which probably resulted from

an excessive aeration of 5.7 L/min per L of must. As shown in Table 1, the phenolic composition of musts changed during enzyme treatment. A significant decrease in total polyphenol index, as well as in flavonoids and hydroxycinnamic acid content were observed in the enzyme treated musts compared with control musts, as noted by Pickering et al. (1999a).

### 3.5. Basic composition of wines from enzyme treated musts

Unlike previous studies, GOX was applied to a fraction of must that it was subsequently blended with untreated must. This winemaking method can avoid an excessive aeration of the total must batch, that could alter its quality. Moreover, this practice is more suitable than blending enzyme-treated acidified wines with untreated ones, as the yeast metabolism is altered at low must pH (Ribéreau-Gayon et al., 2006). Table 2 shows the physico-chemical composition of the wines from untreated musts (A and B) and from the blending of untreated and enzyme treated musts (MA1, MA2, MA3 and MA4). It was decided not to ferment the enzyme treated musts (EA and EB) because the low must pH, mainly in EB, could cause stuck fermentation. Untreated and blending musts fermented to dryness without any difficulty. Neither glucose nor fructose were detected in wines. Moreover, the volatile acidity of the wines was low, pointing out that alcoholic fermentation took place without deviations.

Wines from untreated musts (A and B) were unbalanced, with very high alcohol content and pH. However, GOX wines were more equilibrated, registering lower alcohol content and pH (Table 2). Moderate reductions in alcohol content of 10 and 15 mL/L and in pH were obtained in wines MA1 and MA2, respectively, from untreated must at pH 3.8 (must A) and its corresponding treated must (must AE). The blending of untreated must at pH 4.0 (must B) and treated must (must EB) enabled a greater reduction in alcoholic content (21 and 27 mL/L) and pH values, in line with to those obtained in wines MA1 and MA2. These results indicate that the initial pH of the must is a limiting factor in ethanol depletion by GOX. Röcker et al. (2016) also reported similar reductions in alcohol content of about 20 mL/L but associated with values of wine pH very low (pH 2.8–3.0). The initial must pH was too low (pH 3.1–3.5) to balance the amount of gluconic acid produced by GOX and, therefore, a deacidification step was required before wine consumption.

Although some authors have observed remarkable changes in wine color from enzyme treated musts (Pickering, Heatherbell, & Barnes, 1999b; Valencia et al., 2017), in this study no significant differences in the chromatic properties of control and GOX wines were found. These results are in accordance with those observed for browning index in the musts.

Regarding the phenolic composition, GOX wines had similar level of total polyphenol index than control ones. However, the concentrations of flavonoids and hydroxycinnamic acids were lower in GOX wines than in control wines. Moreover, these concentrations were below than those observed in enzyme treated musts, probably due to the precipitation of phenolic materials during fermentation (Pickering et al., 1999b). A reduction in the concentration of these compounds has been reported in wines from oxygenated musts, increasing their resistance to browning during storage (Cejudo-Bastante, Hermosín-Gutiérrez, Castro-Vázquez, 2011a).

### 3.6. VOCs composition

VOCs composition of control and GOX wines are summarized in Table 3. Forty-eight compounds were identified and grouped in nine families (acetates, ethyl esters, methyl esters, acids, alcohols, C6-alcohols, phenols, aldehydes and ketones). Differences on the concentration of VOCs families among control and GOX wines were detected (Fig. 2). In general, the control wines showed higher concentration of total acetates, total ethyl and methyl esters, total C6-alcohols and total ketones than the GOX wines. Moreover, similar volatile composition was found between MA1 and MA2, and between MB1 and MB2 noting that, within the GOX wines, a higher decrease in alcohol content and pH did not noticeably modify the VOCs composition of wines. Despite the differences observed between the control and GOX wines based on the total concentration of each family of volatiles, a more detailed study of the VOCs of each family is then carried out to determine the effect of GOX treatment on the wine volatile composition.

Concerning acetates, significant differences between the control wines and their corresponding GOX wines were only observed for heptyl acetate with a higher concentration in the control wines. This acetate with floral note was responsible for the highest total acetate concentration found in the control wines (Fig. 2). The composition of the GOX wines and their corresponding control wines was very similar in terms of short-chain ethyl esters characterized by fruity notes (Bakker & Clarke, 2011), with the exception of ethyl hexanoate, which exhibited higher concentrations in the GOX wines, and ethyl octanoate, which displayed higher concentrations in wines A and B. However, enhanced concentrations of long-chain ethyl esters with soapy notes (Bakker & Clarke, 2011), were showed in the control wine A, except for ethyl hexadecanoate. Additionally, the control wine B displayed higher levels of ethyl dodecanoate in comparison to their respective GOX wines. Unlike these results, Pickering, Heatherbell, and Barnes (2001) observed higher concentration of these compounds in GOX wines than in control ones, although only one acetate and two esters were reported from CG-MS analysis of wines. Specifically, the levels of butyl acetate, methyl 11-octadecenoate and 10-undecenoic acid octyl ester were higher in wines from enzyme treated musts than in control ones. Biyela et al. (2009) reported an increase in ethyl lactate in wines from enzyme treated musts but with a decrease in ethyl acetate concentration.

In general, no differences were observed for acids between the control wines and their corresponding GOX wines (Table 3). The volatile acids add complexity to wine, although at high concentrations they can

**Table 2**
Physicochemical properties of control wines and wines from blending of untreated and enzyme treated musts (GOX wines).†

| Samples (n = 3) | Alcohol content (mL/L) | Gluconic acid (g/L) | pH | Total acidity (g/L) | Volatile acidity (g/L) | Colour intensity (AU) | Tonality (AU) | % Red | % Blu | % Yellow | TPI (AU) | Flavonoids (AU) | Hydroxycinnamic acids (AU) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | **135$^{c}$** | **0.07$^{a}$** | **3.65$^{b}$** | **4.65$^{a}$** | 0.35 | 0.20 | 3.90 | 18.7 | 7.60 | 73.7 | 6.3 | **0.97$^{b}$** | **4.50$^{b}$** |
| **MA1** | **125$^{b}$** | **18.9$^{b}$** | **3.30$^{a}$** | **9.90$^{b}$** | 0.30 | 0.20 | 3.40 | 20.7 | 9.10 | 70.1 | 6.4 | **0.41$^{a}$** | **1.29$^{a}$** |
| **MA2** | **120$^{a}$** | **20.3$^{b}$** | **3.25$^{a}$** | **10.8$^{c}$** | 0.39 | 0.26 | 3.25 | 22.3 | 10.9 | 66.2 | 6.9 | **0.48$^{a}$** | **1.40$^{a}$** |
| **B** | **138$^{C}$** | **0.40$^{A}$** | **3.60$^{C}$** | **4.71$^{A}$** | 0.42 | 0.19 | 3.95 | 18.8 | 6.90 | 74.2 | 6.3 | **0.92$^{B}$** | **4.55$^{B}$** |
| **MB1** | **117$^{B}$** | **21.8$^{B}$** | **3.30$^{B}$** | **8.90$^{B}$** | 0.38 | 0.19 | 3.60 | 20.7 | 7.70 | 71.5 | 5.8 | **0.52$^{A}$** | **1.40$^{A}$** |
| **MB2** | **111$^{A}$** | **28.6$^{C}$** | **3.10$^{A}$** | **10.8$^{C}$** | 0.36 | 0.23 | 3.10 | 21.7 | 10.2 | 68.1 | 6.3 | **0.61$^{A}$** | **1.55$^{A}$** |
| ***Sp*** | 3 | 0.5 | 0.04 | 0.2 | 0.04 | 0.2 | 0.2 | 0.8 | 1.4 | 2.1 | 0.8 | 0.03 | 0.1 |

†Values within the same column with different lower case letters indicate significant differences among A, MA1 and MA2 wines and with different capital case letters among B, MB1 and MB2 wines ($P < 0.05$). GOX: glucose oxidase; n: number of replicates; A and B: wines from enzyme untreated musts; MA1 and MA2: wines from blending of untreated and enzyme treated musts A; MB1 and MB2: wines from blending of untreated and enzyme treated musts B; *Sp*: pooled standard deviation; AU: arbitrary units; TPI: Total polyphenol index.

**Table 3**
Volatile organic compounds (VOCs) (mg/L) of control wines and wines from blending of untreated and enzyme treated musts (GOX wines).†

| VOCs | Wines (n = 3) | | | | | | |
|---|---|---|---|---|---|---|---|
| (IUPAC name) | A | MA1 | MA2 | B | MB1 | MB2 | *Sp* |
| ***Acetates*** | | | | | | | |
| 3-methylbutyl acetate | 0.507 | 0.531 | 0.546 | 0.434 | 0.346 | 0.303 | 0.03 |
| 2-ethylhexyl acetate | 0.057 | 0.025 | 0.017 | 0.088 | 0.070 | 0.064 | 0.01 |
| Heptyl acetate | **16.3**$^{b}$ | **7.83**$^{a}$ | **8.53**$^{a}$ | **12.2**$^{B}$ | **7.61**$^{A}$ | **7.11**$^{A}$ | 0.9 |
| ***Ethyl esters*** | | | | | | | |
| Ethyl 2-phenylacetate | 0.042 | 0.060 | 0.037 | 0.048 | 0.019 | 0.015 | 0.01 |
| Ethyl butanoate | 1.02 | 0.553 | 0.219 | 1.54 | 0.942 | 0.654 | 0.3 |
| Diethyl butanedioate | 0.062 | 0.063 | 0.063 | 0.049 | 0.129 | 0.120 | 0.03 |
| Ethyl 3-methylbutanoate | 0.017 | 0.063 | 0.053 | 0.037 | 0.122 | 0.056 | 0.04 |
| Ethyl 4-hydroxybutanoate | 0.064 | 0.037 | 0.019 | 0.054 | 0.035 | 0.033 | 0.007 |
| Ethyl hexanoate | **0.018**$^{a}$ | **0.047**$^{b}$ | **0.020**$^{a}$ | **0.018**$^{A}$ | **0.030**$^{B}$ | **0.023**$^{A}$ | 0.06 |
| Ethyl hex-5-enoate | 0.045 | 0.022 | 0.019 | 0.038 | 0.021 | 0.009 | 0.01 |
| Ethyl octanoate | **0.087**$^{b}$ | **0.016**$^{a}$ | **0.013**$^{a}$ | **0.053**$^{B}$ | **0.016**$^{A}$ | **0.006**$^{A}$ | 0.07 |
| Ethyl dec-9-enoate | **1.25**$^{c}$ | **0.785**$^{b}$ | **0.402**$^{a}$ | 1.42 | 1.40 | 1.25 | 0.1 |
| Ethyl undecanoate | **0.175**$^{b}$ | **0.082**$^{a}$ | **0.080**$^{a}$ | 0.097 | 0.056 | 0.052 | 0.01 |
| Ethyl dodecanoate | **0.593**$^{b}$ | **0.164**$^{a}$ | **0.132**$^{a}$ | **0.387**$^{B}$ | **0.191**$^{A}$ | **0.213**$^{A}$ | 0.01 |
| Ethyl 3-hydroxytridecanoate | **4.07**$^{b}$ | **2.10**$^{a}$ | **0.986**$^{a}$ | 4.58 | 3.57 | 2.54 | 0.5 |
| Ethyl pentadecanoate | **0.104**$^{b}$ | **0.053**$^{a}$ | **0.019**$^{a}$ | 0.060 | 0.039 | 0.041 | 0.01 |
| Ethyl hexadecanoate | 0.030 | 0.043 | 0.026 | 0.040 | 0.033 | 0.033 | 0.004 |
| Ethyl (E)-hexadec-9-enoate | **0.988**$^{b}$ | **0.553**$^{a}$ | **0.593**$^{a}$ | 0.827 | 0.373 | 0.276 | 0.2 |
| Ethyl benzoate | 1.10 | 1.89 | 2.44 | 0.881 | 0.695 | 0.547 | 0.2 |
| ***Methyl esters*** | | | | | | | |
| 3-methylbutyl dodecanoate | 0.022 | 0.026 | 0.023 | 0.024 | 0.021 | 0.020 | 0.003 |
| Methyl decanoate | **75.2**$^{b}$ | **43.2**$^{a}$ | **40.2**$^{a}$ | 52.4 | 39.7 | 35.0 | 4.5 |
| ***Acids*** | | | | | | | |
| 2-methylpropanoic acid | 0.229 | 0.163 | 0.247 | 0.147 | 0.221 | 0.212 | 0.02 |
| Hexanoic acid | 0.605 | 0.682 | 0.392 | 0.514 | 0.217 | 0.186 | 0.04 |
| Octanoic acid | 0.114 | 0.093 | 0.114 | 0.058 | 0.043 | 0.044 | 0.003 |
| Nonanoic acid | **0.299**$^{b}$ | **0.192**$^{a}$ | **0.234**$^{ab}$ | 0.155 | 0.146 | 0.105 | 0.02 |
| Dec-9-enoic acid | 0.020 | 0.029 | 0.020 | 0.026 | 0.031 | 0.031 | 0.008 |
| Decanoic acid | 0.057 | 0.143 | 0.305 | 0.035 | 0.086 | 0.179 | 0.04 |
| ***Alcohols*** | | | | | | | |
| Propan-1-ol | 0.627 | 0.634 | 0.594 | 0.679 | 0.260 | 0.294 | 0.11 |
| 2-methylpropan-1-ol | 12.8 | 20.1 | 10.6 | 11.3 | 22.4 | 19.3 | 6.3 |
| 3-methylbutan-1-ol | 8.71 | 7.45 | 3.06 | 6.21 | 5.21 | 4.35 | 1.4 |
| Decan-1-ol | 0.075 | 0.053 | 0.042 | 0.109 | 0.054 | 0.067 | 0.005 |
| 2-undecanol | 0.031 | 0.027 | 0.023 | 0.025 | 0.026 | 0.021 | 0.003 |
| 2-phenylethanol | **0.072**$^{b}$ | **0.033**$^{a}$ | **0.013**$^{a}$ | 0.048 | 0.050 | 0.048 | 0.003 |
| Phenylmethanol | **0.231**$^{b}$ | **0.090**$^{a}$ | **0.053**$^{a}$ | **0.156**$^{B}$ | **0.052**$^{A}$ | **0.064**$^{A}$ | 0.002 |
| ***Phenols*** | | | | | | | |
| 4-ethenyl-2-methoxyphenol | **0.076**$^{b}$ | **0.032**$^{a}$ | **0.028**$^{a}$ | **0.068**$^{B}$ | **0.042**$^{AB}$ | **0.022**$^{A}$ | 0.01 |
| 2,4-ditert-butylphenol | 0.351 | 0.347 | 0.174 | 0.130 | 0.118 | 0.008 | 0.1 |
| Phenol | 0.042 | 0.048 | 0.048 | **0.039**$^{AB}$ | **0.044**$^{B}$ | **0.034**$^{A}$ | 0.002 |
| ***C6-alcohols*** | | | | | | | |
| Hexan-2-ol | 0.063 | 0.052 | 0.030 | 0.018 | 0.027 | 0.029 | 0.01 |
| Heptan-1-ol | **0.027**$^{a}$ | **0.043**$^{a}$ | **0.072**$^{b}$ | **0.016**$^{A}$ | **0.048**$^{B}$ | **0.079**$^{B}$ | 0.007 |
| (E)-hex-3-en-1-ol | **1.30**$^{c}$ | **0.777**$^{b}$ | **0.489**$^{a}$ | **0.944**$^{B}$ | **0.767**$^{A}$ | **0.618**$^{A}$ | 0.1 |
| ***Aldehydes*** | | | | | | | |
| Acetaldehyde | 0.032 | 0.037 | 0.027 | **0.043**$^{A}$ | **0.054**$^{B}$ | **0.057**$^{B}$ | 0.002 |
| Benzaldehyde | **0.087**$^{a}$ | **0.128**$^{b}$ | **0.103**$^{a}$ | **0.141**$^{A}$ | **0.212**$^{B}$ | **0.205**$^{B}$ | 0.02 |
| Nonanal | 0.343 | 0.336 | 0.283 | 0.273 | 0.426 | 0.354 | 0.09 |
| ***Ketones*** | | | | | | | |
| Octan-2-one | **0.900**$^{a}$ | **1.36**$^{b}$ | **1.47**$^{b}$ | 0.684 | 0.691 | 0.798 | 0.06 |
| Undecan-2-one | **0.192**$^{b}$ | **0.047**$^{a}$ | **0.033**$^{a}$ | **0.108**$^{B}$ | **0.046**$^{A}$ | **0.039**$^{A}$ | 0.01 |
| 2-Methyltetrahydrothiophen-3-one | **0.045**$^{c}$ | **0.027**$^{b}$ | **0.013**$^{a}$ | 0.050 | 0.050 | 0.041 | 0.006 |
| Oxolan-2-one | 0.024 | 0.041 | 0.024 | 0.025 | 0.028 | 0.022 | 0.09 |
| Nonan-2-one | **5.68**$^{b}$ | **0.168**$^{a}$ | **0.047**$^{a}$ | **5.69**$^{B}$ | **1.15**$^{A}$ | **0.347**$^{A}$ | 0.9 |

†Values within the same row with different lower case letters indicate significant differences among A, MA1 and MA2 wines and with different capital case letters among B, MB1 and MB2 wines ($P < 0.05$). GOX: glucose oxidase; n: number of replicates; *Sp*: pooled standard deviation.

have a negative impact (Bakker & Clarke, 2011). Similar results were found by Pickering et al. (2001), who observed higher concentration of nonanoic acid in wines from GOX-treated musts. Nevertheless, Röcker et al. (2016) reported lower levels of isovaleric, hexanoic and octanoic acids in wines from GOX-treated musts.

The total concentration of the family of alcohols was higher in the GOX wines than in their respective control ones, except for the wine MB1 (Fig. 2). However, an individual analysis of the alkyl alcohols indicated that their concentration was similar in all wines. These alcohols at concentrations below 300 mg/L contribute positively to the alcoholic and ethereal notes of wine and to its aromatic complexity (Bakker & Clarke, 2011). In general, other authors have found a lower concentration of alkyl alcohols in GOX wines than in control wines. Biyela et al. (2009) reported a decrease in propan-1-ol in wines from enzyme treated musts compared to control wines, with an increase on 3-methylbutan-1-ol, while Röcker et al. (2016) detected lower concentrations of 2-methylpropan-1-ol and 3-methylbutan-1-ol.

The volatile compounds 2-phenylethanol and phenylmethanol with floral notes had a high concentration in control wines than their corresponding GOX wines, although the variations in the concentration of 2-phenylethanol were not evident between control wine B and wines MB1-MB2. In line with these findings, Röcker et al. (2016) detected also lower

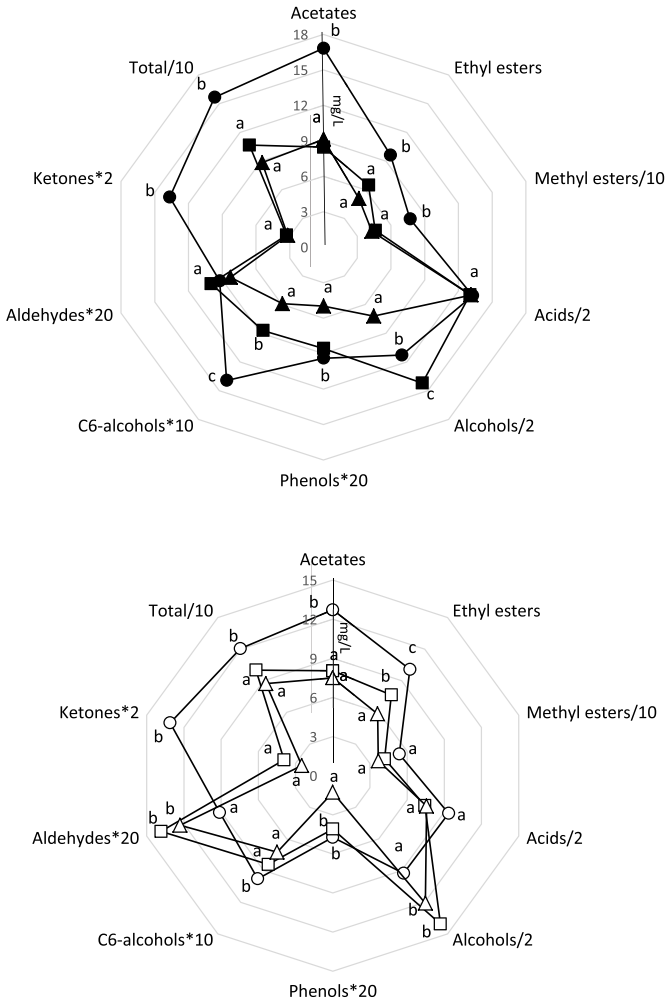


**Fig. 2.** Concentration of volatile organic compounds (VOCs) (mg/L), grouped in families, in wines from untreated and blending musts. The symbols * and/ indicate the factor applied at each volatile family to provide similar range values for all of them. A (●) and B (○): wines from enzyme untreated musts. MA1 (■) and MA2 (▲): wines from blending of untreated and enzyme treated musts A. MB1 (□) and MB2 (△): wines from blending of untreated and enzyme treated musts B. Different letters indicate significant differences among musts ($P < 0.05$).

concentrations of 2-phenyletanol in wines from GOX-treated musts.

Regarding the phenols, 4-ethenyl-2-methoxyphenol (spice note) had a higher concentration in the control wines than in their respective GOX wines while the trend for phenol (phenolic note) was unclear.

There was no evident trend for individual C6-alcohols in the wines. Similar concentration of hexan-2-ol was observed among samples, whereas control wines had lower concentration of heptan-1-ol and higher concentration of (E)-hex-3-en-1-ol than their corresponding GOX wines. However, looking at the total concentration of C6-alcohols (Fig. 2), GOX wines showed lower values than control wines. C6-alcohols are formed by lipooxygenases, with a maximum activity at neutral pH, in presence of polyunsaturated fatty acids and oxygen (Mozzon, Savini, Boselli, & Thorngate, 2016). Although the addition of oxygen to must can increase the concentration of these compounds (Cejudo-Bastante, Hermosín-Gutiérrez, Castro-Vázquez, & Pérez-Coello, 2011), the lower concentration of C6-alcohols observed in the GOX wines could be explained by a decline in lipoxygenase activity, since the pH was lower in the GOX-treated musts than in the control ones. These results are positive as a lower concentration of these compounds could reduce the vegetal notes of the GOX wines (Mozzon et al., 2016; Rodríguez-Nogales, Fernández-Fernández, & Vila-Crespo, 2009).

Enzyme treatment of must also modified the VOCs composition for some aldehydes and ketones which participate in the aromatic complexity of wine (Arias-Pérez, Sáenz-Navajas, De-la-Fuente-Blanco, Ferreira, & Escudero, 2021; Bakker & Clarke, 2011). In the case of aldehydes, the GOX wines exhibited higher concentrations of acetaldehyde (which contributes to a fruity note at low concentrations) and benzaldehyde (imparting an almond note) compared to control wines, with the exception of acetaldehyde in MA1 and MA2 wines. Regarding the ketones, higher concentration of octan-2-one (unripe apple note) was found in the wines MA1 and MA2 than in the wine A. The wine A and B showed high concentration of undecan-2-one (fruity note) and nonan-2-one (floral note), while the wine A reported high levels of 2-methyltetrahydrothiophen-3-one (onion note).

The differences observed in the concentration of VOCs between the control wines and the wines from musts treated with GOX could be caused by various reasons, such as the lowering of must pH and the release of $H_2O_2$ due to GOX activity and/or the contact of the must with oxygen as a result of the aeration. On the one hand, the must pH affects the cell growth of wine yeasts and the pathways involved in the synthesis of individual VOCs that contribute to final wine aroma (Pérez-Torrado et al., 2002; Swiegers, Bartowsky, Henschke, & Pretorius, 2005). It has been observed that the effect of must pH on the biosynthesis of VOCs by yeast is different for some individual compounds and others. According to Liu and Cui (2007), at pH 3.9 the production of ethanol, esters and 2-phenylethyl alcohol was favored, while at pH 3.1 the concentration of higher alcohols was improved, although no effect of must pH on the biosynthesis of fatty acids and aldehydes was observed. The pH can alter in different ways the structural characteristics of the aromatic precursors, as well as the enzyme activities involved in their biosynthesis, modifying the volatile composition of the wine (Bekker, Mierczynska-Vasilev, Smith, & Wilkes, 2016). On the other hand, the presence of $H_2O_2$ in must, given its oxidizing nature, can cause alterations in wine aroma, although the addition of CAT at low concentrations (0.05 mkat/L) sufficiently reduces its content in must (Röcker et al., 2016). Regarding must aeration, the results of the influence of oxygenation on the aromatic characteristics of the wines are contradictory, as its effect depends on the grape variety used, the must composition, the amount of oxygen and the individual VOC studied (Rihak, Prusova, Kumsta, & Baron, 2022). Oxygenation of must enhanced the aromatic intensity of Chardonnay, Riesling, Faberrebe and Parellada wines, however, in Chenin blanc, French Colombard, and Semillon varieties, a decrease in aromatic quality of wines was observed (Du Toit, Marais, & Pretorius, 2006). Cejudo-Bastante, Hermosín-Gutiérrez, Castro-Vázquez, and Pérez-Coello (2011) observed a higher concentration of C6-alcohols, higher alcohols, acetates and fatty acid esters and a lower concentration of acetaldehyde and isoamyl alcohols in wines from oxygenated Chardonnay musts. However, in Airen white wines, oxygenation of must in the pre-fermentation phase did not modify the composition of the major volatiles, such as acetaldehyde, ethyl acetate, 1-propanol, 2-methyl-1-butanol and reduced the concentration of short-chain esters, some long-chain esters and acids (Cejudo-Bastante, Castro-Vázquez, Hermosín-Gutiérrez, & Pérez-Coello, 2011). These differences may be due to the activation or deactivation of different enzymes of the yeast metabolic pathways involved in the biosynthesis of each individual VOC, as a consequence of the addition of oxygen to the must, as well as the oxidation reactions of the aromatic precursors and/or the VOCs.

## 4. Conclusions

These results confirm that the application of GOX to must with high pH (>3.8) allows to produce wines more equilibrated, with lower alcohol content, higher acidity and similar chromatic properties and total polyphenolic index than the control wines. The enzyme treatment of the must did not modify the concentration of some groups of volatiles in the wines, such as short-chain ethyl esters with fruity notes, and acids

and higher alcohols which contribute to the complex aroma of wines. Moreover, as positive effect, the concentration of C6-alcohols, responsible for green-herbaceous aroma, and high-chain ethyl esters with soapy notes was lower in the GOX wines than in their respective control wines. However, the application of GOX reduced the wine concentration of heptyl acetate and some alcohols with floral notes, and ketones with floral and fruit notes. These results are promising, although further studies are needed to evaluate the impact of the application of GOX and CAT on the sensory characteristics of wines and consumer acceptability. On the other hand, more studies would be interesting to contrast these results under different grape varieties.

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## CRediT authorship contribution statement

**Rafael Mangas:** Investigation, Methodology. **María Rosa González:** Writing – original draft, writing-reviewing. **Pedro Martín:** Methodology, Writing – original draft, preparation, writing-reviewing. **José Manuel Rodríguez-Nogales:** Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, preparation, Writing – review & editing.

## Declaration of competing interest

None

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114975.

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