IMPACT OF USING NEW COMMERCIAL GLUTATHIONE ENRICHED INACTIVE DRY YEAST OENOLOGICAL PREPARATIONS ON THE AROMA AND SENSORY PROPERTIES OF WINES

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ABSTRACT

The effect of the addition of a commercial enriched glutathione Inactive Dry Yeast (GSH-IDY) oenological preparation in the volatile and sensory properties of industrially manufactured rosé Grenache wines was evaluated during their shelf-life. In addition, triangle tests were performed at different times during wine aging (among 1 and 9 months) to determine the sensory differences between wines with and without GSH-

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IDY preparations. Descriptive sensory analysis with a trained panel was carried out when sensory differences in the triangle test were noticed. In addition, consumer tests were performed in order to investigate consumers' acceptability of wines. Results revealed significant sensory differences between control and GSH-IDY wines after 9 months of aging. At that time, GSH-IDY wines were more intense in *fruity* aromas (*strawberry*, *banana*) and less intense in *yeast* notes than control wine. The impact of the GSH-IDY in the aroma might be the consequence of different effects that these preparations could induce in wine composition: modification of yeast byoproducts during fermentation, release of volatile compounds from IDY, interaction of wine volatile compounds with yeast macromolecules from IDY and a possible antioxidant effect of the glutathione released by the IDY preparation on some specific volatile compounds.

Key words: Wine, Glutathione, Inactive Dry Yeast Preparations, aroma, sensory analysis

INTRODUCTION

Oxidation processes constitute a serious problem during winemaking and especially in the case of young wines. In general terms, oxidation of young wines, is associated with a rapid loss of the pleasant sensory characteristics of wine, particularly affecting the floral and fruity notes, and the formation of unpleasant new aromas of typical aged wine, as well as atypical aromas associated with wine spoilage [1-3]. Wine oxidation also produces wine browning, which results from the oxidation of phenols to quinones, which in turn polymerise to form macromolecules with a typical yellow-brown hue [4].
The exogenous addition of γ-L-glutamyl-L-cysteinylglycine, named as glutathione (GSH), a tripeptide of non-proteic origin of known antioxidant properties [5], is now being studied by the OIV (International Organisation of Vine and Wine) since it has been shown that it prevents the enzymatic browning of white wines [6,7], and also preserves varietal aroma compounds, reducing the occurrence of aged off-flavor compounds [5]. However, the use of this compound during winemaking is not allowed so far.

In contrast, from the different types of Inactive Dry Yeast (IDY) preparations allowed for different applications during winemaking [8], some of them are claimed to prevent wine oxidation because of their higher content in GSH. Recently, new research performed in our laboratory, has shown a higher level of GSH released into synthetic wines by GSH enriched IDY preparations (GSH-IDY) compared to other non-GSH IDY preparations [9]. In addition, it has been shown that these preparations might reduce terpene oxidation in synthetic wines submitted to accelerated aging conditions [10]. Nevertheless, the impact of glutathione enriched IDY preparations to preserve and/or to improve the sensory characteristics of wines industrially manufactured has not been studied so far. Only the effect of the addition of an IDY preparation in the overall sensory perception of finished wines and their impact on the mouthfeel and taste properties have been studied [11,12]. Keeping these antecedents in mind and taking into consideration the importance of contributing to a better knowledge in the use of these preparations during winemaking, the objective of the present research was to evaluate the effect of a glutathione enriched commercial IDY preparation (GSH-IDY) on the volatile and sensory properties of an industrially manufactured rosé Grenache wine during its shelf-life.

MATERIAL AND METHODS

Description of the wines

Two different types of monovarietal Grenache rosé wines from the 2008 vintage, a control wine and a GSH-IDY wine, were industrially manufactured in a winery from the O.D. Navarra, Spain. To do so, 10,000 L tanks were filled with the same must. GSH-IDY wine was prepared by adding the advised dosage (20 g HL^{-1}) of a commercial glutathione enriched IDY preparation from a yeast autolysate (*Saccharomyces cerevisiae*) specially recommended by the manufacturers to prevent wine aroma oxidation. A control wine was also made from the same must without GSH-IDY addition. To carry out the alcoholic fermentation, the same active dry yeast was inoculated in both types of wines. All the wines were stabilised and clarified in the winery, and sent to our laboratory for the instrumental and sensory analysis. Wines were kept at 12 ºC during 10 months.

General parameters during winemaking (probable alcohol degree in musts, total acidity, volatile acidity and alcohol degree in wines) were determined according to the official methods of wine analysis [13]. From these determinations, it can be concluded that fermentation performance was similar in both types of wines and finished wines had values considered in the normal range for this type of wines (**Table 1**).

Volatile compounds

To determine the effect of GSH-IDY on the volatile profile and its evolution over time, wine volatiles were analyzed after 1, 2, 3 and 9 months of wine aging. To do so, 8 mL of wine spiked with 50 µL of a solution of methyl nonanoate (5 mg L^{-1}) used as internal standard were placed in a 20 mL headspace vial and sealed with a PTFE/Silicone septum (Supelco, Bellefonte, PA). Vials were kept at 40 ºC for 10 min to reach equilibrium before the extraction. The extraction was performed during 20 minutes at 40 ºC under constant stirring (500 rpm), using a StableFlex 85 µm carboxen-polydimethylsiloxane, CAR-PDMS fibre (Supelco). The same fibre was used throughout the study and its performance was periodically checked. After the extraction, the fibre was removed from the sample vial and desorbed in the GC injector port in splitless mode for 10 min. An Agilent 6890N GC system (Agilent, Palo Alto, CA) with a split/splitless injector and interfaced with an Agilent 5973 mass spectrometer was used for sample analysis. The injector was set at 280 ºC. An Agilent MSD ChemStation Software (D.01.02 16 version) was used to control the system. Separation was performed on a Carbowax 10M column (30 m x 0.25 mm i.d. x 0.5 µm). The oven temperature was programmed as follows: 40 ºC as initial temperature, held for 5 minutes. In a first ramp the temperature increased to 60 ºC at 1 ºC min^{-1} and, in the second, to 160 ºC at 5 ºC min^{-1}, then held for 1 minute. In a third ramp the temperature increased to 180 ºC at 20 ºC min^{-1}, then held for 2 minutes. Helium was the carrier gas (7 psi and 1mL min^{-1}). For the MS system, the temperatures of the manifold and transfer line were 150 and 230 ºC respectively; electron impact mass spectra were recorded at 70 eV ionization voltages and the ionization current was 10 µA. The acquisitions were performed in scan mode (from 35 to 450 m/z). Analyses were made in duplicate. The identification was carried out by comparison of the mass spectra of the peaks in the samples with those reported in the mass spectrum libraries, and using the reference compounds when possible. Moreover, linear retention indexes were experimentally calculated with an n-alkane mixture (C5-C30) and compared with those available in the literature. For quantification purposes, the relative area was obtained as the TIC signal of each aroma compound divided by the area of the internal standard. For those compounds whose standards were available, calibration curves in synthetic wines with each of the reference compounds (5 levels of concentration x 2 repetitions) were used, after checking the absence of significant matrix effects for most of the volatile analyzed by the comparison of the slopes of the regression curves obtained in the synthetic and real wines following the same methodology described by Rodriguez-Bencomo and collaborators [14]. A Semiquantification, considering that the response factor of the compound had the same value that the internal standard, was carried out when the reference standards were not available.

Triangle tests during the shelf-life of the wines

Triangle tests were carried out by a panel of 12 judges (6 men, 6 women, aged from 28 to 68) belonging to the staff of the Technical University of Madrid. They were previously trained in detection and recognition of tastes and odours, in the use of scales and in difference and ranking assessments according to the International Organization for Standardization ISO 8586-1 [15].

Three wine samples were presented to the judges identified by three-digit random codes. The order of presentation was randomly assigned for each judge, verifying that for the whole panel, presentation order of the samples was balanced. Wine (25 mL) was served in tulip-shaped ISO tasting glasses at a constant temperature of 12 ºC, and covered with plastic Petri dishes to allow the volatiles to equilibrate in the headspace. Tests were performed in a sensory lab provided with 16 individual booths and complying with usual requirements such as proper light and temperature control and isolation from noises and odours. No information about the aim of the study or about wine samples was given to the judges prior to the tests. Judges were asked to evaluate samples from left to right, looking for differences in aroma and taste. Judges were informed that two samples were identical and one sample was different. They had to select the odd sample. Judges rested between samples, rinsed their mouth with water and ate breadsticks when necessary. Triangle tests were performed throughout the shelf-life of wines, specifically, after 1, 2, 3 and 9 months of wine aging. Judges were given rewards and provided with positive feed back, as motivated judges are more focused and have better performance.

Descriptive analysis

The panel was composed by 3 men and 7 women aged from 24 to 68, belonging to the Technical University of Madrid. All conditions were identical to those described before.

Descriptive analysis of the two types of rosé wines was carried out in three 2-h sessions divided in training, training evaluation and wine evaluation.

Training. In the first training session, 12 representative attributes of Grenache wines were prepared at the highest concentration described in **Table 2** and presented to the judges. During this first training session, judges were first asked to smell the standards corresponding to the 12 attributes to familiarize themselves, and then, they were asked to rate the intensity of the wines for each attribute in an unstructured 15 cm line scale anchored at 1.5 cm from the end points of the line with the words "low" and "high". In this step, judges were introduced to the score card, the rating scale and procedure protocol of evaluation. This training period allowed choosing the attributes most representatives in both wines. At the conclusion of the first training period, 6 attributes were selected (*strawberry*, *peach*, *banana*, *floral*, *yeast*, *acidity*) (**Table 2**). The second and third sessions were focused on refining the standards and training the judges in using the terms consistently. To do so, aromas were presented at random at low and/or high concentration (**Table 2**), together with a form containing an unstructured 15 cm line scale as described before where the corresponding intensity was rated.

Training evaluation. Booths with 2 wine tasting glasses containing each of the 6 standard references at two concentrations (low and high) were prepared as explained before, and properly coded and covered with aluminium paper to avoid the influence of sample colour in the wine tasting evaluation. Judges were asked to determine the attribute and to rate the intensity of the standard in the same unstructured 15 cm line scale as described before. Training evaluation was done in duplicate, therefore each judge rated the 6 attributes at two concentrations twice, with the exception for acidity, for which judges had been previously trained for different sensory studies. Statistical evaluation of performance of the panel was done by two-way ANOVA, in order to discard attributes scores from judges not consistent with the whole panel for the subsequent sessions.

Wine evaluation. Wine evaluation was carried out after training and training evaluation. Both wines were identified by three digit random codes and the presentation order of the samples was randomly assigned and balanced for the whole panel. Judges rated each of the 6 attributes using the same unstructured 15 cm line explained before. First, they were asked to rate the intensity of each aroma attribute in both wines by the orthonasal way. Finally, they were asked to taste the wine and to rate the acidity for both wines.

Consumer tests

Hedonic evaluation of both types of wines (control wine and GSH-IDY wine) were investigated by a panel of consumers (n=64) belonging to the staff of our research institution (CIAL). The selection criteria were focused on consumers who generally enjoy rosé wines, with no ethical or medical reasons for not consuming alcohol. For this study consumers were recruited taking into consideration a balanced distribution by sex (56% male and 44% women). In addition most of them were aged from 21-34 (56%), while consumers aged from 35-49, 50-65 and older than 65 years old represented the 20, 17 and 6%, respectively. No specific information about the samples was given to consumers prior the study. As described before, samples were identified by three-digit random codes at constant serving temperature, using a randomised and balanced serving order across consumers. Consumers were asked to rate each wine for overall liking on a

9 point hedonic scale from “dislike extremely” to “like extremely”. Paper score-sheets were used for data collection.

Statistical analysis

Results corresponding to the concentration of volatile compounds in both types of wines throughout wine shelf-life were submitted to cluster analysis to provide a general view of the main factors involved on data variation (addition of GSH-IDY and aging time). In addition, one-way ANOVA was made to test the effect of aging time in each type of wine. Triangle tests results were analysed as described in ISO 4120 [16]. Data from the training evaluation for each sensory attribute were submitted to two-way ANOVA to determine the effect of the two studied factors (concentration and judges). Consistency of scores among judges was assessed by the interaction concentration x judge in order to guarantee that each attribute was perceived by the whole panel similarly. Data from the wine evaluation were submitted to one way ANOVA, using the t-test when differences in both wines were found. Data from the consumer tests were analysed by a mixed model, considering wines as fixed effect and consumers as random effect [17]. STATISTICA 7.1 (www.statsoft.com) and STATGRAPHICS Plus 5.0 (www.statgraphics.com) were used for data processing.

RESULTS AND DISCUSSION

Evolution of the volatile profile during the shelf-life of the wines

To determine the effect of the IDY-preparation on the volatile profile of the wines, we focused on the evolution of a wide range of volatile compounds (**Table 3**) belonging to different chemical classes: esters (ethyl esters of fatty acids and higher alcohol acetates), alcohols, terpenes, and terpenes derivatives, volatile fatty acids and other compounds such as the norisoprenoids β-damascenone and the aldehyde furfural. Most of them have a fermentative origin, although some terpenes were chosen because of their varietal origin. The concentration, calculated for the volatile compounds, was in agreement with other studies focused on the aroma of Grenache rosé wines [18-20]. As can be seen in **Table 3**, the concentration of many volatile compounds in wines aged 1 month was very similar in both types of wines. However, some esters, such as isoamyl, hexyl and 2-phenyl ethyl acetates and some long chain ethyl esters (octanoate, decanoate, dodecanoate) showed higher concentration values in the GSH-IDY-wine. In addition, the concentration of the three fatty acids (hexanoic, octanoic and decanoic) also showed higher concentration in the wines supplemented with the preparation.

To know if there was a natural grouping of the wine samples based on the addition of GSH enriched IDY during winemaking, a cluster analysis was performed with the data corresponding to the concentration of volatile compounds in both types of wines during their shelf-life (1, 2, 3 and 9 months old wines). The results are shown in **Figure 1**. As can be seen, the dendrogram is showing two separated groups of wines. The first one corresponded to wines of 3 and less than 3 months old, and the second one, included all the wines of 9 months. In addition, within each of these two large groups of samples, the figure is revealing a clear separation between wines depending on the addition or not of the GSH-IDY preparation. These results are showing a major influence of the aging time on wine volatile composition, but also an effect of the addition of the GSH-IDY preparation.

Taking into account these results, one-way ANOVA was made to test the effect of time in the volatile composition in each type of wine (**Table 3)**. As can be seen, differences in the evolution of the volatile compounds during the shelf-life of both types of wines were found. Most of the esters decreased during shelf-life in both type of wines, which might be associated to their slow hydrolysis at wine pH [21]. In addition, specific interactions between some esters with some components from the IDY preparations (glycopeptides) have been shown [22,23]. However, the higher concentration of esters in the 9 moth GSH-IDY wine compared to the 9 month control wines, might be related to the higher pool of these compounds available, because of the promotion of their production during the alcoholic fermentation due to the extra supplementation in nitrogen compounds by the IDY preparation [8,23,24]. In fact, the sum of free amino acids recently determined in the same wines after the alcoholic fermentation was two times higher in the GSH-IDY wine compared to the Control wine [9].

Moreover, the concentration of some terpenes, associated to citric and flowery notes, remained unchanged or even showed a slight increase during the aging of wines. Although during wine aging a slow oxidation of these compounds could have been accounted for, an increase in their concentration may also be possible as a consequence of their spontaneous synthesis from precursors naturally occurring in wines, as has been previously hypothesized [25] or, as in the case of linalool, because it can be formed from other monoterpenoids [26]. The slight increase of linalool during the shelf-life in wines supplemented with the GSH-IDY preparation compared to the control wines may indicate a lower oxidation of these compounds in these wines compared to the control wines. Recent research has also shown the antioxidant properties of the <5000 Da fraction isolated from GSH-IDY against some terpenes in synthetic wines submitted to accelerated aging conditions [10].

Contrary to most of the studied volatile compounds, fatty acids (octanoic and decanoic) increased in the control wines during aging, while remained practically unchanged in the GSH-IDY wines. In addition, significant differences were found between the two types of wines regarding the alcohol content. The concentration of all the alcohols, except benzenemethanol remained constant during shelf life in the GSH-IDY wines, while decreased in the control wines. This could be due to their oxidation to the corresponding aldehydes. Although the role of GSH-IDY preparations on the volatile compounds have not been studied so far, different authors have shown that the addition of glutathione to wines just before bottling at concentration above 20 mg L^{-1} might prevent the decrease of terpenic alcohols such as linalool [27,28] and aromatic esters [28,29] during the storage of wines. Previous research performed with the same wines [9] reported higher concentration of GSH in the GSH-IDY wines compared to the control wines. In fact, GSH-IDY wines showed a concentration of GSH about 16 mg L^{-1}, which was higher than the concentrations of GSH reported to have an antioxidant effect in synthetic wine [28]. However, in the above cited work, it has been showed that most of the GSH released from IDY is rapidly oxidized, so the protective effect of GSH on some volatile compounds might be very limited in winemaking conditions. Nonetheless, GSH released by the IDY preparations may also have had an effect in the must, protecting it from oxidation in the first steps during winemaking. In this case, wines might have a longer shelf-life due to the higher concentration of odour active esters and a better preservation of varietal aromas [30]. However it will be necessary in future works to check this hypothesis by systematically sampling during the fermentation step. Besides the differences noticed in the volatile profile between GSH-IDY and control wines, it was very important to know if these changes are also relevant for the sensory properties of the wines.

Triangle tests during the shelf-life of wines

Triangle tests were performed to find out if there were sensory differences between GSH-IDY and control wines during their shelf-life. Therefore, they were periodically performed (at 1, 2, 3 and 9 months) until sensory differences were perceived. The numbers of correct answers in each triangle test were five, six, four and eight for the 1, 2, 3 and 9 months wines respectively. Therefore, control and GSH-IDY wines were not perceived as different in the just finished wine (1 month wine) ($p \leq 0.05$) and neither during the early shelf-life of the wines (2 and 3 months) ($p \leq 0.05$). This is evidencing a slow evolution in the sensory characteristics of the wines during the first months of aging, which is in agreement with the little evolution of the volatile profile found during the three firsts months of aging (**Figure 1**). These results are indicating that in spite of the supplement in GSH and mainly in nitrogen compounds due to the addition of GSH-IDY preparations into the must [9], the impact of these preparations in the sensory characteristics of wines during the first stages of their shelf-life is relatively low. Different authors have shown that supplementation in nitrogen compounds to the must may affect the production of sulfur compounds [31], medium-chain fatty acid esters and acetic acid [32], whereas other authors claimed that must supplementation with ammonium brings about a decrease in sulphur notes and an increase in the citric flavour

[33]. Although the addition of GSH-IDY may slightly increase the volatile acidity of wines (**Table 1**), it did not provoke sensory differences among IDY wine and control wine after winemaking nor in wines aged 2 and 3 months. Wines were, however, perceived as different after 9 months of aging ($p \leq 0.05$), which also is in agreement with the highest differences found in their volatile profile.

Descriptive analysis

To determine which sensory attributes of Grenache wines were the most affected by the addition of the GSH-IDY preparation into the must, descriptive analysis was performed in the 9-month old wines (since, as was evidenced in the triangle test only after 9 months differences between the control and GSH-IDY wines were statistically significant). To do so, 12 sensory attributes of Grenache wines were selected on the basis of previous studies performed on the sensory characteristics of Grenache wines [34,18,19] and accordingly to the opinion of eight wine sensory experts. All the attributes were typical of rosé young Grenache wines, and they belonged to the fruity (*strawberry*, *peach*, *banana*, *apple* and *lemon* aromas), floral and vegetative (*grassy*) aromas. In addition, other attributes were chosen to evoke sweet aromas, such as *raisin*, *toffee* and *honey* aromas, since they can be found in some oxidized young wines [1,2,35,36]. *Yeast* aroma was also included because it has been associated to wines supplemented with IDY in a previous work [11]. Finally, *acidity* was also evaluated as a taste attribute because is a typical characteristic of young wines.

After the first training session, only those attributes marked above 4, in the 15 cm-scale at least in one of the wines under study were selected. These attributes were *strawberry*, *peach*, *banana*, *yeast* and *floral* aromas, and acid taste. The fact that judges did not score higher than 4 the attributes *honey, toffee* or *raisin,* indicated the low presence of sweet-aroma-related notes and therefore, the low grade of oxidation in these wines.

Once the first training session was concluded, a specific training in the selected attributes at two concentrations was carried out, as has been recommended by Noble and Lesschaeve [37]. .A training evaluation was carried out in order to verify the correct training of the panel, and also to detect those judges who were using an inconsistent term respect to the other subjects. All the data from the training evaluation were submitted to analysis of variance (two-way ANOVA). Interaction plot revealed that judges 1 and 10 did not properly rate the intensity of *strawberry* and *banana* aromas, and consequently, their scores for these attributes were removed from the training and wine evaluations. **Table 4** showed the F-ratios of concentration, judge and concentration x judge of the ANOVA without taking into account the scores of judges 1 and 10 in the attributes *strawberry* and *banana*, respectively. As can be seen, the concentration was significantly different for all the studied attributes, whereas, practically no significant effect was found for judges and concentration x judge. Concentration x judge was not obtained for acidity as the judges evaluate it only once. Therefore, it can be concluded that in general, the two concentrations for each attribute were perceived as different and all the judges used the same part of the scale and rated the attributes in a similar way. Then, the panel was considered as reliable and consistent with respect to all the attributes, thus well-trained in these descriptors to carry out the wine evaluation.

The wine evaluation was performed once (in both types of wines) in a single session once the consistence of the panel was tested. Analysis of variance (ANOVA) was performed in each attribute to determine if wines were perceived as different, and least significant differences between wine means were computed by a t-test. **Table 5** shows F-ratios and p-values of each attribute, discarding the scores for *strawberry* and *banana* of judges 1 and 10. The attributes significantly different in both wines are presented in bold in the table. In addition, the mean intensity rating for control and GSH-IDY wines have been plotted in a cobweb graph to get a sensory profile of each type of wine (**Figure 2**). In this diagram, the centre of the figure represents the lowest intensity with respect to each descriptor increasing to an intensity of 15 at the end of the axes (corresponding to the maximum rating in the 15 cm unstructured scale). As can be seen in **Table 5,** acidity was rated the same in the control and GSH-IDY wine**.** As it can be expected, acidity had the same intensity in both wines, as there was no evidence that the GSH-IDY addition may modify the acidity of wines. In spite of having different concentrations in volatile compounds typically associated to flowery notes, such as 2-phenylethyl acetate [18, 38], both wines presented similar intensities in floral aroma. Regarding fruit attributes, GSH-IDY wine exhibited almost the double intensity in *strawberry* notes (1.98 times more) and also in the *banana* attribute (1.58 times more) than the control wine. These attributes can be related to a higher concentration of esters related to fruity aroma in the 9 months GSH-IDY wine compared to the control wine. For instance, the concentration of isoamyl acetate, a volatile compound typically associated to *banana* flavour was 446 mg L^{-1} in the 9-month GSH-IDY wine while it was of 189 mg L^{-1} in the control wine. However, control wines were more intense in *peach* aroma. The *yeast* aroma attribute was included in this study because it has been previously shown that the sensory profile of IDY preparations might include odorant compounds with *yeast-like* notes [11]. In the above mentioned work, authors showed that *yeast-like* notes may mask some typical varietal aromatic notes in wines. Therefore, its presence in young wines may decrease the aroma quality. However, in the present work, GSH-IDY wines were rated lower in *yeast-like* notes compared to the control wine. The possible release of other odorant molecules, such as pyrazines present in these preparations [11,39] and typically associated to *roasted, toasted, popcorn* aromatic notes may have masked the characteristic typical yeast odour associated to fermentation yeast, although in this work, the amount of IDY added to the musts was not very high (2 mg L^{-1}) and it has been shown that the appearance of the *yeast-like* notes is associated to a higher dose of IDY in wines (150-600 mg L^{-1}) [11]. Finally, it is important to emphasize that during the training, the panel identified the *yeast* aroma as an off-flavor, being related to sulphur-like aroma. Therefore, the higher intensity in *yeast* aroma in the control wine might have been perceived by the panel as a symptom of lower aroma quality compared to the GSH-IDY wine.

Consumer tests

Finally, consumer tests were carried out in order to determine if wine consumers could perceive preferences towards some of the wines. On a 9 point hedonic scale, consumers rated their liking of the control and GSH-IDY wines in 6.12 and 5.92 respectively, which indicated that the acceptability for both types of wines was in general good. However, no significant differences in consumer preferences were found between both types of wines, and neither when the sex or the age of the consumers were taken into consideration (data not shown). These results showed that consumers did not evidence preference patterns towards wines made with GSH-IDY addition. Nevertheless, a greater consumers sample size could improve both, an increase of discrimination power between wines and the representativeness of the consumers population, indicating a future line of research to be explored.

CONCLUSIONS

The addition of glutathione enriched IDY preparations into Grenache musts during winemaking has an impact on the volatile profile of young rosé wines during aging that can be responsible for sensory differences in the later stages of wine shelf-life (above 9 months). In general, wines supplemented with a glutathione enriched IDY preparation are more intense in typical fruity attributes of young rosé wines (*banana, strawberry*), which could be related at least in part by the protection of some aroma compounds against oxidation, likely in the first steps during winemaking. However, the changes in the sensory profile could be also related to other effects linked to the addition of IDYs into wines, such as the release of volatile compounds and/or the effect of yeast macromolecules on aroma volatility. In addition, the influence of IDY in the fermentation might have change yeas metabolic by-products inducing changes in wine sensory characteristics. Nonetheless, the sensory effect is not evident enough to show consumer preferences towards GSH-IDY wines. Finally, although the use of industrial manufacturing conditions has allowed to us a valuable study of the use of GSH-IDY preparations in real winery conditions, new research, using more wine samples with other GSH-IDY preparations and industrially manufactured is necessary, in order to fully understand the chemistry beyond the use of these preparations, during winemaking.

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C-1: Include complete journal titles in all cases?

Done

C-2: Include couple of recent references (last 2 years) from IJFP?

Sorry, but we did not find any recent article published in the IJFP related with the topic of the present manuscript.

Table 1. Evolution of global composition in the must, control wine and in the wine supplemented with the glutathione enriched IDY preparation.

		pH	TA[a]	PAD[b]	AD[c]	VA[d]
Must		3.2	3.7	13.9	-	-
Cont-W	After alcoholic fermentation	3.13	4.2	-	13.8	-
	Stabilized and clarified wine	3.15	3.4	-	13.75	0.16
GSH-IDY-W	After alcoholic fermentation	3.18	4	-	13.6	-
	Stabilized and clarified wine	3.2	3.25	-	13.5	0.22

Cont-W: Control wine; GSH-IDY-W: Wine supplemented with the glutathione enriched IDY preparation.

[a]: Total acidity (g. sulphuric acid/L)
[b]: Probable alcohol degree (% v/v)
[c]: Alcohol degree (% v/v)
[d]: Volatile acidity (g acetic acid/L)

Table 2. Reference standard composition of aroma and taste attributes

Attributes	Reference standard composition[a] Low concentration	High concentration
Strawberry	1.5 g of crushed fresh strawberries	6 g of crushed fresh strawberries
Peach	2 mL of peach nectar	7.5 mL of peach nectar
Banana	¼ 10 mm slice fresh banana	10 mm slice fresh banana
Apple	-	Slice fresh apple, 5 mL apple juice
Lemon	-	5 mL lemon juice, and small peel piece of fresh fruit
Floral	0.2 ml of linalool solution (150 mg/L)	1.5 mL of a linalool solution (150 mg/L)
Grassy	-	1 mL of a cis-3-hexen-1-ol solution (100 mg/L)
Toffee	-	1 toffee candy
Raisin	-	2-3 crushed fresh raisins
Honey	-	8 mL honey
Yeast	0.25 g baker yeast	1 g baker yeast
Acidity	0.2 g/L citric acid in water	0.8 g/L citric acid in water

[a]: references were prepared in tasting glasses filled with 25 mL of rosé base wine, covered by petri dishes, with the exception for acidity that was prepared in water. Attributes in bold were finally selected for the study.

Table 3. Concentration of volatile compounds (mean ± standard deviation, μg L^{-1}) determined in the control wines (Cont-W) and in the wines supplemented with the G-IDY preparation (GSH-IDY-W) at 1, 2, 3 and 9 months of aging (1m, 2m, 3m and 9m, respectively)

					Cont-W				GSH-IDY-W			
	Compounds	RIexp†	Rilit‡	Id§	1 m	2 m	3 m	9 m	1 m	2 m	3 m	9 m
Esters	Ethyl propanoate	920	903	S, R, M	43.9^{b}±2.6	46.3^{b} ±5.3	$39.5^{b,a}$±6.9	26.8^{a} ±2.6	26.5^{a}±0.6	29.3^{a}±1.2	33^{a}±0.1	30.9^{a}±5.1
	Isobutyl acetate	975	953	S, R, M	4.5^{b}±0.4	4.1^{b}±0.94	3.3^{b}±0.7	1.4^{a}±0.2	5.0^{b}±0.3	4.5^{b}±0.0	4.6^{b}±0.1	2.7^{a}±0.3
	Ethyl butanoate	1010	1010	S, R, M	240.7^{b}±12.6	225.2^{b}±41.6	200.7^{b}±38.8	103.2^{a}±22.6	237.8^{b}±7.2	229.9^{b}±4.9	242.0^{b}±2.1	173.2^{a}±18.9
	Ethyl 2-methylbutanoate	1026	1031	S, R, M	2.5^{b}±0.1	2.4^{b}±0.4	2.7^{b}±0.5	2.5^{a}±0.2	1.7^{a}±0.1	$2^{a,b}$±0.1	$2.2^{b,c}$±0.1	2.6^{c}±0.2
	Isoamyl acetate	1115	1117	S, R, M	573.7^{b}±16.6	479^{b}±75.1	390.1^{b}±79.5	188.6^{a}±27.0	811^{c}±22.5	786.2^{c}±1.2	730.6^{b}±3.7	445.9^{a}±17.3
	Ethyl hexanoate	1229	1230	S, R, M	710.3^{b}±6.8	582^{b}±70.8	574.6^{b}±106.8	310.7^{a}±28.6	706^{b}±13.1	722.4^{b}±7.4	716.6^{b}±7.5	467.1^{a}±28.3
	Hexyl acetate	1267	1269	S, R, M	130.7^{b}±2.06	110.1^{b}±14.3	97.7^{b}±19.1	44.9^{a}±4.7	219.6^{c} ±6.3	213^{c}±0.5	194.6^{b}±1.9	114.5^{a}±3.5
	Ethyl heptanoate	1327	1332	R, M	2.1^{b}±0.1	1.8^{b}±0.2	1.9^{b}±0.4	1.1^{a}±0.2	1.4^{b}±0.1	1.5^{b}±0.0	1.4^{b}±0.1	0.8^{a}±0.1
	Ethyl octanoate	1429	1431	S, R, M	1678.8^{b}±306.8	1745.1^{b}±146.2	1788.4^{b}±145.8	666.1^{a}±31.7	2097.7^{b}±8.4	2104.3^{b}±9.1	2197.4^{c}±14.7	1046.1^{a}±13
	Ethyl nonanoate	1530	1541	S, R, M	1.9^{a}±0.7	3.8^{a}±0.2	4.6^{a}±0.2	4.40^{a}±2.4	2.9^{a}±0.1	3.6^{b}±0.0	4.1^{b}±0.2	2.4^{a}±0.4
	Ethyl decanoate	1634	1634	S, R, M	$511.9^{a,b}$±253.0	883.5^{c} ±37	$864.3^{b,c}$ ±47.1	270^{a} ±15	931.6^{b}±55	960.3^{b}±12.7	1045.2^{b}±56.9	398.4^{a}±44.3
	Diethyl succinate	1673	1694	S, R, M	515.3^{a} ±62.7	492.4^{a}±5.8	788^{b} ±97.4	1035.8^{b}±150.8	279.1^{a}±17.1	300^{a}±21.3	436.4^{a}±33.6	800.2^{b}±230.2
	2-Phenyl ethyl acetate	1809	1752	S, R, M	49.4^{b}±1.3	53.3^{c}±0.4	53.6^{c}±0.4	42.6^{a}±1.9	89.4^{a}±5.8	84.2^{a}±3.7	95.6^{a}±0.6	63.7^{a}±23.3
	Ethyl dodecanoate	1840	1833	S, R, M	36.8^{a}±15.0	72^{a}±1.8	49.9^{a}±5.9	97.1^{a}±40.8	82.3^{a}±15.5	65.7^{a}±8.5	52.4^{a}±4.9	63.5^{a}±12.7
Alcohols	1-Butanol	1141	1157	S, R, M	394.8^{b}±9.9	380.6^{b} ±65.7	$343.1^{a,b}$±39.2	226.9^{a}±37.5	333.7^{a}±8.3	310.7^{a}±10.5	361.5^{a}±10.5	322.4^{a}±66.9
	1-Hexanol	1353	1356	S, R, M	1255.6^{b}±100.6	$1122.7^{a,b}$±170.9	$1102.7^{a,b}$±215	756.4^{a}±116.8	864.6^{a}±17.3	718.7^{a}±15.4	877.9^{a}±22.4	893.6^{a}±211.8
	Cis-3-hexenol	1361	1370	S, R, M	44.4^{b}±3.4	$40.7^{a,b}$±5.1	$40.3^{a,b}$±6.9	28.4^{a}±2.5	38.4^{a}±1.1	31.2^{a}±0.2	39.7^{a}±0.3	37.3^{a}±8.8
	Trans-3-hexenol	1378	1370	S, R, M	58.6^{b} ±2.2	61.5^{b}±1.0	57.2^{b}±6.6	39.3^{a}±5.8	69^{a}±0.1	59.6^{a}±1.3	73.0^{a}±1.2	68.6^{a}±15.7
	Benzenemethanol	1880	1834	S, R, M	$79.6^{a,b}$±7.0	68.4^{a}±0.9	$83.6^{a,b}$±9.7	86^{b} ±3.4	77.8^{a}±3.2	71^{a}±6.7	97.4^{a}±8.0	96.9^{a}±33.5
Terpenes	Limonene	1179	1180	S, R, M	0.4^{a}±0.0	0.3^{a}±0.0	0.4^{a}±0.0	1.1^{a}±0.6	0.5^{a}±0.2	0.3^{a}±0.0	0.4^{a}±0.0	0.5^{a}±0.1
	α-terpinene	1494	-	M	1.1^{a}±0.1	1.2^{a}±0.1	$1.40^{a,b}$±0.0	1.6^{b}±0.2	0.8^{a}±0.0	0.7^{a}±0.1	$1.0^{a,b}$±0.1	1.3^{b}±0.2
	Linalool	1547	1541	S, R, M	3.3^{a}±0.7	3^{a}±0.3	3.6^{a}±0.5	3.3^{a}±0.5	2.6^{a}±0.2	2.6^{a}±0.0	$3.3^{a,b}$±0.1	4.3^{b}±0.8
	Citronellyl acetate	1657	1666	R, M	$1.9^{a,b}$±0.5	2.2^{b} ±0.2	$2.1^{a,b}$±0.2	1.4^{a}±0.2	2.3^{b}±0.0	$2.1^{a,b}$±0.6	$2.0^{a,b}$±0.5	1.2^{a}±0.1
	β –Citronellol	1767	1781	S, R, M	4.8^{a}±1.2	4^{a}±0.1	4.5^{a}±0.6	4.8^{a}±0.1	3.9^{a}±0.3	3.3^{a}±0.2	4.2^{a}±0.2	4.0^{a}±0.9
	Isopropyl myristate	2035	2040	R, M	0.3^{a}±0.3	0.3^{a}±0.1	0.3^{a}±0.0	0.1^{a}±0.0	$0.2^{a,b}$±0.0	0.4^{c}±0.0	$0.3^{b,c}$±0.1	0.1^{a}±0.0
Fatty acids	Hexanoic acid	1859	1789	S, R, M	4821.8^{a}±643.4	3411.1^{a}±91.7	4812.9^{a}±683.2	3689.1^{a}±527.2	5097.7^{a}±117.6	4988.2^{a}±152.8	5125.4^{a}±1016	6153.9^{a}±1545.1
	Octanoic acid	2078	1998	S, R, M	2383.2^{a}±188.4	2247.1^{a}±39.7	2858.2^{b}±57.9	3393.4^{c}±191.2	3240.5^{a}±194.5	3335.9^{a}±87.7	3289.6^{a}±226.0	3731.0^{a}±1280.8
	Decanoic acid	2289	2279	S, R, M	438^{a}±4.2	$509.5^{a,b}$±47.4	585.6^{b}±32.2	739.5^{c}±29.1	679.9^{a}±4.6	720.3^{a}±67.0	802^{a}±16.7	597.3^{a}±281.9
Others	2,3 butanedione	937	949	S, R, M	258.7^{a}±51.6	309.1^{a}±61.4	280.8^{a}±59.8	198.1^{a}±17.8	390.2^{c}±1.6	400.3^{c}±21.0	310.5^{b}±24.2	92.8^{a}±21.9
	Furfuraldehyde	1459	1449	S, R, M	3^{a}±0.3	$4.5^{a,b}$±0.4	5.6^{b}±0.1	10.7^{c} ±1.3	2.9^{a}±0.4	3.3^{a}±0.3	3.3^{a}±0.6	4.0^{a}±0.9
	γ -butyrolactone	1613	1595	S, R, M	5644.3^{b}±400.4	3625.8^{a}±401.9	5561.9^{b}±997.3	3579.8^{a}±486.7	3411.7^{a}±433	2785.5^{a}±339.5	3252.8^{a}±552.7	3074.3^{a}±807.8
	Methionol	1709	1714	S, R, M	774.9^{a}±15.4	613.3^{a}±7.7	804.5^{a}±217.4	606.2^{a}±15.7	380.2^{a}±42.7	324.5^{a}±97	493.2^{a}±64.8	381.9^{a}±201.0
	β -damascenone*	1809	1752	S, R, M	6^{a}±0.3	$6.5^{a,b}$±0.4	7.4^{b}±0.3	7.3^{b}±0.7	6.5^{a}±0.4	7^{a}±0.4	$8.^{a}6$±0.1	7.9^{a}±2.2

† Retention index calculated by SPME with an alkane mixture (C5-C30)
‡ Retention index reported in the literature from Flavornet database: http://www.webbook.nis.gov/chemistry
§ Identification method: S, identification by comparison with standard compounds; RI, identified by retention index; MS, identified by mass spectra (NIST libraries)
Different supercripts denote statistical differences ($p<0.05$) in the values in the same row for each type of wine

Table 4. Results from the two-way ANOVA (concentration, judge, concentration x judge) and F-ratios of the sensory terms evaluated by the 10 judges during training in 6 attributes at 2 different concentrations.

Attributes	Concentration	Judge	Concentration x judge
Acidity	162.00***	0.22	-
Banana	1699.54***	1.05	1.53
Floral	1077.5***	1.26	1.68
Peach	98.92***	0.20	1.98
Strawberry	2366.46***	2.78*	9.5***
Yeast	116.55***	1.02	2.28

* and ** *denote significance at $p<0.05$ *and* $p<0.001$ respectively

Table 5. Results obtained on the descriptive analysis by the panel of judges (n=10) of the 6 sensory attributes evaluated in the control (Cont-W) and GSH-IDY wines (GSH-IDY-W) after 9 months of aging

			Mean	
Attributes	F-ratio	p-value	Cont-W-9m	GSH-IDY-W-9m
Acidity	0.00	0.9944	7.72	7.71
Banana	**3.23**	**0.0911**	**4.51**	**7.16**
Floral	0.17	0.6875	7.59	8.24
Peach	**4.07**	**0.0589**	**7.65**	**4.81**
Strawberry	**8.13**	**0.0116**	**4.02**	**7.87**
Yeast	**11.46**	**0.0038**	**4.31**	**1.91**

Judges 1 and 10 not consistent with the whole panel were excluded from data analysis of strawberry and banana attributes**.** Attributes in bold were significantly different between wines.

Figure Captions

Figure 1. Dendrogram resulting from the application of cluster analysis to the data corresponding to the concentration of volatile compounds determined in the wines of different aging time (1, 2, 3 and 9 months) made with or without the addition of a glutathione enriched IDY preparation (G-IDY-W and Cont-W, respectively)

Figure 2. Aroma profiles of Grenache rosé wines in the control wine (Cont-W) and in the wine supplemented with a glutathione enriched IDY preparation (GSH-IDY-W)