ROLE OF GLUTATHIONE ENRICHED INACTIVE YEAST PREPARATIONS ON THE AROMA OF WINES

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RESUMEN

El efecto de preparados comerciales de levaduras secas inactivas ricos en glutatión (GSH-IDY) en la protección del aroma del vino durante su conservación ha sido investigado. Para ello, se elaboraron industrialmente dos vinos rosados a partir de uvas de la variedad Garnacha con y sin adición del preparado de GSH-IDY. La composición volátil se determinó a lo largo de la vida útil del vino (1 a 9 meses). Además en experimentos en vinos modelo, se estudió el efecto de diferentes tipos de preparados sobre compuestos volátiles representativos del vino. Los resultados mostraron que el empleo de los preparados reducen la pérdida de volátiles durante la vida útil del vino. En estos experimentos se observaron diferencias en el comportamiento de los preparados, que pueden ser debidos a la capacidad antioxidante del GSH pero también a la activación de diferentes tipos de reacciones químicas promovidas por la presencia de otros componentes en los preparados de levadura.

ABSTRACT

The effect of commercial glutathione enriched winemaking Inactive Dry Yeast preparations (GSH-IDY) to protect wine aroma during storage has been investigated. To do so, rosé Grenache wines were industrially manufactured with and without the addition of a GSH-IDY preparation. The volatile composition was determined over the shelf-life of the wines (1 to 9 months). In addition, by using experiments in model wines the effect of different types of commercial GSH-IDY preparations on representative wine aroma compounds was carried out. The results showed that these preparations reduced the lost of volatile compounds during the shelf-life of the wines. In the model wines we observed differences in the behaviour of the IDY preparations towards the aroma compounds, which may be due to the antioxidant capacity of the GSH but also on the activation effect of different types of chemical reactions promoted by other components from the IDY preparations.

INTRODUCTION

Currently, the use of winemaking Inactive Dry Yeast preparations (IDY) is gaining interest within the wine industry because of their large amount of potential applications in winemaking. Although they have been mainly used for the improvement of alcoholic and malolactic fermentation, the use of IDY for enhancing wine's sensory characteristics, is one of the most promising and interesting applications (Pozo-Bayon *et al*, 2009a).

The impact of IDY in wine's sensory properties is due to the ability of yeast components to modify wine chemical composition. As a matter of fact, it has been shown that yeast polysaccharides are able to protect wine colour, because of the interaction of yeast mannoproteins with tannins and anthocyanins, therefore, avoiding or minimising polyphenol aggregation and precipitation (Escot *et al*., 2001; Doco *et al*., 2003). In addition, we have shown that some yeast macromolecules released from IDY may affect the volatility of important wine aroma compounds (Pozo-Bayon *et al*., 2009c), which could be related to the sensory differences observed in wines supplemented with these preparations compared to control wines (Comuzzo *et al*., 2006). Moreover, the ability of IDY to release nitrogen heterocyclic volatile compounds, likely formed as a consequence of the thermal reactions accounted for in the last steps during their production has been also shown (Pozo-Bayón *et al.,* 2009b).

Besides of the above mentioned effects of IDY on wine aroma, there are currently in the market, other types of IDYs, which have been claimed to preserve aroma composition during wine storage. The protective effect of these preparations has been associated to the presence of a large amount of glutathione (GSH). This compound is a yeast intracellular tripeptide from non proteic origin, of known antioxidant properties (Penninckx, 2002). Although the use of free glutathione has been shown may avoid the oxidation of some volatile compounds in white wines (Lavigne-Cruege *et al*., 2003), the use of commercial IDY preparations enriched in GSH and their effect on wine aroma composition during wine shelf-life has not been investigated so far.

Therefore, the **objectives** of this work were firstly to study the effect of a commercial GSH enriched IDY preparation on the volatile composition of industrially manufactured rosé wines from Grenache grape variety, and secondly, by using experiments in model wines, to compare the effect of different types of commercial GSH enriched IDY preparations on representative wine aroma compounds.

MATERIAL AND METHODS

Wines and winemaking conditions

Two Rosé wines were made from the same Grenache must in 10000 L tanks in a wine Cellar from *Navarra* O.D. Both wines were from the 2008 vintage. One of the tanks was supplemented with a glutathione enriched yeast preparation (IDY-G) whereas the other tank corresponded to the control wine without IDY (IDY-C). IDY was added to the wines at the concentration recommended by the provider (20 g/Hl), before the alcoholic fermentation took place. Fermentation was carried out by using the same active dry yeast in both tanks. Once alcoholic fermentation was completed, wines were stabilized, clarified and bottled in the own vinery. The wines were storage at 12 ºC during their whole shelf-life.

Model wines

Model wines were prepared by mixing 12% ethanol (v/v) and 4 g/L of tartaric acid. The pH was adjusted to 3.5 with NaOH . Fifty mL of model wine were supplemented with 100 µL of the <3000 Da water fraction previously isolated from 4 g of an IDY preparation. In total three different model wines supplemented with the above

mentioned fractions from IDY were prepared: Two of them with two commercial GSH enriched preparations G1 and G2 (MW-IDY-G1 and MW-IDY-G2) and the third one, was prepared using an IDY usually recommended as a fermentative nutrient (MW-IDY-N1). Control model wines were prepared in the same way but without the addition of the <3000 Da water fraction from the IDY (MW-IDY-C). An aroma solution (0.1 mL) containing typical wine aroma compounds was added into the three types of model wines to obtain the following concentrations of each aroma: geraniol (0.25 mg/L), β-citronellol (0.25 mg/L), ethyl butyrate (0.25 mg/L), ethyl hexanoate (0.15 mg/L), ethyl octanoate (0.15 mg/L), ethyl-decanoate (0.08 mg/L), isobutyl acetate (0.25 mg/L), isoamyl acetate (0.25 mg/L), hexyl acetate (0.5 mg/L) and β-phenyl ethyl acetate (0.25 mg/L). The flasks containing each of the model wines were immediately sealed and kept at 30ºC in stirring conditions until the moment of their analysis to promote their oxidation. The analysis of the aroma compounds was performed after 14 days. All the experiments were performed in duplicated in independent flasks for each type of model wine.

Analysis of volatile compounds by HS-SPME-GC-MS

HS-SPME was used to study the effect of IDY in both, industrially manufactured and model wines, following the method described in Andujar *et al*., (2009) with some modifications. To do so, 8 mL of real or model wines were placed in a 20 mL headspace vial sealed with a PTFE/Silicon septum. For real and model wines methyl nonanoate was used as internal standard. Samples were allowed to reach equilibrium at 40 ºC. The extraction was performed with an automatic autosampler by the exposure of an 85 µm Carboxen-PDMS fiber to the headspace of the sample for 20 minutes at 40 ºC. After the extraction, the fiber was removed from the sample vial and desorbed in splitless mode in the GC injector port for 10 minutes. All the analyses were performed in duplicate.

Separation was performed on a Carbowax 10 M 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 and in the second at 5 ºC/min to 160 ºC then held for 1 minute. In a third ramp the temperature increased to 180 ºC at 20 ºC/min, then held for 2 minutes.

Calibration curves with each of the reference compounds (5 levels of concentration x 2 repetitions) were used for quantitative purposes. The TIC signal of each aroma compound in the headspace of the samples compared to that of the internal standard was used to extrapolate in the calibration curves.

RESULTS

Effect of the addition of glutathione enriched IDY preparations on the volatile composition of wines industrially manufactured.

By using HS-SPME-GCMS, 33 volatile compounds were identified in the wines. Most of them belonged to the 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 TDN and the aldehyde furfural. Most of them have a fermentative origin, although some terpenes identified in the wines (α-terpinene, linalool, etc) came from the grape. All of them also showed concentration values within the normal values for Grenache rosé wines

To know if there was a natural grouping of the wine samples based on the addition of GSH enriched IDY into the wines, 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 wines). The results are shown in **Figure 1**. As can be seen in this figure, the dendrogram is showing two very well separated groups of wines. The first one corresponded to wines of 3 and less than 3 months, 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 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 IDY preparation on these compounds.

Taking into consideration these results, an ANOVA analysis to check the effect of both factors (aging time and addition of IDY) and their interactions was performed with the 33 volatile compounds quantified in the samples. The results showed that most of them were influenced by both factors. **Table 1** lists the volatile compounds which were statistically influenced by one of the two factors (or by the interaction between them). To better understand the effect of the IDY preparation over the time, **Table 1** is showing the ratios (in percentage) corresponding to the change in the concentration of a volatile compound in the 9 months wine compared to the concentration in the 1 month sample. This ratio allowed us to know the effect of the IDY preparation on the volatile composition over the shelf-life of the wine.

The results showed that in general, control wines showed a larger decrease in most of the volatile compounds compared to the wines supplemented with the IDY preparations. This was particularly evident for the compounds belonging to the ester group as it is shown in **Table 1.** The lost of this group of compounds during wine aging, has been associated to their slow hydrolysis at wine pH (Rapp and Mandery, 1986). In addition, some alcohols such as 1-butanol and 1-hexanol also decreased in a greater extend in the control wine than in the wine supplemented with IDY. In addition, the concentration of some terpenes remained unchanged or even showed a slight increased during the aging of control wines. However, this increase was much larger in wines supplemented with the IDY preparation. 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 spontaneous synthesis from a precursor naturally occurring in wines (Jarauta *et al.*, 2005) or, as in the case of linalool, because it can be formed from other monoterpenoids (Pedersen *et al*., 2003). The higher increased of these compounds along the shelf-life in wines supplemented with the IDY preparation compared to the control wines, may indicate a lower oxidation of these compounds in these wines compared to the control wines. Moreover, it is interesting to underline the little change in concentration experienced by some volatile compounds associated to wine oxidation such as furfural in wines supplemented with IDY compared to control wines.

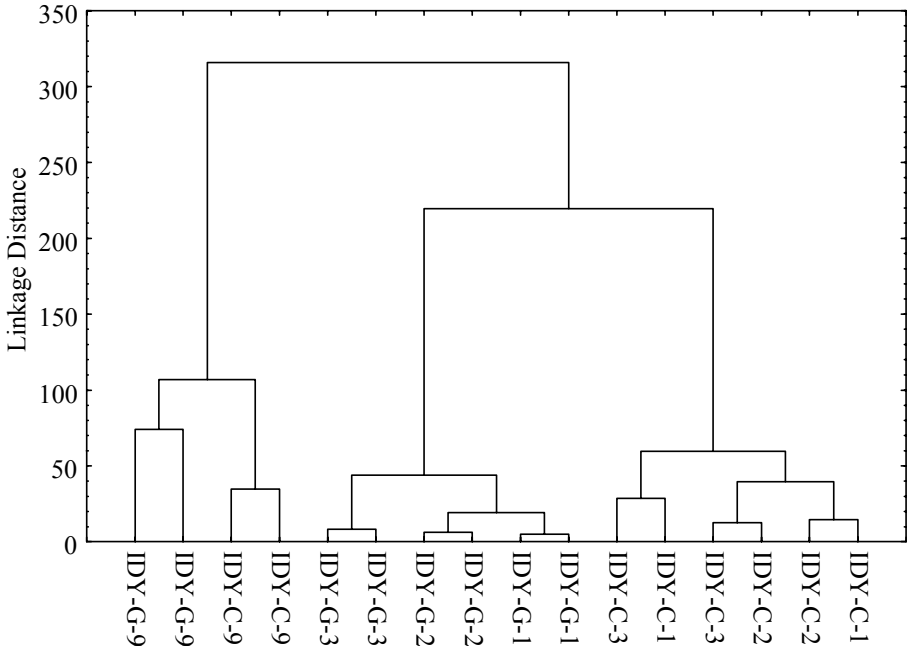

Figure 1. Dendrogram resulting from applying cluster analysis to the data corresponding to the concentration of volatile compounds determined in the wines of different aging time (1, 2, 3 and 9 months) made with or without the addition of a glutathione enriched IDY preparation (IDY-G and IDY-C respectively). (Notice that two repetitions of each wine are included).

Effect of the addition of commercial glutathione enriched IDY preparations on typical wine aroma compounds in synthetic wines under oxidation conditions

Synthetic wines aromatised with typical wine aroma compounds were supplemented with the three types of IDY preparations as was described in the M&M section. Two of them with two commercial GSH enriched preparations (MW-IDY-G1 and MW-IDY-G2) and the third one with a nutrient-type IDY (MW-IDY-N1). A control wine without IDY addition was also prepared. Wines were submitted to oxidation conditions (30 ºC stored for 14 days). The results of this study are presented in **table 2**. In the control wine (MW-IDY-C), a decrease in the concentration of the aroma compounds ranging between 10% (isobutyl acetate) and 91% (ethyl decanoate) and due to the oxidation process was observed. To know the effect of the IDY preparations on each aroma compound, the relative peak area of a compound in the sample with the preparation was compared to the peak area of the same compound in the control sample and the results were expressed as it is shown in the footnote of table 3. Therefore, in table 2, positive ratios means an antioxidant effect, while negative values shows that this compound was more oxidized in the sample with the preparation than in the control wine. As can be seen, the influence of each IDY depended on the type of IDY but also on the physical-chemical characteristics of the volatile compounds. In the MW-IDY-N1 in which the concentration of free GSH from the IDY was lower than 0.01 mg/L, the IDY mainly promoted the oxidation of the three terpenes, which may be due to the presence in the extract of some trace compounds, such as Fe, which could facilitate the oxidation processes (Ferreira et al, 1997). However, in other two model wines, MW-IDY-G1 and MW-IDY-G2, which contained above 2.5 mg/L of free GSH from the IDYs, two

different effects were observed. While preparation IDY-G2 showed antioxidant properties for the three terpenes, IDY-G1 only showed antioxidant effect for geraniol. In the case of esters, ethyl butyrate and isobutyl, isoamyl and β-phenylethyl acetates presented higher concentrations in the three model wines supplemented with the IDY compared to the control wines. However, the change in their concentrations may be also due to hydrolysis or esterification reactions or by hydroxyl radical oxidation reactions (Escudero *et al.*, 2000).

Tabla 1. Values showing the comparison (in percentage) between the concentration of volatile compounds in the 9 months wines compared to the concentration in the 1 month wines. (IDY-C: control wines; IDY-G: wines supplemented with the GSH enriched IDY).

Compounds	Wine type	
	IDY-C	IDY-G
Esters		
Ethyl propanoate	61	100
2-Methyl propanol acetate	31	53
Ethyl butanoate	43	73
2-Methyl etanol butanoate	100	148
Isoamyl acetate	33	100
Ethyl hexanoate	44	66
Hexyl acetate	34	53
Heptyl heptanoate	50	59
Ethyl octanoate	100	50
2-Phenyl etanol acetate	86	100
Alcoholes		
1-Butanol	57	100
1-Hexanol	60	100
cis-3-Hexen-1-ol	67	100
Terpenes		
α-terpinene	146	170
Linalool	100	165
Citronelol acetate	100	53
Fatty acids		
Octanoic	142	100
Decanoic	169	100
Other compounds		
2,3-Butanedione	100	24
Furfural	359	100
γ-Butirolactone	63	100

*Only compounds that showed a significant influence ($p < 0.05$) by one of the two studied factors are included.

Table 2. Results corresponding to the effect of IDY on the aroma composition of model wines submitted to oxidation conditions. Results are expressed in % of change in the concentration of each volatile compared to the control wine

	Model Wines		
Aroma compounds	MW-IDY-N1	MW-IDY-G1	MW-IDY-G2
Terpenes			
β-Citronellol	-13	-2	15
Nerol	-23	-24	21
Geraniol	-8	5	36
Esters			
Isobutyl acetate	21	32	19
Isoamyl acetate	8	12	3
Hexyl acetate	-3	-2	-8
β-Phenylethyl acetate	-4	17	26
Ethyl butyrate	14	22	10
Ethyl hexanoate	-1	-2	-10
Ethyl octanoate	-12	-11	-26
Ethyl decanoate	-43	-49	-53

Values are calculated as: [Area compound in the sample with IDY/ Area compound in the control sample * 100]-100

CONCLUSIONS

The use of GSH-IDY preparations during the winemaking of rosé Grenache wines reduce the lost of volatile compounds along their shelf-life. The studies in synthetic wines also showed lower oxidation of some important aroma compounds such as terpenes when the <3000 Da fraction from GSH enriched IDY preparation was added into the wines. This effect seems to be related to the amount of GSH originally present in the IDY and effectively release into the wines. In addition, an effect of these fractions was also observed on other volatile compounds (esters), which was however, independent of the amount of GSH released into the wines, but that seems to be more related to the catalytic effect of some trace compounds present in the IDY. Therefore, new experiments will be necessary in order to clarify the action mechanisms of GSH-IDY preparations and their truly influence in wine's sensory characteristics.

ACKNOWLEDGMENTS

This work has been funded by the Projects PET2007-0134, AGL2006-04514 and AGL2009-13361-C02-01 from MICINN.

REFERENCES

Andujar-Ortiz, I., Moreno-arribas, M.V., Martín-Álvarez, P.J.; Pozo-Bayón, M.A. (2009). Analytical performance of three commonly used extraction methods for the gas-chromatography-mass spectrometry analysis of wine volatile compounds. *J. Chrom. A*, 1216, 7351-7357.

Comuzzo, P.; Tat, L.; Tonizzo, A.; Battistutta, F. (2006). Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. *Food Chem.,* 99 217-230.

Doco, T., Patrick, V., Cheynier V., Moutonet, M. (2003). Structural modification of wine arabinogalactans during aging on lees. *Am J. Enol. Vitic*., 54, 150-157.

Escot S., Feuillat, M., Dulau, L., Charpentier, C. (2001). Release of polysaccharydes by yeasts and teh influence of the released polysaccharydes on color stability and wine astringency. *Aus. J. Grape Wine Res*., 7, 153-159.

Escudero A, Hernandez-Orte P, Cacho J, Ferreira V. Clues about the role of methional as character impact odorant of some oxidized wines. *J. Agric. Food Chem*, 48 (9), 4268-4272. 2000.

Ferreira V, Escudero A, Fernandez P, Cacho JF. Changes in the profile of volatile compounds in wines stored under oxygen and their relationship with the browning process. *Z Lebensm Unters F A*, 205 (5), 392-396. 1997.

Jarauta., I., Cacho, J., Ferreira, V. (2005) Concurrent phenomena contributing to the formation of teh aroma of wine Turing againg in oak wood: an analytical study. *J. Agric. Food Chem.,* 53, 4166-4177.

Lavigne-Cruege, V., Pons, A., Chone, X., Dubourdieu, D. (2003). Ròle du glutathion sur l'evolution aromatique des vins blancs secs. In : Œnologie VII symposium International d'œnologie. Ed. Tec&Doc, pp. 385-388.

Pedersen, D.S., Capone, D.L., Skouroumounis, G.K., Pollnitz, A.P., Sefton, M.A. (2003). Quantitative analysis of geraniol, nerol, linalool and alpha terpineol in wine. *Anal Bional. Chem*., 375, 517-522.

Penninckx, M.J. (2002). An overview on glutathione in saccharomyces versus non-conventional yeasts. *FEMS Yeast Res.* 2, 295-305.

Pozo-Bayón, M.A., Andujar-Ortíz, I, Moreno-Arribas M.V. (2009a). Scientific evidences beyond the application of inactive dry preparations in winemaking. *Food Res. Int*. 42, 754-761

Pozo-Bayón, M.A, Andujar-Ortíz, I, Moreno-Arribas M.V. (2009b). Volatile profile and potential of inactive dry yeast based winemaking additives to modify the volatile composition of wines *J. Sci. Food Agric.,* 89, 1665-1673.

Pozo-Bayón, M.A., Andujar-Ortíz. I., Alcaide-Hidalgo, JM, Moreno-Arribas, MV. (2009c). Characterization of commercial inactive dry yeast preparations for oenological use based on their ability to release soluble compounds and on their behavior towards aroma compounds in model wines *J. Agric. Food Chem.,*57, 10784-10792.

Rapp, A., Mandery, H. (1986) Wine aroma. *Experientia*, 42, 873-884.