VOLATILE COMPOSITION OF SULPHITE-FREE WHITE WINES OBTAINED AFTER FERMENTATION IN THE PRESENCE OF CHITOSAN

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Introduction

Oxidation of wine is one of the main problems to be faced by winemakers during vinification due to the alteration of both phenolic and volatile compounds. Particularly, in white wines, oxidation leads to a decrease of the overall attractiveness of final products. Volatile compound are deeply involved in the oxidative decay of wines, being reduced the aromatic character due to the loose of grape-derived odoriferous compounds(Bueno *et al.*, 2010).

Among all the preservatives used in oenology sulphur dioxide has demonstrated to possess the most powerfull antioxidant and antimicrobial properties, controlling undesirable fermentations and preventing from oxidative spoilage in white and red wines (Ribéreau-Gayon *et al.*, 2006) .However, its use has been questioned due to the adverse effect to the human health, causing asthma, dermatitis, bronchoconstriction, urticaria or anaphylaxis. Due to the disadvantages, substitution or reduction of the employment of sulphur dioxide in wine is one of the objectives pursued by winemakers.

Despite all the efforts made to replace sulphur dioxide in wines, including physical, chemical or biological treatments, none of them has demonstrated enough effectiveness to completely substitute sulphur dioxide so a decisive alternative to sulphites is still unknown.

Chitosan is the deacetylated product of chitin, a homopolymer of n-acetyl-glucosamine, extracted from shell, insects of fungal sources. Its features like metal chelation, multifaceted antioxidant and radical scavenging activities against hydroxyl and superoxide radicals makes this natural products very attractive to use in food and agriculture sciences. Recently, the use of chitosan has been authorized in must and wine for microbial stabilization, or metal and protein removal('Commission Regulation (EU) 53/2011 of 21 January 2011') but its use as an antioxidant is still scarcely studied.
The aim of this work was to study the effects of the fermentative addition of chitosan on fixed and volatile compounds of sulphites free white wines. Studies of the difference between sulphite-free white wines and those added with sulphur dioxide in fermented must and after 12 months of storage were carried out.

Material and Methods

Results and discussion

Fermentation and oenological parameters

The evolution of fermentation was monitored by following the weight loss of fermentors. The fermentation of samples added of 1 g/L insoluble chitosan showed a 24 hours extended lag phase (data not shown). This was somehow expected since chitosan has already been reported to variably

interfere with *Saccharomyces ssp.* growth kinetics (Escudero-Abarca *et al.*, 2004) and have been suggested to be linked to the contents in polyunsaturated free fatty acids of cells plasma membrane that, in turn, influence membrane permeabilization. However, at day 8 and thereafter, their weight loss was similar to SO_2 or control samples and all the fermentations were completed in 10 days.

At the end of fermentation, chitosan samples had a decreased content in organic acids, with consequent higher pH values (augmented by 0.08 units) and lower titrable acidity (lessened by 1.1 g/L). In particular the grape-derived tartaric and malic acids were reduced of about 0.30 g/L and 0.50 g/L respectively while, in the same wines, succinic acid amount was 0.25 g/L lesser.

This feature is due to the electrostatic interaction between the positively charged amino groups of glucosamine and the anions coming from dissociated acids (Mitani *et al.*, 1995)(Scheruhn, Wille and Knorr, 1999) . Succinic acid, however, is produced by yeasts during alcoholic fermentation, and its residual presence in Kt wines could be, in principle, the result of both the adsorption by chitosan or a reduced fermentative excretion.

	Control	SO2	KT
Alcohol (% v/v)	12,07 a	11,99 a	11,97 a
Titratable Acidity (g/L)	6,52 a	6,23 ab	5,25 b
Volatile Acidity (g/L)	0,39 a	0,36 b	0,42 a
pH	3,11 b	3,11 b	3,19 a
Total SO_2 (mg/L)	1,92 a	48,7 b	2,56 a
Total phenolics (mg/L)	42,3 a	42,3 a	40,7 a
O. D. 420 nm	0,092 a	0,082 b	0,085 ab
Citric acid (g/L)	0,20 a	0,19 a	0,18 a
Tartaric acid (g/L)	2,94 a	3,03 a	2,67 b
Malic acid (g/L)	2,23 a	2,14 a	1,68 b
Lactic acid (g/L)	0,18 a	0,23 a	0,18 a
Succinic acid (g/L)	0,95 a	0,93 a	0,69 b
Acetic acid (g/L)	0,36 a	0,39 a	0,41 a
Glycerol (g/L)	9,37 a	9,74 a	9,30 a

Table 1: Enological parameters of wines at the end of alcoholic fermentation. In the same row, significant differences at p<= 0.05 are flagged with different letters.

Volatile composition

Esters

Volatile esters content of wines are of great interest, because of their key role in the sensorial profile, being responsible of fruitness, floral and “sweet-like” notes in white wines. Chitosan seems to enhance the esters production, particularly isoamyl acetate (banana), phenylethyl acetate (floral) and medium chain fatty acids (MCFA) ethyl esters, ethyl n-caproate, ethyl octanoate, ethyl decanoate and ethyl 3-hydroxybutanoate (Table 2). This fact is directly correlated with MCFA production, being the latter the substrates for the synthesis of the former (Saerens *et al.*, 2008). The lesser content of ethyl lactate, ethyl malate, mono and diethyl succinate found in KT samples after fermentation can be justified to the decreased content of organic acids after fermentation in chitosan-treated wines (Table 1), being

these ester compounds the products of esterification of the respective organic acid. During 12 months of storage, as expected, acetate esters drastically decreased while ethyl esters increased to various extents (Table 2) in accordance with previous findings.

Fatty acids

Three of the medium chain fatty acids (MCFA) hexanoic, octanoic and decanoic acid were influenced positively in treatments with chitosan (Table 2). This increase in MCFA content may be due to an augmented permeability of yeast membranes caused by chitosan by means of an interaction between positive charged glucosamine units of chitosan and anionic negative charged components of cell surface(Zakrzewska *et al.*, 2005). This electrostatic interaction induces changes in the properties of membrane thus modifying, among other, cell permeability (Hadwiger *et al.*, 1986). According with sensory studies, the latter C6 to C10 fatty acids, can contribute to the volatile quality of wine by imparting pleasant aroma at concentrations of < 10 mg/L. However, at levels beyond 20 mg/L, their impact on wines becomes negative(Shinohara, 1985). In our samples, MCFA concentration at the end of fermentation did non exceed that limit.

Alcohols.

Pre-fermentative addition of chitosan seemed not to particularly influence the alcohols content, except for the lower levels of isobutyl alcohol and 3-methylthio-1-propanol, both derived from aminoacid metabolism. This finding may be related to the protein binding capacity of chitosan in musts and hence, reducing amino acid availability(Chagas, Monteiro and Boavida Ferreira, 2012). After 12 months of storage, an increase of total amount of alcohols has taken place mostly due to 3-methyl-1-butanol and 2-phenetyl alcohol, without significant differences among samples. The majority of other compounds remained unchanged in quantity except 3-methylthio-1-propanol, benzyl alcohol and tyrosol (4-hydroxy-benzenethanol) that decreased similarly to what has been already observed in previous works(Garde-Cerdán and Ancín-Azpilicueta, 2007).

	Wines					
	End of fermentation			12 months of storage		
	Test	SO_2	KT	Test	SO_2	KT
			Esters			
isoamyl acetate	0.77 b	0.69 b	1.11 a	0.21 a	0.22 a	0.20 a
ethyl hexanoate	0.23 b	0.21 b	0.52 a	0.34 b	0.31 b	0.60 a
ethyl pyruvate	0.04 b	0.06 a	0.05 b	0.11 b	0.17 a	0.08 b
ethyl octanoate	0.15 b	0.16 b	0.43 a	0.64 b	0.50 b	1.15 a
ethyl-3-hydroxybutyrate	0.05 b	0.06 b	0.10 a	0.05 b	0.09 a	0.09 a
ethyl decanoate	0.03 b	0.04 b	0.15 a	0.11 b	0.09 b	0.34 a
diethyl succinate	0.34 a	0.39 a	0.27 b	13.33 a,b	15.56 a	9.35 b
ethyl 4-hydroxybutanoate	2.93 b	3.47 a	1.31 c	0.23 a,b	0.30 a	0.19 b
2-phenylethyl acetate	0.33 b	0.32 b	0.75 a	0.07 b	0.08 b	0.15 a
diethyl malate	0.26 a	0.31 a	0.17 b	5.26 b	8.75 a	5.45 b
ethyl hydrogen succinate	11.65 a	11.96 a	8.87 b	48.97 a	56.65 a	61.40 a
Total esters	*16.78 a*	*17.68 a*	*13.73 b*	*69.31 a*	*82.71 a*	*79.00 a*
			Acids			
isobutyric acid	1.12 a	1.02 a	0.53 b	0.95 a	0.81 a	0.41 b
n-butyric acid	0.31 b	0.34 b	0.39 a	0.21 c	0.29 b	0.33 a
pentanoic acid	1.91 a	1.90 a	1.07 b	1.86 a	1.85 a	0.87 b
hexanoic acid	1.42 b	1.46 b	2.43 a	1.39 b	1.43 b	2.57 a
octanoic acid	3.11 b	3.11 b	5.67 a	2.65 b	2.69 b	5.45 a
decanoic acid	0.75 b	0.63 b	2.74 a	0.58 b	0.51 b	1.93 a
dodecanoic acid	0.16 a	0.17 a	0.14 a	0.03 b	0.04 b	0.08 a
benzenacetic acid	0.12 b	0.20 a	0.07 c	0.05 b	0.10 a	0.06 b
Total acids	*8.90 b*	*8.83 b*	*13.04 a*	*7.72 b*	*7.70 b*	*11.69 a*
			Alcohols			
Isobutyl alcohol	5.06 b	7.06 a	3.35 c	6.88 a	5.11 b	3.69 b
n-hexanol	0.04 c	0.10 a	0.07 b	0.10 a	0.08 a	0.10 a
3-methyl-1-butanol	38.13 b	49.97 a	38.07 b	68.92 a	56.61 a	69.59 a
2-hexanol	0.04 a	0.04 a	0.04 a	0.01 a	0.01 a	0.01 a
4-methyl-1-pentanol	0.02 c	0.03 b	0.04 a	0.02 b	0.03 a	0.03 a
n-hexanol	0.11 a	0.11 a	0.08 b	0.09 a	0.10 a	0.07 b
3-ethoxy-1-propanol	0.10 a	0.06 b	0.09 a	0.10 a	0.04 c	0.08 b
3-hexen-1-ol	0.01 b	0.02 a	0.01 a,b	0.01 a	0.01 a	n.d
3-methylthio-1-propanol	0.95 a	1.05 a	0.36 b	0.56 a	0.58 a	0.23 b
Benzyl alcohol	0.12 a,b	0.18 a	0.06 b	0.05 a	0.06 a	0.04 a
2-mercaptoethanol	n.d	0.02 a	n.d	n.d	n.d	n.d
Phenethyl alcohol	30.83 a	30.36 a	29.61 a	49.84 a	56.86 a	59.55 a
4-hydroxy-benzenethanol	25.24 a	25.35 a	28.20 a	17.29 a	23.77 a	25.70 a
Total alcohols	*100.65 a*	*114.34 a*	*99.98 a*	*143.87 a*	*143.27 a*	*159.11 a*

Table 2. Concentration of the quantified volatile compounds (mgL-1) in wines at the end of the alcoholic fermentation and after one year of storage. In the same row, different letters indicate significant differences according to Tukey's test ($p<0.05$)

Conclusion

Results suggested that chitosan can influence fermentative kinetics, delaying the yeasts lag-phase. In addition, it affected the volatile composition of wines, increasing medium chain fatty acids content or esters such as isoamyl acetate or b-phenylethyl acetate.

Further, some organic acids were adsorbed by this polysaccharide, slight reducing the titratable acidity of the treated wines.

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Abstract

Fermentations of sulphite-free white must in the presence of chitosan were carried out. Fixed and volatile composition of the final wines have been analysed and compared with wines obtained after fermentation with addition of sulphites. Evolution of wines during a period of 12 months of storage were also studied.