Volatile composition of sulphite-free white wines obtained after fermentation in the presence of chitosan

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Introduction:

- Oxidation of wines is one of the main reactions responsible of **altering phenolic and aromatic** profile, leading to less attractive and spoilage final products.
- **sulphur dioxide** is the most powerful antioxidant and antimicriobial additives used in wines, but a number of **problems to human health** have been demonstrated**. One focus of the SOSTINNOVI project (funded by POR-FESR Emilia-Romagna 2014-202 program) is the reduction of SO_2 in wines.**
- To date, none of the techniques proposed as SO_2 alternative (ascorbic acid, glutathione, lysozyme, UV, ultrasounds...) is able to completetly **substitute sulphur dioxide in wines.**
- **Chitosan** is a deacetylated natural product of chitin, with some interesting activities (metal chelation, antimicrobial capacity, **antioxidant** and radical scavenging capacity).
- As its use is accepted in wines for clarifing, eliminate OTA and *Brettanomyces* spp., the aim of this work was to study the behaviour of chitosan as **antioxidant during fermentation of white musts and its effect on volatile profile on final wines after fermentation and a 12 months storage period.**

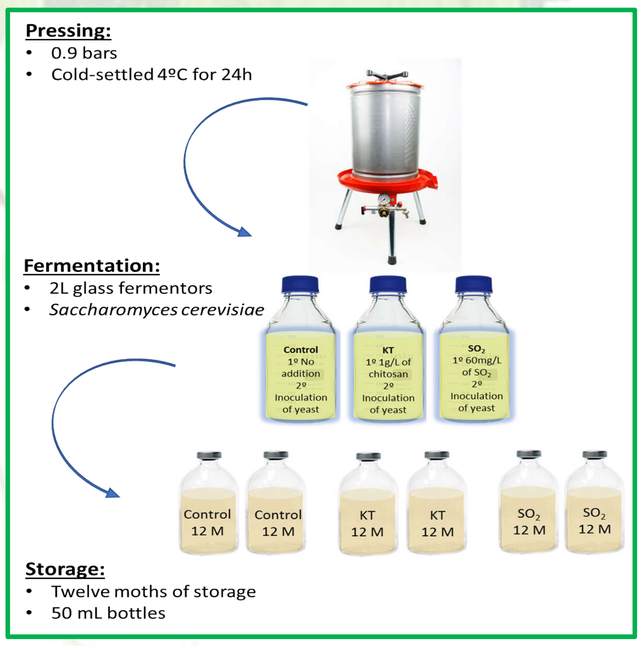

Figure 1. Diagram of winemaking process (KT= Chitosan, SO_2= sulphur dioxide, 12M = 12 months)

Material & methods

Samples: Three different fermentation were carried out with Cv. Trebbiano grapes, and stored during one year as shown in figure 1. **Volatile Extraction and CG/MS Analysis**: Volatile compounds were analyzed after and SPE extraction on Lichrolut EN cardtridges ad described by Lopez et al. (2002). GC-MS analysis was undertaken in a Trace GC ultra gas chromatograph equipped with a Trace DSQ mass selective detector (Thermo Fisher Scientific, Milan, Italy) and a fused silica capillary column Stabilwax DA (Restek, Bellefonte, PA, USA; 30 m, 0.25mm i.d., and 0.25 µm film thickness). Analysys were done in duplicate and data were collected by means of Xcalibur software (Thermo Fisher Scientific, Milano, Italy). **Statistical analysis:** Statistical analysis of the entire dataset was performed using the XLSTAT Software package (Version 2013.2, France). One-way analysis of variance (ANOVA) followed by a post hoc comparison and Principal Component Analysis (PCA) were carried out.

Volatile composition

The most significant compounds identifyed in wines at the end of fermentation and after storage period are shown in Table 1 grouped as chemical families:

	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
methyl lactate	0,02 b	0,03 b	0,05 a	n.d	n.d	n.d
ethyl lactate	0,51 b	0,53 a	0,42 c	1,65 a	1,44 b	1,46 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
methyl salicylate	0,01 a	0,01 a	0,01 a	n.d	n.d	n.d
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
diethyl tartrate	n.d	n.d	n.d	0,58 b	1,00 a	0,35 b
ethyl hydrogen succinate	11,65 a	11,96 a	8,87 b	48,97 a	56,65 a	61,40 a
Total esters	*17,31 a*	*18,25 a*	*14,21 b*	*71,54 a*	*85,15 a*	*80,81 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 1. Concentration of the quantified volatile compounds (mgL-1) in wines at the end of the alcoholic fermentation and after one year of storage

- **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[1]. 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 1). This fact is directly correlated with MCFA production, being the latter the substrates for the synthesis of the former[2]. The lower content of ethyl lactate, ethyl malate, mono and diethyl succinate found in KT samples after fermentation can be justified due to the decreased content of organic acids after fermentation in chitosan-treated wines (Table 2), 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 1) in accordance with previous findings[2].

- **Acids**

Three of the medium chain fatty acids (MCFA) hexanoic, octanoic and decanoic acid were influenced positively in treatments with chitosan (Table 1). 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[3]. This electrostatic interaction induces changes in the properties of membrane thus modifying, among other, cell permeability[4]. 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[5]. 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 isobubtyl 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[6,7]. After 12 month 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[8].

Fermentation

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. This is was somehow expected since chitosan has already been reported to variably interfere with *Saccharomyces ssp.* growth kinetics[9].

These differences in fungi responses have been suggested to be linked to cells plasma membrane composition where higher contents in polyunsaturated free fatty acids (as is the case of *Saccharomyces cerevisiae*) corresponds to enhanced fluidity, membrane permeabilization and increased intracellular oxidative stress because of the chitosan entrance in the plasma.

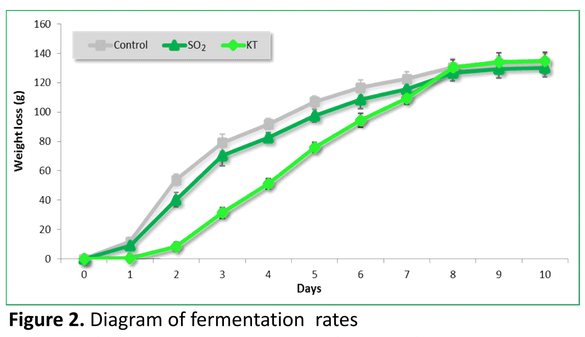

Figure 2. Diagram of fermentation rates

However, at day 8 and thereafter, their weigh loss was similar to SO2 or control samples (Figure 2) and all the fermentations were completed in 10 days.

Oenological parameters

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 of 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, whose pKa and hydroxyl content may also play[10,11]. Hence, this would be the reason for our findings on native organic acids decrease during the 10 days of fermentation. 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 2. Oenological parameters and organic acids at the end of fermentation

Conclusions

Results suggested that chitosan does not adversely affect the aromatic profile of the wine, reinforcing the floral and fruity character by increasing the compounds responsible for these aromatic notes such as isoamyl acetate or β-phenylethyl acetate and appear to maintain the previous characteristics of the product over the time. However, attention should be paid to fixed compounds, in particular organic acids, whose adsoption by chitosan, may reduce the overall acidity of final products.

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