Chemical and technological alternatives to the use of sulfur dioxide during winemaking process

Antonio Castro Marin (email: antonio.castromarin2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
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Tutor: Fabio Chinnici; Co-tutor: Claudio Riponi

1. State of the art

During winemaking process, a number of wine components are susceptible to oxidation, being the polyphenols the pivotal protagonist for the development of browning phenomena (Singleton *et al.,* 1995). Browning is one of the main problems encountered in wimenaking because it adversely affects both the sensory properties (color loss, aromatic defects, increase of astringency) and the nutritional value of the final product. (Waterhouse *et al*., 2006)

Sulfur dioxide (SO_2) is among the most used and powerful antioxidant in food industry, especially in low pH food such as fruit juices and fermentable drinks. However, it has been shown that sulfites can cause adverse effects to human health such as allergic reaction (Vally *et al*., 2003). Asthmatics who are steroid dependent or have a higher degree of airway hyper-reactivity may be at greater risk of experiencing a reaction to sulfite containing foods (Vally *et al*., 2009). From a technological point of view, however, reduced doses of SO_2 do not ensure adequate stability to the wine against oxidation and microbial growth. Therefore, there is a great interest in developing new healthier technologies to replace or reduce the use of sulfur dioxide in wines while ensuring wine stability all along the production process (Santos *et al*., 2012). Among the alternative practices studied like addition of compounds (DMDC, bacteriocins, phenolic compounds, lysozyme, ascorbic acid) or physical methods (pulsed electric fields, ultrasound, UV, high pressure), none of them is capable to completely replace sulfur dioxide, so there remains the challenge of using new alternative techniques to it use.

Chitosan is a polysaccharide derived from chitin, the second most abundant polymer in nature next to cellulose. Is also known for its biocompatibility, biodegradability and low toxicity, and has been recently admitted as wine additive (Luo *et al* 2013). In food industry, chitosan is increasingly used thanks to its properties, included the antioxidant and antiradical activities. However, there is a lack of scientific investigations on its effects against phenolic oxidation and reduction of wines browning which, in turn, could intriguingly fuel the researches on sulfite-free wines.

2. Bibliography

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3. Research development

PhD research has been developed by following five main topics:

1) Antioxidant and antiradical activity of chitosan in model and white wine. The scavenging activity of chitosan against the radical specie relevant to wine environment will be investigated both in model and real white wines by means of the ESR technique.
2) Study of the aromatic profile in wines fermented in the presence of chitosan. Laboratory scale fermentations were carried out in the presence of chitosan in order to evaluate its influence on the volatile profile of final wines.

3) Evaluation of the effect of chitosan during the stabilization of white wine at laboratory scale. Chitosan was applied either in must before fermentation or in wines after fermentation in order to study the quality and stability of wines
4) Effect of chitosan during the "prise de mousse" in sparkling wines. The influence of chitosan in bottle during "prise de mousse" was monitored by analysing the fixed and volatile composition of wines treated.
5) Effect of chitosan during flotation and stabilization of wines at semi-industrial scale.

Table 1. Gantt diagram of PhD research project

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Preliminary studies on chitosan***	■	■	■															
	1) Bibliographic review	▒	▒																
	2) Antioxidant activity of chitosan	▒	▒	▒															
A2)	***Study of the aromatic profile of chitosan***	■	■	■	■	■	■	■	■										
	1) Studies of the effect of chitosan during fermentation	▒	▒	▒	▒	▒	▒												
	2) Effect of chitosan in stabilization process			▒	▒	▒	▒	▒	▒										
A3)	***Evaluation of the effect of chitosan during stabilization process***				■	■	■	■	■	■	■								
	1) Vinification process and general analysis of rosè wines				▒	▒													
	2) Studies of development of organic and phenolic compounds						▒	▒	▒	▒	▒								
	3) Studies of aromatic profile of wines during storage period								▒	▒	▒								
A4)	***Effect of chitosan during "prise de mousse"***											■	■	■	■	■	■	■	■
	1) Studies of chitosan properties											▒	▒	▒	▒	▒	▒	▒	▒
	2) Effect of chitosan in red and sparkling wines											▒	▒	▒	▒	▒	▒	▒	▒
A5)	***Evaluation of the effect of chitosan at semi industrial scale***											▒	▒	▒	▒	▒	▒	▒	▒
A6)	***Thesis development and article writing***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Main results

1) Antioxidant and antiradical activity of chitosan in model and white wine.

Experiments on model and real white wines were carried out in order to study the antioxidant effect of chitosan. Oxidation development was followed by monitoring different parameters: 1) Generation of oxidation intermediates such as glyoxilic acid and acetaldehyde by HPLC, 2) Scavenging effect against hydrogen peroxide, 3) Metal content by ICP, 4) Scavenging of hydroxyl and hydroxyethyl radical by Electron Paramagnetic Resonance, carried out in collaboration with Institute de Chemie Radicalaire of the University of Marseille (FR) 5) Evolution of browning, 6) monitoring oxidation products of (+)-catechin.

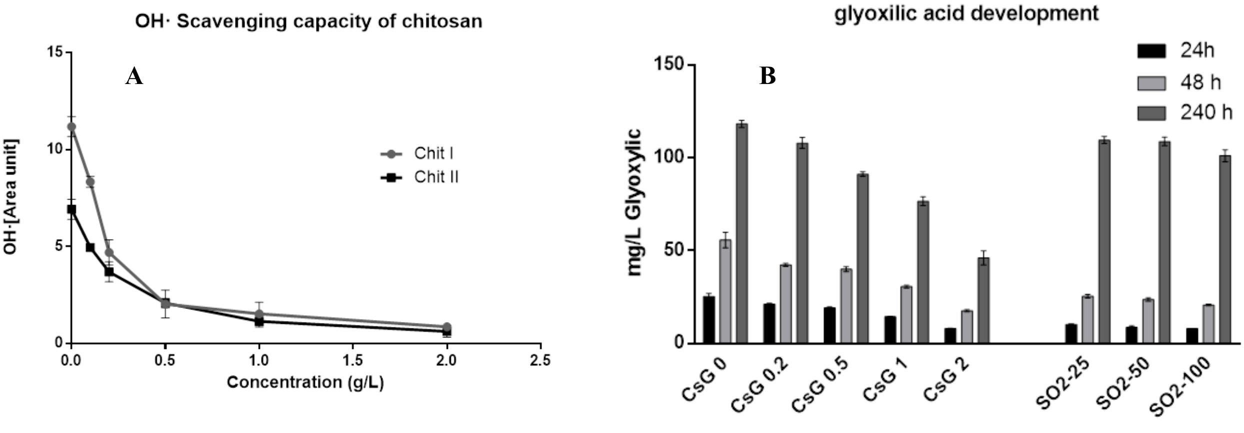

Figure 1. Effect of chitosan against hydroxyl radical in model wine and on the development of glyoxylic acid in white wine.

All the results showed a dose response effect of chitosan against oxidation phenomena. At maximum doses (2g/L) chitosan exhibit a 90% of inhibition of hydroxyl radical production (Figure 1A), 70% of glyoxylic acid reduction (Figure 1B) 80% of browning (Figure 2) and 72% of chelation effect (not shown). Based on these results, chitosan demonstrated to be an effective alternative to control oxidation in wine by its interaction with the oxidation precursors (metals, hydrogen peroxide, hydroxyl radical), intermediates (glyoxylic and acetaldehyde) and products of oxidation (Xanthillium cations).

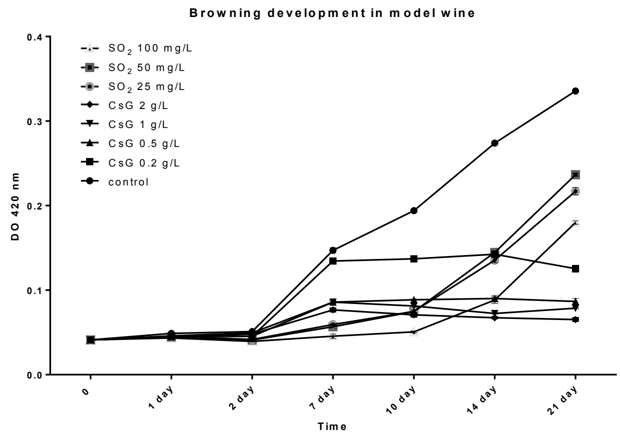

Figure 2. Effect of chitosan and sulphites on the development of browning in model matrix

2) Study of the aromatic profile in wines fermented in the presence of chitosan

In this work, distinct fermentations of white musts either in the presence of chitosan or sulfur dioxide were carried out in order to compare the volatile and fixed composition of the wines produced and evaluate the impact of chitosan as an alternative to sulfur dioxide.

Chitosan promoted a 24 h extended lag-phase and diminished the titratable acidity of wines by about 1 g L^{-1} as a consequence of the absorption of tartaric and malic acids onto the polymer surface. The volatile composition of wines was analyzed at the end of the alcoholic fermentation and then after 12 months of storage in glass bottle. Hexanoic, octanoic and decanoic acids were significantly higher in chitosan added wines, which further contained an increased amount of ethyl and acetate esters. Results demonstrated that, when added before the alcoholic fermentation, chitosan may affect both the acidic and volatile composition of wines, likely due to its polycationic behavior and interaction with yeast wall constituents. This also suggests that attention to wine acidic balance should be paid before its use in other vinification steps such as must clarification or wine fining.

3) Evaluation of the effect of chitosan during the stabilization of white wine at laboratory scale

During this work, studies on the influence of chitosan during stabilization have been made. This experiment has been divided into two objectives, in one of them the stabilization has been carried out before alcoholic fermentation (on white musts), and the other one after fermentation. Then, during storage period the wines have been bottled with ascorbic acid or glutathione. We observed how stabilization with chitosan caused a retention of phenolic compounds (absorbance at 280 nm) led to a similar protection against browning than the samples treated with SO_2 during bottling period (absorbance at 420 nm)

Another of the relevant effects observed after the stabilization with chitosan in must and wines are the retention of organic acids, thus contributing to a different gustative profile (**Table 2**).

		Tartaric	Piruvic	Malic	Shikimic	Citric	Lactic	Acetic	Succinic
Stabilization of must	**KT**	3.518 ^{bb}	-	1.56 ^{bb}	0.4375 ^{aa}	0.265 ^{bb}	-		
(Objective 1)	**SO2**	3.803 ^{aa}	-	1.74 ^{aa}	0.5 ^{aa}	0.355 ^{aa}	-		
Stabilization of wine	**KT**	2.447 ^{aa}	191 ^{bb}	1.25 ^{bb}	5.945 ^{bb}	0.232 ^{bb}	0.217 ^{bb}	0.147 ^{aa}	0.403 ^{aa}
(Objective 2)	**SO2**	2.803 ^{aa}	307 ^{aa}	1.47 ^{aa}	8.49 ^{aa}	0.3 ^{aa}	0.305 ^{aa}	0.167 ^{aa}	0.472 ^{aa}

Table 2. Effects on organic acids concentration during stabilization with chitosan in either must or wine

4) Effect of chitosan during the "prise de mousse" in sparkling wines

"Prise de mousse" was carried out in the presence of chitosan in order to study the development of wine during the second fermentation. Samples treated with chitosan experience a reduction in the polyphenolic content due to an adsorption by means of chitosan. However, results showed a minor development of browning on those wines, demonstrating that chitosan was able to control the oxidation of the wine. In addition, from a sensory point of view the wines produced in the presence of chitosan presented a greater sensory complexity than those not treated. These results indicate that chitosan could represent an interesting alternative in the control of second fermentations that could take place by the permanence of the wine in bottle in the production of sparkling wines.

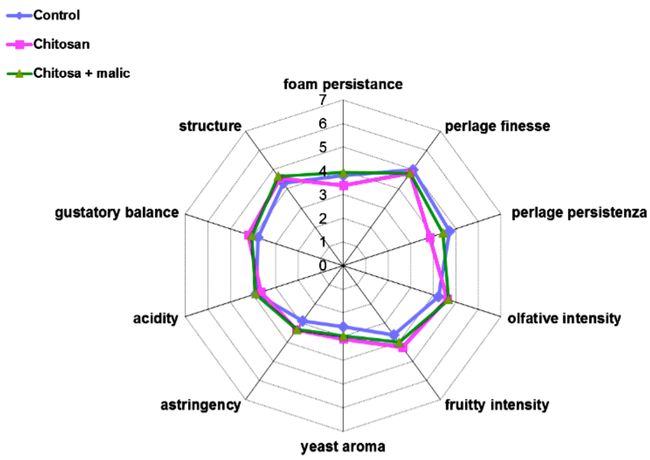

Figure 3. Effect of chitosan on the aromatic profile during "prise de mousse"

5) Effect of chitosan during flotation and stabilization of wines at semi-industrial scale

Based on the results obtained at the laboratory scale, chitosan was evaluated in different stages of the winemaking process at a semi-industrial scale. Specifically, the effect was studied during: 1) flotation of must, 2) stabilization of wines before bottling.

The results showed that treatments with chitosan caused a reduction of the polyphenolic content, in agreement with what has already been obtained in previous works. In addition, it was observed that the samples treated with chitosan experienced not only a reduction in colour, but also a greater stability against browning with respect to the control. Based on the results presented in the first study explained above, this phenomenon could be due to different mechanisms: 1) direct adsorption of xhantyllium cations, 2) reduction of metal content, 3) hydroxyl radical scavenging by means of chitosan

In our samples, spontaneous malolactic fermentation occurred during alcoholic fermentation. During the malolactic fermentation the hydrolysis of the hydroxycinnamic acids (caffeic, fertaric, cutaric) into their cinnamic derivatives (caffeic, ferulic, p-coumaric) could be produced. This activity is known as *"cinnamoyl esterase"* and is carried out, among others, by some microorganisms such as lactic acid bacteria. As can be seen in the figure, this activity is more enhanced in the samples that were treated with chitosan, so it could be hypothesized that a microbiological selection has been made by the chitosan, selecting those strains with greater activity cinamoil esterase.

5. Publications during PhD

Castro-Marín A, Buglia A, Riponi C, Chinnici F (2018) Volatile and Fixed Composition of Sulphite-Free White Wines Obtained after Fermentation in the Presence of Chitosan, LWT. 93:174–80.

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