POLYSACCHARIDES DERIVED FROM FUNGAL EXOSKELETON CLARIFICATION EFFECTS ON WHITE MUST COMPONENTS FROM GRENACHE VARIETY

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Abstract: Large molecular weight chitin, chitosan and derivatives of cationic character derived from fungal and shrimp chitin were tested for the clarification of white must.
The Grenache white must used in this study contained 280 mgGAE/l of total phenosls expressed as gallic acid equivalent, 203,7 g/L of sugars, 4,40 gH_2SO_4 total acidity, a pH of 3.29, and turbidity of 3670 NTU. The fermentation was conducted with Lalvin BM45 *Saccharomyces cervesiae* yeast at 22°C.
The concentrations used for chitin, chitosan and derivatives treatment were 0.5 and 2g/l.
With the highest concentration treatment:
• The turbidity was reduced to 91.8% of its initial value with hydrolysed chitin-glucan; the corresponding values for chitosan, chitin and chitin glucan were 93.6% with, 79.7% and 67% respectively.
• Total phenol content was reduced to 45.4% of its initial value with hydrolysed chitin-glucan, to 20.2% with chitosan, 54.7% with chitin and to 46.2% with chitin glucan.
• The protein content determined by Bradford method was reduced to 56.7% with hydrolysed chitin-glucan, 66.2% with chitosan, 45.3% with chitin and 46.2% with chitin glucan.
• The concentrations of individual phenolic compounds (p-coumaric acid, procyanidin dimer B3 and epicatechin) analysed by HPLC-UV in the white must after fermentation process were not affected by the different treatments. With hydrolysed chitn-glucan a the concentration of epigallocatechin was reduced to 92.2% of its initial value. Chitosan treatment reduced the concentration of caftaric acid by 83.4%. Chitin glucan was the only treatment to reduce the concentration of catechin (71.5%) and caffeic acid (54.5%).
Chitin, chitosan and derivatives appear to be interesting candidate products for the clarification of grape musts during winemaking. In the future it will be interesting to complete this study with a investigation of the effects of chitin, chitosan and derivatives on the organoleptic effects of the finished wine.

Introduction

Chitin, chitosan and derivatives are known biodegradable polymers based on polysaccharides, which are extracted from various animals and plants. Chitin exists widely in the cell walls of some microorganisms such as fungi, molds and yeast and in the exoskeletons of invertebrates such as

crustaceans, mollusks, crabs, shrimps, lobster, squid and insects. Chitosan exists in only a few species of fungi. Chitin and chitosan consist of 2-acetamido-2-deoxy-ß-D-glucose and 2-amido-2-deoxy-ß-D-glucose, respectively as repeating units. Chitin is chemically identical to cellulose except that secondary hydroxyl group on the alpha carbon atom of the cellulose molecule is substituted with acetoamide groups. Chitosan is the N-acetylated form of chitin and exhibits the deacetylation reaction of chitin. It is also a non-toxic and biodegradable carbohydrate polymers. Numerous studies have demonstrated that chitosan and its derivatives have various biological actions such as antimicrobial and antitumor activities in addition to immuno-enhancing effects. An interesting innovation purposed by Kitozyme is the possibility to obtain these polysaccharides (chitin, chitin-glucan, chitin glucan hydrolysate and chitosan) from fungal sources after a specific industrial hydrolysis process.
Because of the apparition of BSE, oenological products of vegetable origin are preferred to those of animal origin are. This paper reports the application of new polysaccharide compounds (chitin, chitin-glucan, hydrolysed chitin-glucan and chitosan) in the clarification of musts prior to fermentation.

Material and Methods

Materials
The samples used in these experiments were south of France Grenache noir red must and Grenache blanc white must.
The novel polysaccharide products (chitin, chitin-glucan, hydrolysed chitin-glucan and chitosan) used for tests were produced by Kitozyme SA Herstal, Belgium:
Treatments
The fermentation was carried out with Lalvin BM45 Saccharomyces cervesiae yeast at a temperature of 22°C.
For each sample of must 10 or 50g/hl of the polysaccharide were added to 10 litres of must at the fermentation mid-point.

Methods of analysis
All the measurements were carried out in triplicate on representative samples of must.
Oenological parameters were analysed according to the methods described by the Organisation International de la Vigne et du Vin (1990).
Must turbidity was determined using a Hach turbidimeter, model 1200N (Hach Co. Loveland, CO) prepared for coloured samples.

Colorimetric determination of total phenolics was based on the procedure of Singleton and Rossi (1965), using a standard curve of gallic acid. Results are expressed as milligram per litre gallic acid equivalents (GAE).

Protein content determination was based on the procedure of Bradford (1976), using a bovine serum albumin standard curve. Results are expressed as milligram per litre bovine serum albumin(BSA).

Individual phenolic compounds were analysed by HPLC analysis with UV detection using a Hewlett-Packard model 1090 with three low-pressure pumps and a diode array detector coupled to a Hewlett-Packard Chem. Station ® for solvent delivery and detection. A Hewlett-Packard column packed with Nucleosil 100 C_{18} (250 x 4mm x 5µm dp) was used as the stationary phase with an eluent flow rate of 0.5 ml/min. The solvents used for a separation were as follows: solvent A 200mM orthophosphoric acid adjusted to pH 1.5 with ammonia; solvent B was 20% solvent A with 80% acetonitrile; solvent C was 50mmol/l ammonium dihydrogen phosphate adjusted to pH 2.6 with orthophosphoric acid.

Elution was performed with a gradient previously described by Carando *et al.* (1999).

Results and Discussion

The results obtained indicate that chitosan, chitin-glucan and hydrolysed chitin-glucan have a positive clariflying effect on grape must, at treatment varying from 10g/hl to 50g/hl. The turbidity was reduced to 91.8% of its initial value with hydrolysed chitin-glucan; the corresponding values for chitosan, chitin and chitin glucan were 93.6% with, 79.7% and 67% respectively
The enological parameters was not affected by different treatment.

The protein content determined by Bradford method was reduced to 56.7% with hydrolysed chitin-glucan, 66.2% with chitosan, 45.3% with chitin and 46.2% with chitin glucan. This is an interesting observation since although proteins and peptides are a minor constituents of wine , they make a significant contribution to its quality: proteins can create a number of technological problems during vinification and may be responsible for the appearance of turbidity in bottled wine.

Total phenol content was reduced to 45.4% of its initial value with hydrolysed chitin-glucan, to 20.2% with chitosan, 54.7% with chitin and to 46.2% with chitin glucan, and it is likely that this decrease occurs during the fermentation process.

The concentrations of individual phenolic compounds (p-coumaric acid, procyanidin dimer B3 and epicatechin analysed by HPLC-UV in the white must after fermentation process) were not affected by the different treatments. With hydrolysed chitn-glucan a the concentration of epigallocatechin was reduced to 92.2% of its initial value. Chitosan treatment reduced the concentration of caftaric acid by 83.4%. Chitin glucan was the only treatment to reduce the concentration of catechin (71.5%) and caffeic acid (54.5%).

The concentrations of gallic acid and vanillic acid determined by HPLC-UV in red must after fermentation were not affected by the different treatments..
With hydrolysed chitin-glucan treatment the concentration of dimer B1 was reduced to 21% of its initial value. Chitin-glucan treatment reduced the concentration of this compound to 77%, delphinidin to 96% and peonidin to 73%. Chitin treatment reduced the concentration of dimer B1 to 84% and delphinidin to 96%. With chitosan treatment no reduction in the concentration of any of these compounds was observed

Chitin, chitosan and derivatives are interesting candidate compounds for the clarification of must during winemaking. The polysaccharides derived from fungal exoskeletons produce an effect similar to that of bentonite, at a lower dose (10-50 g/hl) as opposed to (50-100 g/hl) in the case of bentonite.

In the future it will be interesting to complete this study with an investigate of the effects of chitin, chitosan and derivatives on the organoleptic properties of the finished wine.

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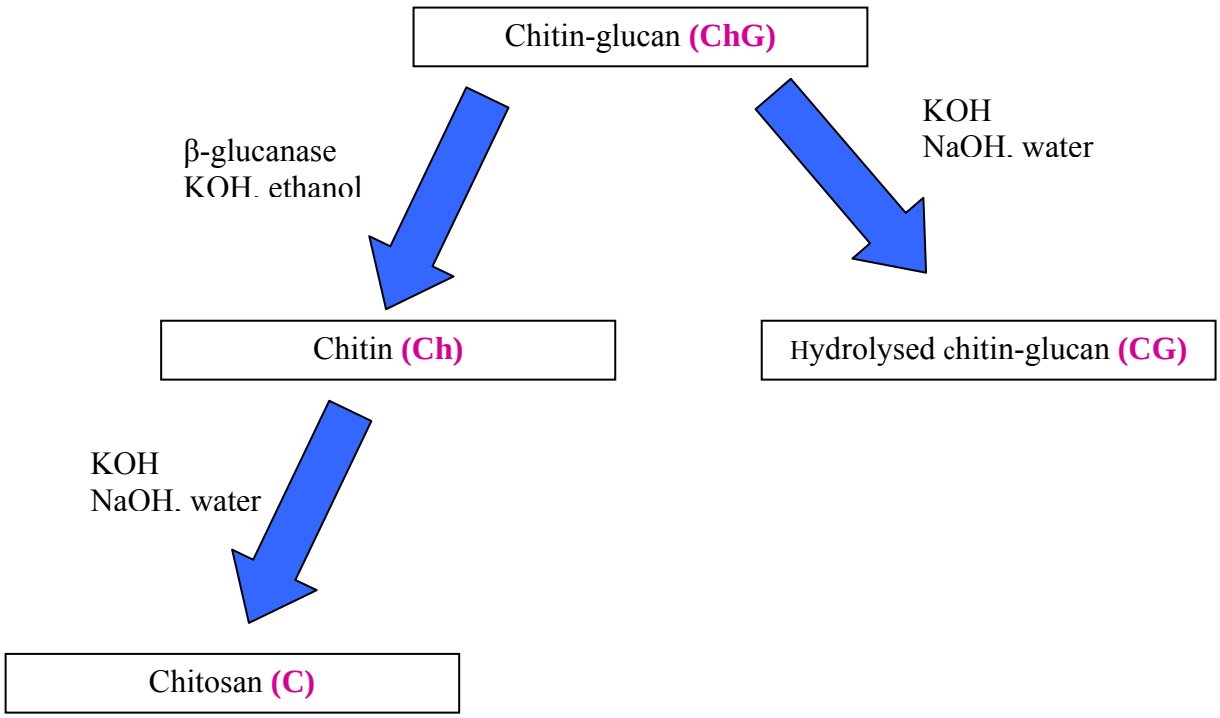
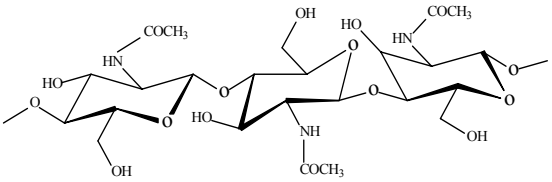
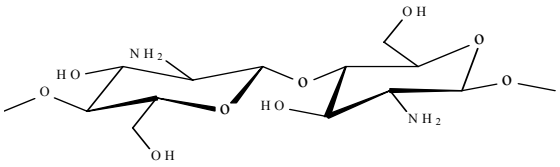


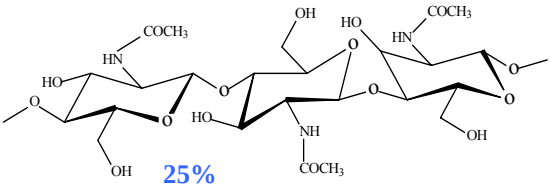
Figure 1 : Process extraction chitin and derivatives by KITOZYME

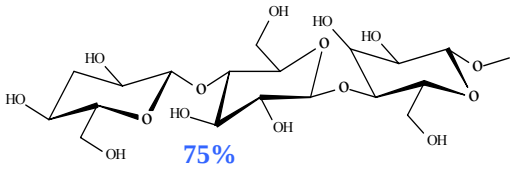


Chitin (Ch)
Polysaccharides linéaire with repeating units N-acetyl D glucosamine

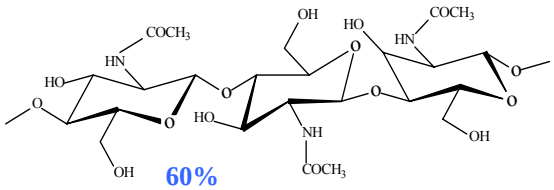


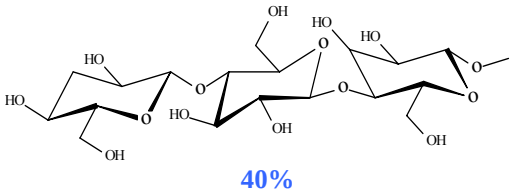
Chitosan (C)
N-acetylated form of chitin





Hydrolysed chitin-glucan (CG)
Short copolymer of chitin and β-glucans





Chitin-glucan (ChG)
Copolymer of chitin and β-glucans

Figure 2 : Stucture of chitin and derivatives

		TAV %vol	**Ac.T.** (g/l H2SO4)	**Ac.V.** (g/l H2SO4)	**SO2 T.** (mg/l)	**pH**
Red must	Grenache noir	0,22	2,94	0,04	75	3,86
White must	Grenache blanc	0,08	4,40	0,05	59	3,29

Figure 3 : Analytical characteristics of must