CHITOSAN, CHITIN GLUCAN AND CHITIN EFFECTS ON MINERALS (IRON, LEAD, CADMIUM) AND ORGANIC (OCHRATOXIN A) CONTAMINANTS IN WINES

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Abstract : Large molecular weight chitin, chitosan and derivatives of cationic character derived from fungal and shrimp chitin were tested for removal of minerals (iron or lead and cadmium) and organics (Ochratoxin A : OTA) contaminants in wines. Preliminary, wines (red, white,sweet) are spiked in minerals (iron 20 mg/L and lead 500 µg/L and cadmium 20 µg/L) or (Ochratoxin A 5µg/L) organics contaminants.
For wine enriched with minerals contaminants, treatments with chitosan, chitin or chitin glucan at the dose of 0,1g/L or 0,5g/L or 2g/L were realized. After a 2 days experimental period with agitation of wines, the levels of iron, lead and cadmium were assayed on Flame and Graphite Furnace Atomic Absorption Spectrophotometer. For red wine, iron is reduced to 90% of its initial value with chitosan; the corresponding values for chitin and chitin glucan were 80%, and 73% respectively. Cadmium is decreased to 29% of its initial value with chitosan; the corresponding values for chitin and chitin glucan were 27% and 57% respectively. Lead is decreased to 74% of its initial value with chitosan, the corresponding values for chitin and chitin glucan were 51% and 33% respectively.

In the case of white wine, iron is reduced to 91% of its initial value with chitosan, the corresponding values for chitin and chitin glucan were 34% and 32% respectively. Cadmium is decreased to 11% of its initial value with chitosan, the corresponding values for chitin and chitin glucan were 23% and 17% respectively. Lead is decreased to 65% of its initial value with chitosan, the corresponding values for chitin and chitin glucan 50% and 58% respectively.

For wine enriched in organic contaminant (Ochratoxin A), treatments with chitosan, chitin or chitin glucan were realized with doses of 2g/L or 5g/L. After a 2 days experimental period with agitation , the levels of Ochratoxin A in wines were analyzed by HPLC with fluorimetric detection. For red wine, the levels of OTA are reduced until 83,4% with chitosan, 66,7% for chitin and 56,7% with chitin glucan. In the case of white wine, the levels of OTA are reduced until 53,4% with chitosan, 53,4% for chitin and 64,5% with chitin glucan.

Our results indicates a real interest in the use of chitosan, chitin glucan and chitin for wine clarification to reduce levels of iron, heavy metals (Pb, Cd) and mycotoxins (Ochratoxin A) to improve the hygienic quality of wine in the frame of food safety.

Introduction

Chitin, chitosan and derivatives are known biodegradable polymers based on polysaccharides, which are extracted from various animals and plants. Chitin exists widely in cells walls of some microorganisms such as fungi, molds and yeast and in the exoskeletons of invertebrates such as crustaceans, mollusks, crabs, shrimps, lobster, squid ad insects. Chitosan exist only in 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 as repeating units respectively. 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 shows the deacetylation reaction of chitin. It is also one of the nontoxic and biodegradable carbohydrate polymers. Numerous studies have demonstrated that chitosan and its derivatives have various biological activities such as antimicrobial activity, antitumor activity and immune-enhancing effects. An interesting innovation purposed by Kitozyme is the possibilty to obtain these polysaccharides molecules : chitin, chitin-glucan, and chitosan from fungal origin after some specific industrial hydrolysis process. Because of their high toxicity, minerals (lead and cadmium) or organic mycotoxin contaminants (ochratoxin A) need to be quantified and reduced as low as possible in beverages and in wine in particular to improve food safety. Possible haze formation in wine, can be due to iron and is a well known common problem. The level of this compound need also to be reduced to protect wine from possible haze formation. In this work, we used new polysaccharides molecule (chitin, chitin-glucan, chitosan from fungal origin, Kitozyme Firm) in wines to remove lead, cadmium, iron and ochratoxin A .

Material and Methods

Materials

The samples used in our tests were semiprocessed, bottled wine from 2 cellars in southern France, namely: Chardonnay white wine (W), Merlot red wine (R), Grenache-macabeu natural sweet wine (S). The novel polysaccharide adsorbent auxiliary used for tests were produced by Kitozyme S.A.Herstal, Belgium : chitin Ch, chitin-glucan ChG, chitosan C

Stabilization treatments

For each sample of wine, 100 mg , 500 mg , 2000mg, or 5000 mg of the adsorbent were added to 1000 mL of the wines and slightly stirred at 20°C with a contact time up to 48 h. The solutions were analyzed after centrifugation at 3000 g. Subsequently, 50 mL aliquots of each of the 3 wines were used for analysis.

Methods of analysis

All analyses were carried out in triplicate an representative sample of wine, and effected on the wine before and after treatment with absorbent.. Wines (W,R,S) are spiked in minerals (iron 20 mg/L and copper 2 mg/L or lead 500 µg/L and cadmium 20 µg/L) or (Ochratoxin A 5µg/L) organics contaminants. For wine enriched with minerals contaminants, treatments with polysaccharides derived from fungal at the dose of 0,1g/L or 0,5g/L or 2g/L were realized. After a 2 days experimental period with agitation of wines, the levels of iron, copper, lead and cadmium were assayed on Flame and Graphite Furnace Atomic Absorption Spectrophotometer. Iron measurement is realized after a suitable dilution of the wine and removal of alcohol. Iron is determined, by the

OIV validated method, directly by atomic absorption spectrophotometry at wavelength of 248.3 nm in comparison with standards measurements. Lead measurement is obtained, by the OIV validated method, after a suitable dilution of wine Lead is determined directly in wine by graphite furnace atomic absorption spectrophotometry with a selected wavelength of 283.3 nm in comparison with standard measurements. Cadmium is determined, by the OIV validated method,directly in the wine by graphite furnace atomic absorption spectrophotometry with a selected wavelength of 228.8 nm in comparison with standards measurements. For wine enriched in organic contaminant (Ochratoxin A), treatments with F1 were realized with doses of 2g/L or 5g/L. After a 2 days experimental period with agitation, the levels of Ochratoxin A in wines were analyzed by using an immunoaffinity column and HPLC with fluorimetric detection. The validated method used for determining ochratoxin A (OTA) in red, white, sweet wines, including special wines, in concentrations ranging up 10 µg/L using an immunoaffinity column and high performance liquid chromatography (HPLC) with fluorescence detection in comparison with standards. Wine samples are diluted with a solution containing polyethylene glycol and sodium hydrogen carbonate. This solution is filtered and purified on the immunoaffinity column. OTA is eluted with methanol and quantified by HPLC in inverse state with fluorimetric detection (4).

Results and Discution

Instability of wine can be found with 4 types of hazes formation. Excessive iron levels in wine (10 to 20 mg/L or more) with oxidation process (ferric form) can generate a precipitation of colored pigments materials (Blue haze) or with orthophosphate ions (White haze). Also the level of iron in wine is controled prior to bottling. In our experiments at the highest treatment dose 200g/HL, for red wine (R) , iron was reduced to 90% of its initial value with chitosan, for white wine, iron was reduced to 91% of its initial value with chitosan , and for sweet wine, iron was reduced to 98% of its initial value with chitosan. The toxic effects of cadmium are due to its inhibition of various enzyme systems. it is able to inactivate enzymes containing sulphydryl groups and it can also produce uncoupling of oxidative phosphorylation in mitochondria. Cadmium may also compete with other metals such as zinc and selenium for inclusion into metallo-enzymes and it may compete with calcium for binding sites on regulatory proteins such as calmodulin. A best effect treatment for cadmium removal was at the dose 200g/hl. The polysaccharides eliminate hightest of cadmium is chitin-glucan for red wine. Our results indicates that chitin glucan decreased cadmium for red wine (R) to 57% of its initial value, to 17% for white wine (W) to 25% for sweet wine (S). No real dose effect was found between the treatments at 10, 50 and 200g/Hl for white and sweet wines . Lead perturbs multiple enzyme systems, as in most heavy metals, any ligand with sulfhydryl groups is vulnerable. The best-known effect is that on the production of heme. Lead interferes with the critical phases of the dehydration of aminolevulinic acid and the incorporation of iron into the protoporphyrin molecule; the result is a decrease in heme production. Because heme is essential for cellular oxidation, deficiencies have far-reaching effects. Lead is renally excreted, but the elimination rate varies, depending on the tissue tha absorbed the lead. The reduction of lead source in foods and beverages is a necessity to improve food safety. In our experiment, a best effect treatment for lead removal was at the dose 200g/hl for chitin-glucan. We found for red wine that lead is decreased until 33% with chitin-glucan, 58% in the case of white wine, 38% in the case of sweet wine.

Ochratoxin A is a toxic metabolite produced by several molds of the Aspergillus flavus and Penicillium genera, including Aspergillus ochraceus. The fungal species has the potential to produce ochratoxin A, a known nephrotoxin and carcinogen. It has been frequently detected in human foods (mainly in cereal products) but also in beverages. In humans, exposure to ochratoxin A has been linked with Balken endemic nephropathy, a chronic kidney disease associated with tumors of the renal system, including Aspergillus ochraceus. Wine and grape juice have been identified as a possible source of ochratoxin A (mycotoxin).The main fungi responsible in grapes and wines have been identified to be Aspergillus section Nigri, in particular Aspergillus carbonarius and Aspergillus nigri. The European Community established, with regulation 123/2005 dated 26th

January 2005, the maximum allowable concentration of Ochratoxin A in wine, must and grape juice.Starting from April 2006, it will be forbidden to market batches that will not satisfy the maximum limit of 2 micrograms/kg (ppb). Our results showed a dose dependance with highest removal of OTA with the treatment dose of 500 g/HL. Removal of OTA is twice at 500 g/HL in comparison with 200 g/HL. For red wine (R), levels of OTA are reduced until 83,4% with chitosan, for white wine (W) OTA level are reduced until 64.5% with chitin-glucan, and for sweet wine (S) OTA level are reduced until 43.5%with chitin glucan.

Lead and cadmium in wine are 99,9 % on the forms Pb^{2+} and Cd^{2+}. The chitin glucan used is able to chelate lead and cadmium. The possible explanation of iron , lead and cadmium removal can be the result of Fe^{2+}, Pb^{2+} and Cd^{2+} chelation with hydroxyls groups located in position C-3 of chitin, and the binding of hydroxyls groups located in position C-3 of ß-glucan part. Possibly an alternative to explain high adsorption abilities of chitin glucan particles for heavy metals ions could be attributed to the deposition of metal hydroxide aggregates in pores of chitin glucan particles. To explain adsorption abilities of chitin glucan particles for OTA, it could be attributed to OTA aggregation with deposition in pores of chitin or chitin-glucan. Our results indicate a real interest in the use of the fungal biopolymer chitin-glucan and derivates, as technology auxiliary for wine clarification to reduce levels of iron , heavy metals (Pb, Cd) and mycotoxins (Ochratoxin A) to improve the hygienic quality of wine in the frame of food safety.

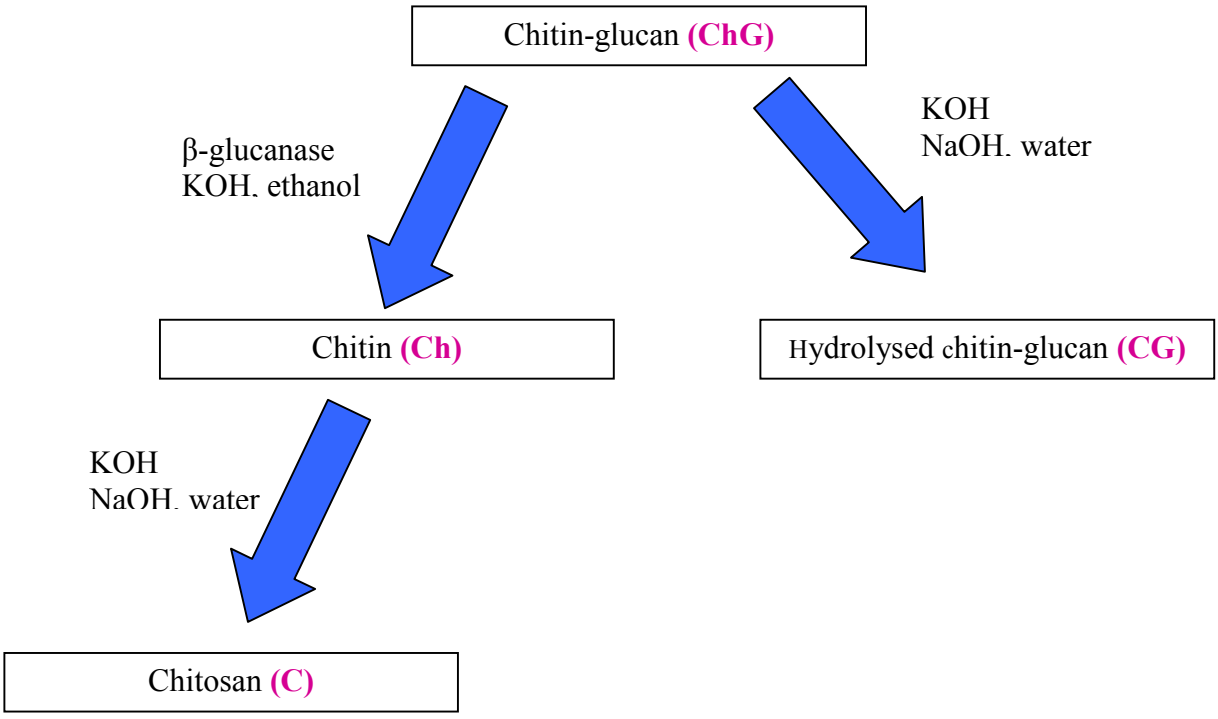
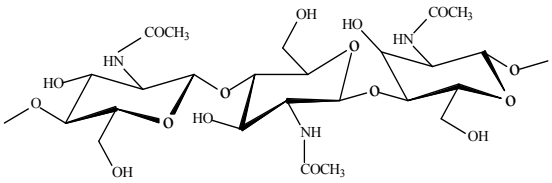
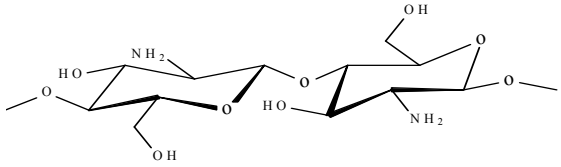


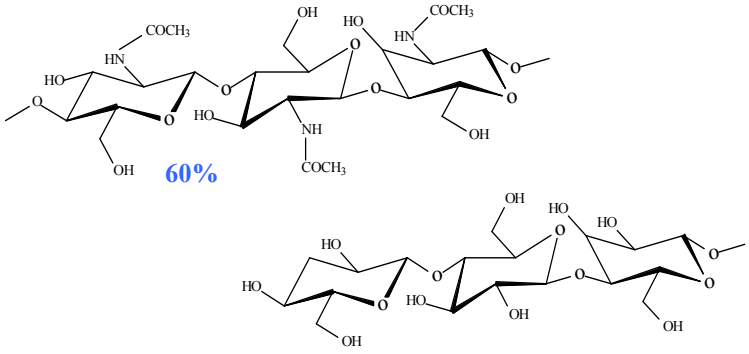
Figure 1 : Process extraction chitin and derivatives by KITOZYME.



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



Chitosan (C)
N-acetylated form of chitin



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

Figure 2 : Stucture of chitin and derivatives

		CPT (mg GAE/l)	**TAV** (%vol)	**Ac T.** (g/l H2SO4)	**Residual sugar** (g/l)	**SO2 T.** (mg/l)	**pH**
Red wine	Merlot	2075,0	13,55	2,8	2,3	82	3,88
White wine	Chardonnay	273,3	12,85	3,0	2,5	121	3,55
Sweet wine	Grenache-macabeu	370,8	15,95	2,6	121,0	127	3,86

CPT: Total phenol content in mg acid gallic equivalent/L; TAV: alcohol level %vol.; Ac.T.: Total acidity in gH2SO4/L; SO2 T.: Total sulfite in mg/l

Figure 3 : Analytical characteristics of wines

	Pb (µg/l)			Cd (µg/l)			Fe (mg/l)		
	Red wine	White wine	Sweet wine	Red wine	White wine	Sweet wine	Red wine	White wine	Sweet wine
Initial level	**150**	**111**	**110**	**19**	**18**	**10**	**23**	**6**	**5**
ChG 200g/hl	**101**	**47**	**68**	**8,8**	**15,2**	**7,0**	**6**	**4**	**1**
ChG 50g/hl	104	79	82	8,5	16,0	9,0	6	4	3
ChG 10g/hl	118	100	75	8,2	14,8	8,8	7	5	4
Ch 200g/hl	**73**	**55**	**110**	**15,2**	**15,0**	**8,4**	**4**	**4**	**2**
Ch 50g/hl	89	62	94	14,1	13,9	8,4	6	5	4
Ch 10g/hl	89	81	64	16,6	14,6	8,0	7	4	5
C 200g/hl	**39**	**38**	**18**	**14,4**	**16,6**	**8,4**	**2**	**0,5**	**0,3**
C 50g/hl	51	53	51	13,9	17,2	7,8	3	2	0,6
C 10g/hl	94	63	58	14,3	16,1	9,6	5	5	2

Figure 4 : Levels of lead, cadlium and iron in wine for 3 doses treatment by chitin-glucan, chitin and chitosan.

	OTA (µg/l)		
	Red wine	White wine	Sweet wine
Initial level	**3,7**	**4,3**	**4,7**
ChG 500g/hl	**1,3**	**1,6**	**2,6**
ChG 200g/hl	2,6	3,3	3,6
Ch 500g/hl	**1,0**	**2,1**	**3,0**
Ch 200g/hl	2,4	3,6	3,3
C 500g/hl	**0,5**	**2,1**	**3,4**
C 200g/hl	2,8	3,4	2,8

Figure 5 : Levels of Ochratoxin A for 2 doses treatment by chitin-glucan, chitin and chitosan.

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