

Summary

Chitosan is a naturally occurring carbohydrate polymer that is widely distributed in nature (crustacean shells, fungal cell walls). It is a non-allergenic, biodegradable polysaccharide of glucosamine and N-acetylglucosamine that is derived from chitin. Fungal chitosan is obtained by deacetylation of chitin present in the cell walls of non-genetically modified *A. niger* mycelium.

Fungal chitosan from *A. niger* is proposed for use as a processing-aid in the manufacture of wine, beer, cider and spirits, as well as grain and beet derived food grade ethanol. They are proposed for use in stabilizing through their antimicrobial effect on a number of economically important microbial contaminants such as *Brettanomyces* as well as for flotation, clarification to reduce cloudiness and the content of unstable colloids, for use as fining agent in the treatment of wine, for stabilization of the color, for riddling of sparkling wine, for reducing organic and mineral contaminants in wine and spirit, and for encapsulation of fermentation yeast, lactic bacteria and nutrients. Two fungal chitosans are proposed which differ slightly in their residual glucan contents as well as in their granulometry.

Fungal chitosan as a processing aid does not fall in any of the categories of processing aids listed in FSANZ standard 1.3.3.

Evidence that the form and the amount of the processing aid perform the intended function has been demonstrated through a thesis, laboratory and industrial experimentations. These assessments have been conducted in particular during two years consecutively experiments (year 2008-2009 and 2009-2010) under derogation of the DGCCRF (the French official body supervising regulations), in the frame of the Regulation EC 423/2008 (Art. 44) and Regulation EC 606/2009, respectively.

As fining agent in the treatment of wine, for stabilization of the color, for riddling of sparkling wine, clarification, and removal of mineral and organic contaminant, fungal chitosan is added at the end of the alcoholic fermentation.

For flotation clarification from must, chitosan can be added before or during the alcoholic fermentation. In spirits, it is used before filtration and bottling to remove mineral contaminants.

For microbiological stabilization in wine, cider, and beer, chitosan can be added during all process of winemaking.

Regardless of the technological purpose, the sediments that contain the chitosan are removed from the wine, must, or spirits at the end of the treatment by physical separation processes such as racking, centrifugation and/ or filtration. Since chitosan is insoluble at slightly acidic to neutral pH levels, as well as in aqueous and ethanol solutions, it is unlikely that any residual chitosan will remain in the treated

products. High-performance liquid chromatography (HPLC) analyses for residual chitosan in wine processed with chitosan indicate that the final product is free from chitosan carry-over products up to the limit of detection of the analysis method (10 mg/L).

Therefore, the estimated intake of chitosan from all proposed technological uses can be considered as negligible. Chitosan derived from *A. niger*, was shown to be chemically and structurally equivalent to shellfish derived chitosan. The principal difference between the two chitosan preparations is the presence of small quantities of *beta*-1,3-glucans in *A. niger* sources of chitosan that are not present in shellfish chitosan. Therefore, data establishing the safety of shellfish-derived chitosan are considered relevant to the safety evaluation of fungal chitosan for the proposed food uses described herein.

Shellfish derived chitosan is widely available in the food supply through use in dietary supplement products, industrial, pharmaceutical, agricultural, and cosmetic applications, and background exposures to chitosan are therefore expected to exceed those occurring from the proposed food uses of fungal chitosan. Thus, based on the absence/trivial exposure to chitosan under the proposed food uses, calculation of estimated intakes was not deemed necessary in the assessment of the safety of the material under the proposed food uses in wine/alcoholic beverage processing for the GRAS determination.

A number of animal, human, and *In vitro* studies relevant to the safety of shellfish chitosan, which has a long history of safe use in the food supply, have been published. Published studies examining the metabolism and kinetics; acute, subchronic, and chronic toxicity; reproductive toxicity in animals; and safety in human of shellfish-derived chitosan or chitosan oligosaccharides are presented in the dossier.

Shellfish derived chitosan has a long history of safe use in the food supply. It is currently approved for use as a natural food additive for general food use in Japan and Korea (Japan Food Chemical Research Foundation, 2011; KFDA, 2011), and has widespread use as a dietary supplement product in the United States, the European Union, and other regulatory jurisdictions throughout the world. Finally, fungal chitosan (derived from *Agaricus bisporus* and *Aspergillus niger* sources) has been granted Novel Food approval by the European Commission, for use in supplement products in the European Union based on its substantial equivalence to existing shellfish derived chitosan products that are currently in the market¹.

Resolutions permitting the use of fungal chitosan in winemaking as a fining agent and contaminant treatment have been granted by the International Organisation of Vine and Wine (OIV/OENO 336A/2009; 337A/2009; 338A/2009; 339A/2009) (OIV, 2011) (Attachments 1-4).

A monograph for fungal chitosan has been added to the International Oenological Codex by decision of the OIV general assembly dated July 2009 considering the works of the group of experts "Specifications of Oenological Products" (OIV/OENO OIV/OENO 368/2009).

The corresponding approval for use of fungal chitosan in wine products marketed within the European Union has been issued by the European Commission (EU, 2011). Since 2011, chitosan from fungal origin is therefore approved as oenological practices for clarification according to ANNEX I of REGULATION (EU) No 53/2011 and for treatment of wines.

Fungal Chitosan (from *Aspergillus niger*) is GRAS self-affirmed for use as a processing-aid in the manufacture of alcoholic beverages. This GRAS self-affirmation was notified to the offices of the U.S. Food and Drug Administration (FDA), without objection from the Agency (FDA 2011, GRAS NOTICE No. 397).

The application of fungal chitosan in the winemaking process has been approved by the National Authority of Argentina.

The European Union requested the addition of chitosan and chitin-glucan of fungal origin to the Annex of the Wine Agreement in November 2010. Provisional approval was granted for the use of these products in European wine exported to Australia under the Wine Agreement.

