FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting Document 1

Risk and Technical Assessment Report – Application A1077

Fungal Chitosan as a Processing Aid

Executive Summary

This Application seeks approval to use fungal chitosan as a processing aid for the production of wine, beer, cider, spirits and food grade ethanol. Fungal chitosan is produced by chemical deacetylation of the polysaccharide chitin derived from the fungus *Aspergillus niger*. Chitosan derived from crustaceans has a history of safe use as a processing aid for the production of fruit juices and is also used at high oral doses (grams per day) as a weight loss supplement. Chitosan derived from *A. niger* has been recently approved for use as a wine processing aid in Europe.

The Applicant has clearly articulated the technological function of fungal chitosan when used as proposed. The available data indicate that fungal chitosan is an efficacious treatment of wine and alcoholic beverages as a processing aid to improve clarity and stability of the products by removing unwanted components during production and that it does not perform a technological function in the final food.

Animal toxicity studies on chitosan preparations of various molecular weights and degrees of acetylation did not show any treatment-related adverse effects following oral administration at high doses. A published review of human data from 13 clinical trials of up to 6 months duration found no adverse effects associated with oral chitosan (average daily dose 3.5 g) as a weight loss supplement. In view of the absence of adverse effects at high chitosan doses, a group Acceptable Daily Intake (ADI) “not specified” was established for chitosan derived from fungi.

Information was provided indicating negligible levels of fungal chitosan in wine following processing. Negligible levels would also be expected in beer and cider, while no residual fungal chitosan would be expected in alcoholic products derived from distillation.

The overall conclusion of this Risk and Technical Assessment is that the use of fungal chitosan as a processing aid for the production of wine, beer, cider, spirits and food grade ethanol is technologically justified and raises no public health and safety issues for consumers.

Table of Contents

1. Introduction

1.1 Background

On 20 September 2012, Food Standards Australia New Zealand (FSANZ) received an Application from the Winemakers' Federation of Australia, seeking an amendment to the Table to clause 14 of Standard 1.3.3 – Processing Aids in the *Australia New Zealand Food Standards Code* (the Code) to permit the use of fungal chitosan as a processing aid for the production of wine, beer, cider, spirits and food grade ethanol.

1.2 Risk Assessment Questions & Scope

The following questions are addressed in this Risk and Technical Assessment Report:

- Is the use of chitosan as a processing aid for the production of wine, beer, cider, spirits and food grade ethanol technologically justified?

- Are the products made using chitosan as a processing aid safe for consumption?

This Risk and Technical Assessment Report addresses the above questions in order and comprises the following components:

(1) Food Technology Assessment, which describes the chemical properties of chitosan and considers whether the use of chitosan as a processing aid is technologically justified.

(2) Risk Assessment, which evaluates the intrinsic toxicity of chitosan and the potential risk to consumers from residual chitosan in alcoholic beverages produced through its use.

2. Food Technology Assessment

2.1 Chitosan Characteristics

The following information regarding the identity and chemical and physical properties of the processing aid chitosan has been taken from the Application and various references.

2.1.1 Chemical structure and identity

Chitosan is a linear copolymer comprised of randomly repeating glucosamine and N-acetylglucosamine units connected by β→(1,4) type linkages. The chemical structure is represented by Figure 1.

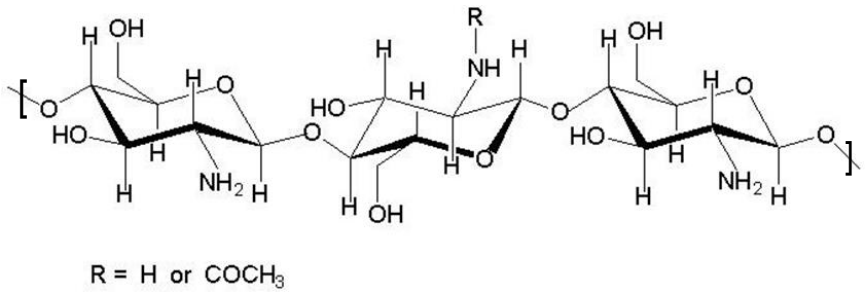

Figure 1 *Chemical structure of chitosan*

Common name:	Chitosan
Molecular formula:	$(C_6H_{11}NO_4)_n (C_8H_{13}NO_4)_m$
CAS register number:	9012-76-4
Molecular weight range:	10-15 kDa as determined by viscometry
Degree of acetylation:	0-30% on a molar basis

2.1.2 Chemical and physical properties

The chemical and physical properties of chitosan vary depending on molecular weight and degree of acetylation. Ranges for these parameters for the fungal chitosan product of this Application are reported above (2.1.1). The fungal chitosan preparation is an odourless, off white to slightly brownish, fine, free-flowing powder with a settled density ≥ 0.7 g/cm^3. It is insoluble in ethanol and in aqueous media at slightly acidic to neutral pH.

2.1.3 Production

Source of chitosan and differences between crustacean and fungal derived chitosan

Chitosan is obtained by the deacetylation of chitin, a carbohydrate polymer that is widely distributed in nature, notably in crustacean shells and fungal cell walls. The chitosan of this Application is derived from chitin extracted from the cell walls of the fungus *Aspergillus niger.* The Application included spectroscopic data indicating that the structures of chitosan from crustacean and fungal sources are closely similar. However, the identity of the chitosan origin can be determined from three characteristics: the residual content of β-1,3-D-glucans, the viscosity of a 1% solution and the settled density. The differences are noted in the OIV (International Organisation of Vine and Wine) monograph (including specification – see Section 2.1.4) for fungal derived chitosan (OIV chitosan monograph, Resolution 368, 2009).

β-1,3-D-glucans are present in larger amounts in the chitosan product from *A. niger* than from shellfish, with levels greater than 2% w/w in the OIV specification. β-1,3-D-glucans are a major constituent of fungal cell walls as well as yeast cell walls, such as Baker's yeast and the yeasts used to produce alcoholic beverages.

Manufacture of chitosan sourced from *A. niger*

The chitosan preparation of this Application is derived from the post-fermentation biomass of *A. niger* used to produce citric acid. Chitosan is obtained from the partial de-acetylation (hydrolysis of acetyl groups) of chitin extracted from the cell walls of *A. niger* using sodium hydroxide and heat. Further treatment involves washing steps, solubilising using acetic acid and then re-precipitation using sodium hydroxide, filtration, washing, concentration, drying and milling.

2.1.4 Specifications

There is no specification for chitosan in either of the primary sources of specifications in Standard 1.3.4 – Identity and Purity (i.e. not in the JECFA (Joint FAO/WHO Expert Committee on Food Additives) Combined Compendium of Food Additive Specifications, nor the Food Chemicals Codex). A secondary source of specifications in Standard 1.3.4, the *International Oenological Codex* of the OIV, has a monograph (OIV/OENO 368/2009) on chitosan obtained from fungal sources including *A. niger* (this Application). Therefore, a specification for the substance is not required to be written into the Standard.

Although the chitosan requested for approval in this Application meets the current OIV specification, FSANZ has noted that there are some details within the OIV specification for chitosan that need addressing and future amendment by the OIV:

- Although a method for determination of acetylation degree is given in the OIV specification, the required range is not. The fungal chitosan of this Application is stated to have a degree of acetylation range of 0-30% on a molar basis. As the degree of acetylation is an essential defining chemical attribute of chitosan preparations, this needs inclusion in any proposed updates to the specifications.

- There are two references to "chitin-glucan" within the chitosan specification (see Sections 4.1: Aspect and solubility, and 4.2: Purity and soluble residues); however, there is a separate OIV specification for chitin-glucan. Hence, this needs correcting to delete inappropriate references to chitin-glucan in the chitosan specification.

- Chitosan is referred to as a white, odourless and flavourless powder; however, the product that the Applicant is seeking approval for in this Application states that fungal chitosan products are off-white to slightly brownish.

The above technical issues do not give rise to public health and safety concerns; however, FSANZ has notified the Applicant in order that they can approach the OIV to address these issues in a future update to the chitosan specification.

2.2 Technological function

Fining of wine is the act of adding a product to wine to remove suspended solids. Most of the suspended solids in wine have an electrical charge. Chitosan performs this function by carrying a positive charge and attracting particles of opposite charge, resulting in the formation of insoluble aggregates which sink to the bottom of the wine as sediment. Chitosan (positive charge) is especially popular in clearing white wines, since it does not require the aid of tannins to clear, as do some fining agents like gelatine. When used with negatively-charged Kieselsol (silicon dioxide) it is an effective remover of most suspended proteins and solids. Chitosan and Kieselsol are often sold as a set in sealed liquid envelopes as fining A (negatively charged Kieselsol) which is added to the wine first and then fining B (positively charged chitosan) added about a day afterwards. The resulting sediment is removed from the wine usually by filtration.

Chitosan has good affinity for polyphenolic compounds such as catechins, proanthocyanidins, cinnamic acid and their derivatives that can change the initial straw-yellow colour of white wines into deep golden-yellow colour due to their oxidative products (Shahidi *et al* 1999).

The Application references the various OIV resolutions relevant to the use of chitosan during wine production. These OIV resolutions include specific technological functions performed by chitosan. These are summarised below:

336A – 2009 (Musts – Fining using Chitosan)
Facilitate settling and clarification
Prevent protein haze

337A – 2009 (Wines – Fining using chitosan)
Reduce turbidity by precipitating particles in suspension
Prevent protein haze by partial precipitation

338A – 2009 (Wines – Treatment using chitosan)
a) Reduce heavy metal content, notably iron, lead, cadmium and copper
b) Prevent haze due to presence of iron and copper

c) Reduce possible contaminants, especially ochratoxin A
d) Reduce microorganism contamination, especially *Brettanomyces.*

Some, if not all, of these technological functions relevant to the production of wine are also applicable to the manufacture of other alcoholic beverages (beer, cider, spirits) and food grade ethanol.

An alternative approach to explaining the technological function of chitosan during the production of alcoholic beverages is to differentiate when chitosan is added in the production process.

Addition at the end of fermentation for:
fining of wine
for colour stabilisation of wine
riddling (traditional term, consolidate sediment prior to removal) of sparkling wine
clarification of wine and beer
removal of mineral and organic contamination in wine and spirits.

Addition before or during fermentation for:
flotation clarification of must.

Addition before filtration and bottling:
to remove mineral contaminants in spirits.

Addition during all production processes for:
microbiological stabilisation of wine, cider and beer.

Evaluation of efficacy of technological function

The Application contains an assessment report performed by the manufacturer of fungal chitosan (KitoZyme, Belgium) on the technological efficacy for must and wine production during 2008-2009 and 2009-2010 in France (Kitozyme 2010). The study was performed on various commercial wine productions during these two consecutive years of production, with a collective volume of greater than 44,000 litres of treated red wine.

The reported results were mainly directed at investigating the effect of microbiological contamination (levels of the spoilage yeast contaminant *Brettanomyces*) on treated wine compared to untreated. The conclusion was stated that fungal chitosan was shown to be effective as a microbiological stabilisation agent by eliminating the presence of *Brettanomyces*, irrespective of the initial rate of contamination. This is a major positive attribute of chitosan treatment for wine producers.

The study also found that fungal chitosan had no negative impact on colour, colour intensity or taste compared to the control untreated samples. Where a taste difference was noted, the treated sample was preferred compared to the untreated control.

Chitosan has been identified in the literature and is used commercially in many countries, sometimes with other substances and treatments for the production of wine and other alcoholic beverages.

For all treatments chitosan remains insoluble in the alcoholic beverages and chitosan along with the adsorbed unwanted components from the liquids are removed using physical processes such as filtration and racking. Negligible chitosan residues are expected in the final treated alcoholic beverage. Therefore no analytical methods are required to check for chitosan residues.

Individual alcoholic beverage companies (and industries) will conduct their own efficacy studies to determine if the use of fungal chitosan is commercially warranted as an alternative or additional treatment for their products.

2.3 Food Technology Conclusion

Investigations of the literature indicate that chitosan is an efficacious treatment of wine and alcoholic beverages as a processing aid to improve clarity and stability of the products by removing unwanted components during production and that it does not perform a technological function in the final food.

3. Risk Assessment

3.1 Introduction

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

Chitosan derived from *A. niger* is chemically and structurally equivalent to shellfish derived chitosan. However, 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 present only at negligible levels in shellfish chitosan. 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).

The Application included unpublished and published *in vitro*, animal and human studies on chitosan derived from crustacean and fungal sources of chitin. Several additional relevant studies were located in the published literature. Studies using non-oral routes of administration (e.g. dermal, subcutaneous and intravenous) were not considered in this Risk Assessment. The chitosan preparations used in the evaluated studies covered a range of molecular weights and degrees of acetylation. As indicated in Section 2.1.3, the Application included spectroscopic data supporting the chemical and structural similarity of chitosan derived from *A. niger* and crustaceans. Therefore, data relevant to the safety of crustacean derived chitosan are considered relevant for the safety evaluation of chitosan derived from *A. niger*.

3.2 History of Use

Chitosan derived from crustaceans has a history of use as a processing aid for the production of fruit juices/nectars and wine (see Overseas Approvals), and in over-the-counter products marketed for weight loss and improvement of blood lipid profiles. In 1998, the Australian Complementary Medicines Evaluation Committee recommended that chitosan derived from crustaceans was suitable for use as an active ingredient in Listable medicines without limits (TGA 1998). Products containing chitosan at up to 600 mg per tablet/capsule are on the Australian Register of Therapeutic Goods.

3.3 Overseas Approvals

Fungal chitosan is an approved wine processing aid in the European Union (EU 2011). Chitosan from *A. niger* is Generally Recognized as Safe (GRAS) under US FDA regulation (FDA 2011). Chitosan is listed as a processing aid in the Codex General Standard for Fruit Juices and Nectars (Codex 2005), and is an approved Food Additive in Japan (JFCRF 2011).

3.4 Absorption, Distribution, Metabolism and Excretion

There are limited data on the absorption, distribution, metabolism and excretion (ADME) of chitosan following oral administration. Studies in mice and rats have reported systemic exposure to chitosan labelled with a fluorescent dye (Chae et al 2005; Zeng et al 2008a). While the authors reported the presence of fluorescent tagged material in plasma, with peak concentrations 0.5 to 1 hour post-dose, it is most likely that this is due to absorption of short chain oligomers and/or monomers already present in the administered test material.

Orally administered glucosamine, the major monomeric constituent of chitosan, is poorly absorbed in the animal species tested, with reported bioavailabilities of 10% in dogs, 2.5-6% in horses, and as high as 20% in rats (Simon et al 2011).

3.5 Toxicity

3.5.1 Acute toxicity

Single gavage doses of chitosan (2000 mg/kg bw) or vehicle control (distilled water) were administered to female Sprague-Dawley rats (6/group) and the animals were observed for 14 days. The chitosan test article, derived from the edible mushroom *Agaricus bisporus*, had an average molecular weight (MW) of 67 kDa and degree of acetylation of 16% on a molar basis. No deaths occurred during the study. No clinical signs related to the administration of chitosan were observed. Body weight gain was similar between treated and control animals. Macroscopic examination of selected organs/tissues at the end of the study did not reveal any treatment-related changes (Seguier 2008).

Single gavage doses (1000, 2150, 4640 or 10000 mg/kg bw) of oligomeric chitosan (MW 1.86 kDa; derived from shrimp chitosan with degree of acetylation 15%) or vehicle control (distilled water) were administered to Kunming mice (5/sex/group) and the animals were observed for 7 days. There were no deaths and no clinical signs related to the administration of oligomeric chitosan (Qin et al 2006).

3.5.2 Sub-chronic toxicity

Oligomeric chitosan (MW < 1 kDa; degree of acetylation and source not provided) was administered by gavage to Sprague-Dawley rats (9/sex/group) at doses of 0 (vehicle identity not stated), 500, 1000 or 2000 mg/kg bw/day for 28 days. Observations regarding mortality and clinical signs were not reported. No statistically significant between-group differences were observed with respect to food consumption, body weight. Statistically significant differences between groups were reported for several urinalysis, clinical chemistry and haematology parameters; however these differences were either not dose-dependent and/or occurred in only one sex, or the altered parameter was still within the normal range. These statistically significant differences and are therefore not considered to be treatment-related. There were no statistically significant differences between groups for organ weights (absolute and bw relative; testis, ovary, kidney, spleen, liver, lung). Gross pathology observations were not reported. There were no histopathology findings considered related to treatment. The no

observed adverse effect level (NOAEL) was therefore considered to be the high dose of 2000 mg/kg bw/day (Kim et al 2001).

Sprague-Dawley rats (10/sex/group) were fed diets containing oligomeric chitosan at concentrations of 0, 0.75, 1.5 and 3.0% w/w. Diets and water were provided ad libitum for 30 days. The oligomeric chitosan test article had an average MW of 1.86 kDa and was derived from shrimp chitosan with a degree of acetylation of 15%. There were no deaths and no treatment related clinical signs or effects on food consumption, body weight, clinical chemistry, haematology, organ weights (heart, liver, kidney, spleen, thymus, testis; absolute and bw relative), gross pathology and histopathology (liver, kidney and small intestine examined). The no observed adverse effect level (NOAEL) was therefore considered to correspond to the high dietary concentration of 3.0% w/w which is equivalent to a calculated dose of 1500 mg/kg bw/day (Qin et al 2006).

F344 rats (10/sex/group) were fed diets containing oligoglucosamine at concentrations of 0, 0.04, 0.2 or 1.0% w/w. Diets and water were provided ad libitum for 90 days. The test article was stated to be a mixture of D-glucosamine and its dimer, trimer, tetramer, pentamer and hexamer, and was produced by the hydrolysis of chitosan (source not stated), followed by purification. There were no deaths. There were no treatment related clinical signs in the 0.04% and 0.2% groups. In the 1% group, erythema and swelling of the snout and forelimbs and loss of forelimb fur were observed in both sexes. The study authors suggested that these findings might be due to dermal responses to oligoglucosamine adhering to the skin and fur, which are easily soiled with saliva during grooming. In the 1% group, food consumption decreased ($p < 0.01$; significantly different from the control group), resulting in reduced body weight gain ($p < 0.01$); however, the reductions were substantially greater in magnitude in males. Over the 90 days, body weight gain in 1% males was only 60% of controls, while in 1% females it was 90%. It is possible that the topical lesions on the snout and forelimbs adversely affected feeding resulting in reduced body weight gain. In males of the 1% group, neutrophils were increased while lymphocytes and platelets were decreased ($p < 0.01$ for each). These changes might be related to the dermal inflammation. Abnormalities in urinalysis (proteinuria, ketone bodies, increased bilirubin) and clinical chemistry (decreased calcium, albumin, cholesterol, triglycerides, glucose), as well as small thymus, small spleen, dark spots or areas on the glandular stomach mucosa, pale Harderian glands and small testes, were observed in males in the 1% group. These changes may be due to malnutrition resulting from reduced food intake. The no observed adverse effect level was determined to be 0.2% w/w corresponding to doses of 124 mg/kg/day in males and 142 mg/kg/day in females (Naito et al 2007).

Kunming mice (10 females/group) were fed diets containing one of four chitosan preparations of various average molecular weights and degrees of acetylation. A single dietary concentration of 1.05% w/w was examined equivalent to a calculated dose of 500 mg/kg bw/day. Diets and water were provided ad libitum for 90 days. The average molecular weight and degree of acetylation of the four chitosan preparations were as follows: (i) 760 kDa, 14.5%; (ii) 32.7 kDa, 14.8%; (iii) 990 Da, 14.3%; (iv) 39.1 kDa, 47.3%. There were no deaths or clinical signs. Food consumption and body weight gain were unaffected by treatment. Clinical chemistry, haematology and urinalysis investigations were not conducted. There were no gross pathology or haematology findings related to treatment (heart, liver, kidney, spleen, thymus and lung were examined). Thymus weight/bw ratio was decreased ($p < 0.05$) in animals receiving chitosan preparation (iv), however there were no associated histopathology findings. Tissue levels of iron, zinc and copper were examined for heart, liver, spleen, and kidney. Statistically significant differences from the control group were only observed with chitosan preparation (ii), for which increased iron was observed in liver and spleen, increased zinc was observed in liver, spleen and heart, and increased copper was observed in liver ($p < 0.05$ for all increases). The study authors suggested that chitosan

preparation (ii) may act to increase the bioavailability from the diet of the metals examined (Zeng et al 2008b).

3.5.3 Chronic toxicity and carcinogenicity

The US National Toxicology Program (NTP) has conducted a 26 week feeding study with chitosan in Sprague-Dawley rats (10/sex/group). A study report is not yet published, however some tabulated data are publically available, namely data on survival, bodyweight, and individual animal data on non-neoplastic observations (NTP 2009). The dietary concentrations of chitosan (source, average MW and degree of acetylation were not provided) were 0, 1, 3 and 9% w/w, equivalent to calculated doses of 0, 500, 1500 and 4500 mg/kg bw/day. There were no deaths. Body weight gain was decreased by about 10% in both sexes at the high dietary concentration of 9%, with no effect evident at the lower concentrations (statistical analysis not available). An analysis of the non-neoplastic data is not yet available (as at May 2013).

3.5.4 Genotoxicity

Chitosan preparations have been tested in several *in vitro* and *in vivo* genotoxicity assays as summarised in the Table 1. There was no evidence of genotoxicity. Negative and positive controls were used in all assays and gave expected results.

Table 1: Genotoxicity assays

Test type	Test system	Test article	Concentrations /doses	Result	Reference
Bacterial reverse mutation	*Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100, and *E. coli* WP2 uvrA pKM 101 (±S9)^	Chitosan (average MW 67 kDa, degree of acetylation 16%. Derived from *Agaricus bisporus*) dissolved in 0.9% saline	10 – 1000 µg/plate	Negative	Vivotecnia (2008)
Bacterial reverse mutation	*S. typhimurium* TA 97, TA 98, TA 100, TA 102 (±S9)^	Chitosan oligomer (average MW 1.86 kDa. Derived from shrimp chitosan with degree of acetylation 15%) dissolved in distilled water	0.5 – 5000 µg/plate	Negative	Qin et al (2006)
Micronucleus induction	Bone marrow cells from Kunming mice (5/sex/group)	As above	0, 1250, 2500, 5000 mg/kg bw (two gavage doses 24 h apart)	Negative	Qin et al (2006)
Micronucleus induction	Bone marrow cells from female ICR mice (20/group)	Chitosan (MW ≤ 10000, degree of acetylation 10%, source not stated)	0, 0.01, 0.1 and 1% w/v in drinking water for 7, 60 and 180 days	Negative	Yoon et al (2005)

Chromosomal aberration	Bone marrow cells from female ICR mice and offspring of 3 subsequent generations (20/group)	As above	As above	Negative	Yoon et al (2005)

^ The bacterial reverse mutation assays were performed both in the presence and absence of rat liver microsomes as a metabolic activation mixture (±S9).

3.5.5 Reproductive and developmental toxicity

Female B6C3F1 mice (15/group) were placed on a standard control diet or a high fat diet for 4 weeks followed by administration of chitosan by gavage at 480 mg/kg bw/day for 4 days. Mice were then treated with gonadotropin by intraperitoneal injection to induce superovulation. The chitosan test article was stated to be water soluble with an average MW of 300 kDa and degree of acetylation <10% (source not stated). Chitosan treatment had no effect on oocyte production rates and fertilization rates in animals fed a standard control diet. In contrast, chitosan treatment was associated with a small statistically significant increase ($p < 0.05$) in these parameters in mice fed a high fat diet (Choi et al 2002).

Kunming mice (5 males/group) were administered chitosan oligomer by gavage at doses of 0, 1250, 2500, 5000 mg/kg bw/day for 5 days. The chitosan oligomer test article had an average MW of 1.86 kDa and was derived from shrimp chitosan with degree of acetylation 15%. Microscopic examination of sperm (n=1000/animal) indicated no statistically significant differences in morphological parameters between treated groups and the negative control group. A positive control group gave a statistically significant ($p < 0.001$) elevation of abnormal sperm heads compared with the negative control (Qin et al 2006).

No studies on developmental toxicity were located.

3.5.6 Human studies

Oral chitosan as a weight loss treatment has been examined in a large number of clinical trials. The Cochrane Collaboration reviewed data from 13 trials that provided quantitative data on numbers of adverse events and found that there was no clear difference between intervention and control groups in terms of frequency of adverse events (Jull et al 2008). The average chitosan dose and study duration in these studies was 3.5 g per day and 8.5 weeks, respectively. The dose administered in the longest duration study (6 months) was 4.5 g per day. EFSA recently published a scientific opinion on food-health relationships related to chitosan, however this opinion did not consider safety aspects (EFSA 2011).

3.5.7 Potential allergenicity

Fungal chitosan is stated to contain a low concentration of proteins (~0.5%; Kitozyme 2011) which are carried over during production from the source organism, *Aspergillus niger*. However, residual levels of *A. niger* proteins in products derived using fungal chitosan as a processing aid would be expected to be extremely low.

As *A. niger* is a widely distributed fungal contaminant commonly detected in a range of foods, consumption of the organism is expected to occur in the diet of most individuals (EFSA 2010). In addition, a number of approved enzyme processing aids are produced using *A niger* as a source organism. No reports were identified in the medical literature of allergic reactions to foods attributable to proteins derived from *A. niger*.

The allergenic potential of products derived using fungal chitosan as a processing aid is therefore considered to be negligible.

3.6 Hazard Characterisation

Animal toxicity studies on chitosan preparations of various molecular weights and degrees of acetylation did not show any adverse effects. A published review of human data from 13 clinical trials of up to 6 months duration found no adverse effects associated with oral chitosan (average daily dose 3.5 g) as a weight loss supplement. No specific toxicity studies are available on the particular fungal chitosan product for which approval is being sought, however fungal chitosan has no physicochemical or compositional attributes that would suggest a hazard profile that differed from other chitosan preparations. In view of the absence of any adverse effects at high chitosan doses a group Acceptable Daily Intake (ADI) "not specified" was established for chitosan obtained from fungal sources.

3.7 Residual Levels in Food

Fungal chitosan is stated to be insoluble in ethanol and in aqueous solutions at slightly acidic to neutral pH levels, enabling physical removal from treated products by filtration, centrifugation, or racking. Negligible residual chitosan is therefore expected in the final products. High performance liquid chromatography of wine processed with fungal chitosan indicated that the final product was free from chitosan at the limit of detection (10 mg/L). Negligible levels of chitosan would also be expected in beer and cider produced using chitosan, while no residual chitosan would be expected in products derived from distillation (spirits and food grade ethanol).

The Application states that β-1,3-D-glucans (10-15% w/w maximum) are present in fungal chitosan as residues from the manufacturing process. No information was provided on expected levels of β-1,3-D-glucans in alcoholic beverages that have been produced using fungal chitosan, however β-1,3-D-glucans are present as structural components of edible fungi and vegetables, including mushrooms, oats, soybean, banana, apple, pear, celery, carrot and radish, with levels of up to 20% of total carbohydrate by mass (Ko and Lin, 2004). Additional dietary exposure to β-1,3-D-glucans from the consumption of alcoholic beverages produced using fungal chitosan would be negligible.

3.8 Dietary Exposure

No dietary exposure assessment was conducted because experimental data indicates that residual levels of fungal chitosan in alcoholic beverages are expected to be negligible.

3.9 Discussion

Chitosan derived from crustaceans has a history of safe use as a processing aid for the production of fruit juices and is also used at high doses (grams per day) as a weight loss supplement. Chitosan derived from *A. niger* has been recently approved for use as a wine processing aid in Europe. The chitosan molecules derived from crustacean and fungal sources are chemically equivalent in that they are copolymers of glucosamine and N-acetylglucosamine connected by β→(1,4) linkages. Small potential differences in the toxicokinetic behaviour of chitosan preparations may be observed due to differences in molecular weight and degree of acetylation.

Owing to its molecular size (> ~10 kDa) intact fungal chitosan is not expected to be absorbed following oral exposure. In the gastrointestinal tract, extensive degradation to oligomeric and monomeric chemical components by endogenous enzymes and colonic bacteria is expected,

followed by fermentation to common metabolites such as short chain fatty acids. At high doses, such as when used as a weight loss supplement, it is likely that a proportion of chitosan passes through the gastrointestinal tract substantially unchanged.

Animal toxicity studies on various chitosan preparations show no treatment-related adverse effects and no target organ of toxicity has been identified following oral administration. Genotoxicity studies have been uniformly negative. Limited data on reproductive toxicity indicate no adverse findings. A published review of human data from 13 clinical trials of up to 6 months duration found no adverse effects associated with oral chitosan (average daily dose 3.5 g) as a weight loss supplement. No specific toxicity studies are available on the fungal chitosan product for which approval is being sought, however fungal chitosan has no physicochemical or compositional attributes that would suggest a hazard profile that differed from that of other chitosan preparations. Because of a lack of adverse effects in animal and human studies with chitosan, an Acceptable Daily Intake (ADI) “not specified” is considered appropriate.

Experimental data was provided indicating that wine processed using fungal chitosan contained no more than 10 mg of residual chitosan per litre, the limit of detection of the analytical method. Negligible levels would also be expected in beer and cider, while no residual fungal chitosan would be expected in alcoholic products derived from distillation. Because of this, no dietary exposure assessment was conducted.

Chitosan preparations derived from *A. niger* contain non-covalently linked β-1,3-D-glucans which are negligible in crustacean derived preparations. Normal dietary exposure to β-1,3-D-glucans occurs through the consumption of fruit and vegetables; any additional dietary exposure from the consumption of alcoholic beverages produced using fungal chitosan would be negligible.

3.10 Risk Assessment Conclusion

The use of fungal chitosan as a processing aid for of the production of wine, beer, cider, spirits and food grade ethanol as proposed in this Application raises no public health and safety concerns.

4. References

Aam BB, Heggset EB, Norberg AL, Sørlie M, Vårum KM, Eijsink VG (2010) Production of chitooligosaccharides and their potential applications in medicine. *Marine Drugs* **8**(5):1482-1517.

Chagas R, Monteiro S, Ferreira RB (2012) Assessment of potential side effects of common fining agents used for white wine protein stabilization. *American Journal of Enology and Viticulture* **63**(4):574-578.
Chae SY, Jang MK, Nah JW (2005) Influence of molecular weight on oral absorption of water soluble chitosans. *J Control Release* **102**(2):383-394.

Choi HG, Kim JK, Kwak DH, Cho JR, Kim JY, Kim BJ, Jung KY, Choi BK, Shin MK, Choo YK (2002) Effects of high molecular weight water-soluble chitosan on in vitro fertilization and ovulation in mice fed a high-fat diet. *Arch Pharm Res.* **25**(2):178-183.

Codex (2005) Codex General Standard for Fruit Juices and Nectars (CODEX STAN 247-2005). Available at: http://www.codexalimentarius.org/standards/list-of-standards/

Dutta PK, Dutta J, Tripathi VS (2004) Chitin and chitosan: chemistry, properties and applications. *J Sci Indust Res.* 63:20-31.

EFSA (2010) Scientific Opinion on the safety of 'Chitin-glucan' as a Novel Food ingredient. *EFSA Journal* **8**(7):1687

EFSA (2011) Scientific Opinion on the substantiation of health claims related to chitosan and reduction in body weight (ID 679, 1499), maintenance of normal blood LDL-cholesterol concentrations (ID 4663), reduction of intestinal transit time (ID 4664) and reduction of inflammation (ID 1985) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. European Food Safety Authority. *EFSA Journal* **9**(6):2214.

EU (2011) Commission Regulation (EU) No 53/2011 of 21 January 2011 amending Regulation (EC) No 606/2009 laying down certain detailed rules for implementing Council Regulations (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. *Off J Eur Union* 54(L19):1-6. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:019:0001:0006:EN:PDF

FDA (2011) US Food and Drug Administration, Generally Recognized as Safe (GRAS) substance under the US FDA regulation. GRAS Notice No. GRN 000397, FDA response letter, 19 December 2011. Available at: http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=397

http://www.winemakermag.com/stories/techniques/article/indices/12-clarityfiltration/26-a-clearer-understanding-of-fining-agents

Ibrahim A, Gilzad-kohan MH, Aghazadeh-Habashi A, Jamali F (2012) Absorption and bioavailability of glucosamine in the rat. *J Pharm Sci.* **101**(7):2574-2583.

JFCRF (2011) Japan Food Chemical Research Foundation. List of Existing Food Additives. Available at: http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/pages/list-exst.add

Jull AB, Ni Mhurchu C, Bennett DA, Dunshea-Mooij CA, Rodgers A (2008) Chitosan for overweight or obesity. *Cochrane Database Syst Rev.* Jul 16;(3):CD003892.

Kean T, Thanou M (2010) Biodegradation, biodistribution and toxicity of chitosan. *Adv Drug Deliv Rev.* **62**(1):3-11. Review.

Kim SK, Park PJ, Yang HP, Han SS (2001) Subacute toxicity of chitosan oligosaccharide in Sprague-Dawley rats. *Arzneimittelforschung* **51**(9):769-774.

Kitozyme (2010) Report on the industrial test of chitosan from fungal source as a technological

auxiliary on must and wine (2008/SA/0150). Chitosan from fungal source. Assessment of 2 years experiments (2008-2009, 2009-2010). Unpublished report.

Kitozyme (2011) Chitosan GRAS Notice. Submitted to US FDA, 3 Aug 2011. http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=397

Ko YT, Lin YL (2004) 1,3-beta-glucan quantification by a fluorescence microassay and analysis of its distribution in foods. *J Agric Food Chem.* **52**(11):3313-3318.

Kurtbay HM, Bekçi Z, Merdivan M, Yurdakoç K (2008) Reduction of ochratoxin A levels in red wine by bentonite, modified bentonites, and chitosan. *J Agric Food Sci.* **56**:2541-2545.

Muzzarelli RA (1997) Human enzymatic activities related to the therapeutic administration of chitin derivatives. *Cell Mol Life Sci.* **53**(2):131-140.

Naito Y, Tago K, Nagata T, Furuya M, Seki T, Kato H, Morimura T, Ohara N (2007) A 90-day ad libitum administration toxicity study of oligoglucosamine in F344 rats. *Food Chem Toxicol.* **45**(9):1575-1587.

NTP (2009) National Toxicology Program. Testing Status of Agents at NTP. Chitosan. Study no. C20226. Available at: http://ntp.niehs.nih.gov/?objectid=BD3BB7C6-123F-7908-7BB099AF2C319611

OIV (2009) International Organisation of Vine and Wine Resolutions. OIV-OENO 336A-2009; OIV-OENO 337A-2009; OIV-OENO 338A-2009; OIV-OENO 339A-2009; OIV-OENO 368-2009. Available at: http://www.oiv.int/oiv/info/enresolution

Qin C, Gao J, Wang L, Zeng L, Liu Y (2006) Safety evaluation of short-term exposure to chitooligomers from enzymic preparation. *Food Chem Toxicol.* **44**(6):855–861.

Seguier S (2008) Kiomedine-Cs Chitosan. Acute oral toxicity in the rat. Study no. TAO423-PH-08/0064. Unpublished study conducted by Phycher Bio Développement, 33611 CESTAS, France.

Shahidi F, Arachchi JKV and Jeon YJ (1999) Food applications of chitin and chitosan. *Trends in Food Science and Technology*, **10**, 37-51.

Simon RR, Marks V, Leeds AR, Anderson JW (2011) A comprehensive review of oral glucosamine use and effects on glucose metabolism in normal and diabetic individuals. *Diabetes Metab Res Rev.* **27**(1):14-27.

Spagna, G., Pifferi, P. G., Rangoni, C., Mattivi, F., Nicolini G., & Palmonari, R (1996). The stabilization of white wines by adsorption of phenolic compounds on chitin and chitosan. *Food Research International* 29, 241-248.

TGA (1998) CMEC Meeting 07, 3 August 1998. Complementary Medicines Evaluation Committee. Extracted ratified minutes. Available at: http://www.tga.gov.au/archive/committees-cmec-resolutions-07.htm

Vivotecnia (2008) Final Report, Ames Test. B-00561: Chitosan (Cs). 7 April 2008. Unpublished study conducted by Vivotecnia Research S.L., Madrid, Spain.

Xia W, Liu P, Liu J (2008) Advance in chitosan hydrolysis by non-specific cellulases. *Bioresour Technol.* **99**(15):6751-6762.

Yoon HJ, Park HS, Bom HS, Roh YB, Kim JS, Kim YH (2005) Chitosan oligosaccharide inhibits $^{203}HgCl_2$-induced genotoxicity in mice: micronuclei occurrence and chromosomal aberration. *Arch Pharm Res.* **28**(9):1079-1085.

Zeng L, Qin C, Wang W, Chi W, Li W (2008a) Absorption and distribution of chitosan in mice after oral administration. *Carbohydrate Polymers* **71**:435–440.

Zeng L, Qin C, He G, Wang W, Li W, Xu D (2008b) Effect of dietary chitosans on trace iron, copper and zinc in mice. *Carbohydrate Polymers* **74**:279-282.