Food and Agriculture Organization of the United Nations

87th JECFA - Chemical and Technical Assessment (CTA), 2019

MANNOPROTEINS FROM YEAST CELL WALLS

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1. Summary

This Chemical and Technical Assessment (CTA) summarizes data and information on yeast mannoproteins submitted to the 84th and 87th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) upon requests by the 48th and 50th Codex Committee on Food Additives (FAO/WHO, 2016, FAO/WHO, 2018). At its 87th meeting, JECFA was asked to revise the specifications of the additive according to the information provided on the chemical characterization of the additive, content of impurities, and concentration of mannoproteins in wine.

Yeast extracts containing mannoproteins have not been evaluated by JECFA before its 84th meeting (2017). Yeast mannoproteins have been approved for use in wine as a stabilizer in the following countries: Argentina, Australia, Canada, EU, New Zealand and the USA.

Argentina has approved the use of yeast mannoproteins as a food additive for wine stabilization (Resolución C 19/2006). It is also approved by the Organization Internationale de la Vigne et du Vin (OIV).

Australia and New Zealand have approved the use of yeast mannoproteins as a food additive for wine stabilization in Standards 1.3.1 – Food Additives (listed in Schedule 5 Technological functions which may be performed by food additives) and 4.5.1 – Wine Production Requirements (Australia only) of the Australia New Zealand Food Standards Code. It is approved at amaximum permitted level of 400 mg/kg that is considered to be appropriate, which takes into account both added mannoproteins and naturally occurring mannoproteins in wine and aligns with the Agreement between Australia and the European Union on Trade in Wine, and Protocol (1994).It allows for the use of preparations of yeast cell wall, up to 400 mg/litre for wines originating in Australia and separately for wines originating in the European Union.

Health Canada on 6 January 2015 also published a Proposal to enable the use of a new food additive, yeast mannoproteins, to inhibit crystal formation in wine in the List of permitted food additives with other generally accepted food uses, which came into force on 15 June 2015 at a maximum level of use of 0.04% (400 parts per million).

In the USA, a Generally Recognized as Safe (GRAS) notice for baker's yeast mannoprotein (GRN 000284) was submitted to the US Food and Drug Administration (FDA) for review on March 6, 2009. In a response letter dated August 28, 2009, the US FDA had no further questions regarding the petitioner's determination that its baker's yeast mannoprotein was GRAS for use as a stabilizing agent in wines, at levels ranging from 50 – 400 mg/litre, to prevent tartaric acid precipitation. The OIV International Oenological Codex (OIV Codex) includes a specification for yeast mannoproteins in OIV Resolution

Oeno 26/2004. This specification indicates that yeast mannoproteins can be used for tartaric and/or protein stabilization of wine. In Europe Regulation (EC) No. 1493/1999 as amended by Regulation (EC) No. 2165/2005, permitted "the addition of yeast mannoproteins to ensure the tartaric and protein stabilization of wines."

This Chemical and Technical Assessment discusses published information relevant to yeast cell wall extracts containing mannoproteins, production methodologies, and specifications. Based on the information received at the 87th JECFA, the name was changed from "yeast extracts containing mannoproteins" to the more suitable "Mannoproteins from yeast cell walls", the specifications were revised and the tentative status was removed.

2. Description

Yeast mannoproteins are extracted from purified yeast (*Saccharomyces cerevisiae*) cell walls by enzymatic treatment with β-glucosidase or by physicochemical extraction with thermal treatment. Yeast mannoprotein represents a large family of natural compounds in which polysaccharide chains are bound to proteins and peptides by covalent and non-covalent linkages (e.g. ionic interactions). The structures and molecular weights of mannoproteins vary depending on the degree and type of glycosylation. The polysaccharide chains consist almost exclusively of mannose units linked by α-linkages forming a long α-1→6 linked backbone containing short α-1→2 and α-1→3 linked side-chains. Several of the side chains may have phosphodiester linkages to other mannosyl residues. The molecular weight of yeast mannoprotein ranges from 20 kDa to more than 450 kDa (Food Standards Australia New Zealand 2008). Yeast mannoproteins are a white or beige, odourless powder (powder form), or yellow, translucent colloidal solution (liquid form). In liquid form, yeast mannoproteins precipitate when one volume of ethanol is added. Yeast mannoproteins decompose on excessive heating. Storage temperatures should be 4 - 20° for the powder form and 4 – 12° for the liquid form.

3. Manufacturing

Yeast mannoproteins are extracted from purified yeast (*S. cerevisiae*) cell walls, using an approved enzyme such as glucan 1,3-β-glucosidase (EC 3.2.1.58), or by physico-chemical extraction. During enzyme hydrolysis of the yeast cell wall, the mannoproteins are solubilized. Figure 1 depicts the production scheme. The *S. cerevisiae* cell walls, all media and equipment used in production of yeast mannoprotein are food grade. Hydrolysis conditions are monitored throughout the enzymatic process. Each batch of yeast extract containing mannoproteins is sterilized by ultrafiltration or pasteurization and analysed to certify the levels of chemical and microbiological contaminants.

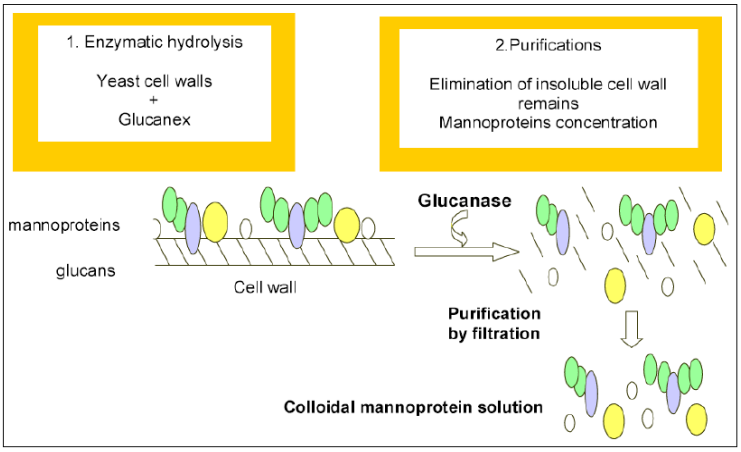

Figure 1. Enzymatic extraction of mannoproteins from Saccharomyces cerevisiae cell walls.

Likewise, thermal treatment of yeast hulls can also break the links with β-glucans to allow solubilisation of the mannoproteins, which are then separated from the insoluble cell wall material, concentrated and

eventually micro- or ultra-filtered. Figure 2 depicts the scheme of this operation. Again, all used in this production process are food grade and hydrolysis conditions are monitored throughout the thermal process. Again, each batch of yeast extracts containing mannoproteins manufactured in this manner is sterilized by pasteurization and analysed to certify the levels of chemical and microbiological contaminants.

Yeast mannoprotein products are commercialized in solid form or in solution. Both products have a long shelf-life; both products are stable for a minimum of two years if kept sealed and in a cool dry location at or below 12°.

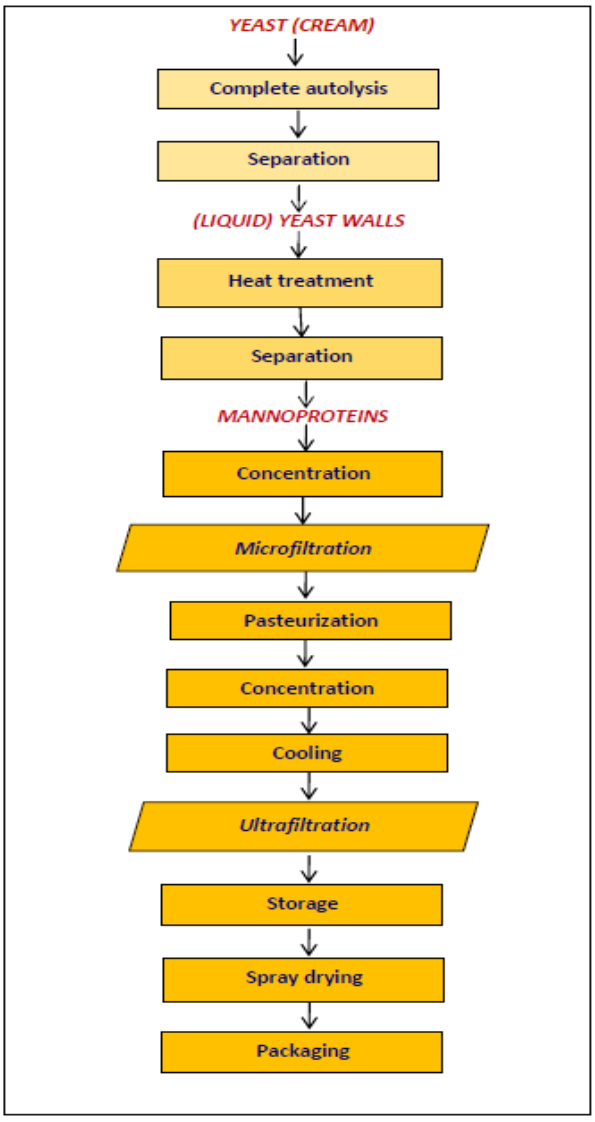

Figure 2. Thermal extraction process of mannoproteins from *S. cerevisae* cell walls.

4. Chemical characterization

4.1 Composition of the food additive

The external protein layer of the cell wall of *S. cerevisiae* may at any time consist of at least 20 different glycoproteins and the composition of this protein layer may vary depending on growth conditions (Klis et al., 2006). Yeast mannoproteins have different structures depending on their molecular weights and the degree and type of glycosylation. Their molecular weight ranges from 20 kDa to more than 450 kDa. Many

mannoproteins carry *N*-linked glycans with a core structure of Man10–14GlcNAc2-Asn. Outer chains present on many yeast *N*-glycans consist of 50 to 200 additional α-linked mannose units, with a long α-1,6-linked backbone decorated with short α-1,2- and α-1,3-linked side chains. There are often several large *N*-glycans per glycopeptide, so that *N*-linked sugar can add 50-100 kDa to the size of the mannoproteins. Phosphorylation of the mannosyl side chains gives yeast its anionic surface charge.
Commercial yeast extracts containing mannoproteins contain more than 60% polysaccharides on the dried basis, more than 70% mannose of the total polysaccharides, and 0.5-7.5%of total nitrogen on the dried basis.

4.2 Possible impurities and microbial contamination

- Lead: < 2 mg/kq
- Total aerobic mesophile flora: < 10,000/g
- Coliforms: < 10 CFU/g
- *Staphylococcus aureus:* None in a 1 g sample
- *Salmonella:* None in a 25 g sample
- *Escherichia coli:* None in a 25 g sample
- Moulds: < 50 CFU/g
- Yeasts: < 10^2 CFU/g

5. Analytical methods

A method for the determination of total polysaccharides in yeast mannoproteins was proposed; mannose content is determined spectrophotometrically after treatment with concentrated sulfuric acid and phenol against a standard solution containing free mannose. The sponsors also proposed a spectrophotmetric enzymatic method for the determination of % mannose in total polysaccharides in the yeast extracts.

All other methods in the specifications for yeast extracts containing mannoproteins are based on standard methods, published in the Combined Compendium of Food Additive Specifications FAO JECFA Monographs 1, Vol 4 (JECFA, 2006).

5.1 Rationale for proposed specifications

The identity assay for yeast mannoproteins is intended to define the identity of the products of commerce as verified by visual inspection and solubility. The purity of the product of commerce is established by determination of loss on drying, specific rotation, total ash, lead, and presence of microbiological contaminants. The specifications are consistent with the specification for yeast mannoproteins in the OIV International Oenological Codex (OIV Codex), OIV Resolution Oeno 26/2004. This specification indicates that yeast extracts containing mannoproteins define the product of commerce used for tartaric and/or protein stabilization in wine.

6. Functional use

6.1 Technological function

Yeast mannoproteins contribute to the chemical stabilization of wine by preventing crystallization of tartrate salts (potassium bitartrate crystallization) (Feuillat et al. 1998, Gerbaud et al. 1996; Moine et al. 1997) and protein haze (Dupin et al. 2000; Gonzalez-Ramos et al. 2008; Gonzalez- Ramos and Gonzalez 2006; Gonzalez-Ramos et al. 2009, Waters et al. 1994). Accordingly, mannoproteins falls into a class of colloids termed "protective colloids", other examples include gum arabic, and β-glucan derived from *Botrytis cinerea* infection of grapes (Ribereau-Gayon et al. 2006). Protective colloids function by coating the site of crystallization or aggregation. Mechanisms for this process have been postulated but are not yet fully elucidated.

Wine clarity refers to the absence or presence of suspended particles or sediments in wine. Consumers usually reject wine bottles containing crystalline and cloudy precipitates (Ferreira et al. 2004), thereby reducing their commercial value (Bayly and Berg 1967, Hsu and Heatherbell 1987, Waters et al. 1991, Waters et al. 1992, Dupin et al. 2000, Lomolino and Curioni 2007), although most of the precipitates do not affect the sensory characteristics of the wine (Ferreira et al. 2004, Lomolino and Curioni 2007).

6.1.1 Tartrate stability

Tartrate crystals (potassium hydrogen tartrate and calcium tartrate) develop naturally in wine and are the major cause of sediment in bottled wines. Accordingly, before the delivery for domestic or international trade, particularly white wine and sparkling wines have to be stabilized against tartrate salt precipitation, arising from a natural grape source. The concentration of tartaric acid itself in the wine, alcohol, colloids, calcium, and potassium, the pH value of the wine, the duration and temperature of storage, and the surface (roughness) of the storage container all have an impact on tartrate salt precipitation in wine (Pilone and Berg 1965). Tartaric acid predominantly precipitates as the potassium salt (potassium bitartrate) followed by calcium tartrate. Mannoproteins inhibit the crystallization of potassium bitartrate.

Mannoproteins are naturally released from yeast during the fermentation process and are able to reduce tartrate crystallization, such that barrel-aging white wines on yeast lees for several months often provides sufficient tartrate stability to overcome the need for further stabilization. The macromolecules responsible for inhibiting crystal formation have been shown to be glycosylated proteins released by enzymatic degradation of the yeast cell wall (Llauberes et al. 1987; Dupin et al. 2000).

The addition of a purified yeast cell wall preparation exploits the supposed ability of these mannoproteins to act as protective colloids by coating the site of crystallization or aggregation and hindering access to nearby particles.

6.1.2 Protein stability

Wines contain varying amounts of different nitrogenous substances, amongst which are proteins.

These wine proteins do not contribute significantly to the nutritive value of wines since their concentration varies typically from 15-300 mg/litre (Ferreira et al. 2002, Waters et al. 2005). The majority of the wine proteins derive from the grape pulp and include chitinases, thaumatin-like proteins and osmotins (Monteiro et al. 2001, Waters et al. 1998), which are particularly stable under winemaking conditions (low pH value and proteolytic enzymes), therefore passing selectively into the wine (Ferreira et al., 2000). The slow denaturation of residual amount of unstable wine proteins, possibly resulting from unfavourable storage conditions leads to protein aggregation and flocculation into a hazy suspension, which results in the appearance of a haze or deposit in the bottled wine. For example, it has been shown that that all the major wine protein fractions are present in wine hazes and all have been shown to be heat unstable (Waters 1991, Waters and Høj 1999, Waters et al. 1990, Waters et al. 1991, Waters et al. 1992).

Yeast mannoproteins have been shown to possess haze-protective properties, while also positively impacting the sensorial properties of the product. Such mannoproteins are released into the wine during the wine making process, at amounts measured at the order of 100 to 200 mg/litre. Yeast mannoproteins do not prevent haze-inducing proteins in wine from precipitating but compete with complex formation with other compounds, thereby reducing the size of haze particles to the threshold level of human detection (Waters et al. 1993, Dupin et al. 2000). The yeast mannoprotein preparation is added to the wine after fining, just before the final stage of filtration prior to bottling. The correct dose is determined by preliminary testing each wine, with the recommended dosage being the lowest concentration at which no crystallization appears plus 50 mg/litre. Addition of excess mannoproteins can reduce the stabilizing effect. The dose of yeast extracts containing mannoproteins depends on factors such as the amount of tartrate and mannoproteins naturally present in the wine, and may typically range between 200 and 400 mg/litre.

7. Food categories and use level

Levels of yeast mannoproteins used in food or expected to be used in food based on technological function and the range of foods are as follows:

Food categories: wine, sparkling wine and fortified wine

The amount of mannoproteins from yeast cell wall to be added to wine will range between 50–400 mg/litre as determined by the winemaker, with a recommended dose of 200 mg/litre and a maximum recommended dosage of 400 mg/litre depending on the amount of naturally occurring mannoproteins and tartrate in the wine.

8. Reactions and fate in foods

Mannoproteins from yeast cell wall are stable for two years in a sealed container < 12°. As they may decompose at excessive temperatures, they are recommended to be stored at 4 – 12°.

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