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March 6, 2009

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Via Federal Express

Office of Food Additive Safety (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: GRAS Notification for Mannoprotein derived from an extract of bakers yeast (*Saccharomyces cerevisiae*)

Dear Sir or Madam:

As counsel for DSM Food Specialties (DSM), we are submitting under cover of this letter three copies of DSM's GRAS notification for Mannoprotein derived from an extract of bakers yeast (*Saccharomyces cerevisiae*). DSM has determined through scientific procedures that Mannoprotein is generally recognized as safe for use in the food industry as a stabilizing agent to prevent tartaric acid precipitation in wines.

This use of Mannoprotein derived from an extract of *S. cerevisiae* is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

If for any reason the agency has any questions or requires any additional information to aid its review of DSM's conclusion, please contact me at your earliest convenience.

Sincerely,

Gary L. Yingling

cc: DSM Food Specialties

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GRAS NOTIFICATION FOR MANNOPROTEIN DERIVED FROM AN EXTRACT OF BAKERS YEAST (*SACCHAROMYCES CEREVISIAE*)

March 6, 2009

DSM Food Specialties
Regulatory Affairs
P.O. Box 1
2600 MA Delft

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GRAS NOTIFICATION FOR MANNOPROTEIN DERIVED FROM AN EXTRACT OF BAKERS YEAST (*SACCHAROMYCES CEREVISIAE*)

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1. General Introduction

DSM Food Specialties ("DSM") manufactures a Mannoprotein which can be marketed either as a dry powder preparation or a ready-to-use colloidal solution. The Mannoprotein is derived from an extract of a classical strain of bakers yeast (*Saccharomyces cerevisiae*). This document will refer to this ingredient as "Bakers Yeast Mannoprotein." The trade name for the product is Claristar™.

The Bakers Yeast Mannoprotein is added to wines to stabilize them during storage and to prevent the development and subsequent precipitation of tartrate crystals that are naturally formed in wine.

Pursuant to the regulatory and scientific procedures established by proposed regulation 21 C.F.R. § 170.36, DSM has determined that its Bakers Yeast Mannoprotein from *Saccharomyces cerevisiae* is a generally recognized as safe ("GRAS") substance for the intended food application and is, therefore, exempt from the requirement for premarket approval. Information on the Bakers Yeast Mannoprotein and bakers yeast (*Saccharomyces cerevisiae*) providing the scientific basis for this GRAS determination is described in the following document.

General and specific information providing the name and address of the notifier, identifying and characterizing the Bakers Yeast Mannoprotein, its applicable conditions for use, DSM's basis for GRAS determination, and the availability of supporting information and reference materials for FDA's review can be found in Section 1.

A description of the Bakers Yeast Mannoprotein, its composition, a description of the manufacturing process, and the ingredient's specifications are provided in Section 2.

The level of use is provided in Section 3.

A discussion of the scientific data establishing the GRAS status of the Bakers Yeast Mannoprotein is discussed in Section 4.

The conclusions are summarized in Section 5.

A list of Annexes is provided in Section 6, and a list of References is provided in Section 7.

1.1 Name and Address of the Notifier

DSM Food Specialties
Alexander Fleminglaan 1
2613 AX Delft
The Netherlands

1.2 Common or Usual Name of the Substance

The common or usual name of the substance that is the subject of the GRAS Notification claim is "Bakers Yeast Mannoprotein."

The commercial name of the product is Claristar™.

1.3 Applicable Conditions of Use

The Bakers Yeast Mannoprotein can be added before or after the final filtration of the wine, just before bottling.

1.3.1 Foods In Which the Substance Is To Be Used:

The Bakers Yeast Mannoprotein is intended to be used in all types of wines, such as white, rosé and red wines, as well as sparkling wines.

1.3.2 Levels of Use

As explained in further detail below, when Mannoprotein is added to wine, it forms Mannoprotein-tartrate complexes and prevents formation of tartrate crystals. Thus, the dosage of the Bakers Yeast Mannoprotein depends on, among other factors, the amount of tartrate and Mannoprotein already naturally present in the wine.

The amount of Bakers Yeast Mannoprotein to be added will range between 50 – 400 mg/l as determined by the winemaker, with a recommended dose of 200 mg/l and a

maximum dosage of 400 mg/l. Winemakers will either use the recommended dose or perform an application test in order to determine the required dose.

The Bakers Yeast Mannoprotein colloidal solution preparation has an average dry matter content of 20%. The average dosage of the Bakers Yeast Mannoprotein colloidal solution will therefore be 1 ml/l wine.

The maximum dosage of 400 mg Mannoprotein/l wine (e.g., 2 ml Mannoprotein colloidal solution/l wine) complies with the maximum dosage established by the Organisation Internationale du Vin (OIV) in Resolution OENO 26/2004 (see Annex 1). This Resolution is adopted in the Wine Regulation of the European Union (Council Regulation (EC) No 2165/2005).

1.3.3 Purpose of Use

The purpose of the use of Bakers Yeast Mannoprotein is to stabilize wines during storage by preventing the development and subsequent precipitation of tartrate crystals, which are naturally formed in wine.

In its normal state, wine is supersaturated with potassium bitartrate salts. These salts can form crystals, which precipitate out of the wine. Although this has no effect on the taste, the presence of the crystals may be perceived as unpleasant. Consequently, wine manufacturers try to limit the presence of the crystals in the bottled wine.

Current methods to achieve stabilization of wines include induction of tartrate precipitation by high energy consuming cold treatment followed by filtration, or delaying the crystallization by treatment with the polymer meta-tartaric acid or various types of colloidal solutions of chemicals. The addition of Bakers Yeast Mannoprotein to wine can be used to replace such treatments.

It is known that wine that is aged on yeast lees (old dead yeast and yeast residues) has improved tartrate stability (Ribérau-Gayon et al., 2000). Research has established that this is due to the Mannoprotein derived from the yeast cells (Gerbaud et al., 1997, Llauberes et al., 1987; Lubbers et al., 1993).

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Although the exact mechanism is not known, it is assumed that the Mannoprotein attaches to the developing tartrate crystals (crystal nucleation site), thereby preventing

them to grow. Consequently, the Mannoprotein-tartrate complexes remain in solution in the wine, and the crystallization and precipitation process is delayed by several months to years, depending on the storage conditions and wine properties.

The method to measure the efficiency of Mannoprotein in wine is described in the OIV Resolution (see Annex 1). DSM developed its own method to measure the efficiency of the Bakers Yeast Mannoprotein, which is presented in Annex 2.

The results of application trials performed with Bakers Yeast Mannoprotein from DSM are described in Annex 3.

1.3.4 Population Expected to Consume the Bakers Yeast Mannoprotein

The Bakers Yeast Mannoprotein is expected to be consumed by adults that are drinking wines. It should be emphasized, however, that Mannoprotein naturally occurs in the bakers yeast *Saccharomyces cerevisiae* and is, thus, also consumed via various yeast-containing and yeast-derived products such as bread and yeast extract.

1.4 Basis for the GRAS determination

Pursuant to 21 C.F.R. § 170.30, DSM has determined, through scientific procedures, that Bakers Yeast Mannoprotein derived from bakers yeast (*Saccharomyces cerevisiae*) is GRAS for the intended use set forth in this document.

1.5 Availability of Information for FDA Review

The data and information that are the basis for DSM's GRAS determination are available for the FDA's review, and copies will be sent to FDA upon request. Requests for copies and arrangements for review of materials cited herein may be directed to:

Name	J. Reuvers
Title	Regulatory Affairs Manager
Address	DSM Food Specialties Regulatory Affairs P.O. Box 1 2600 MA Delft The Netherlands
Email	jack.reuvers@dsm.com
Telephone	+31152793739

2. Identity of the notified substance

2.1 <u>Chemical name</u> Mannoprotein

2.2 <u>CAS Registry Number</u> Not applicable

2.3 <u>Empirical Formula</u> Not applicable

2.4 <u>Structural formula</u>

Mannoprotein represents a large family of natural compounds in which polysaccharide chains are bound to proteins and peptides by covalent and non-covalent linkages (i.e., ionic interactions). The structures and molecular weights of Mannoprotein vary, depending on the degree and type of glycosylation. Their molecular weight ranges from 20 kDa to more than 450 kDa (Food Standards Australia New Zealand, 2008). The polysaccharide chains consist almost exclusively of mannose units linked together by α-links, with a long α-1→6 linked backbone containing short α-1→2- and α-1→3 linked side chains (Lipke and Ovalle, 1998). Several of the side chains may have phosphodiester linkages to other mannosyl residues.

Mannoprotein is found in yeasts where it forms, together with glucan, the main component of the cell walls (Lipke and Ovalle, 1998). Apart from the cell wall, yeast cells also contain some periplasmic Mannoproteins, such as invertase (Dupin et al., 2000; Whitehurst, 2002).

The Mannoprotein described in this notification is derived from an extract of bakers yeast (*Saccharomyces cerevisiae*).

The carbohydrate content of this Mannoprotein is at least 70%, of which at least 60% is composed of mannose. The Bakers Yeast Mannoprotein and the glycosidic linkages have been identified by NMR (see Annex 4).

2.5 Quantitative Composition

The chemical composition on a basis of 100% dry matter Bakers Yeast Mannoprotein is presented in the Table below:

	Method	Unit	Limit	Batch (b)(4)	Batch [redacted]	Batch [redacted]	Batch [redacted]
Ashes	550 – 600 °C	%	≤ 8	5.3	4.6	4.9	4.5
Nitrogen[1]	Kjeldahl or equivalent	%	0.5 – 7.5	5.9	5.7	6.3	4.6
Carbohydrates	Phenol/sulfuric acid	%	≥ 60	65	60	67	63
Fraction of mannose in carbohydrates	HPLC after TFA hydrolysis	%	≥ 70	95	96	n.d.	n.d.

The Bakers Yeast Mannoprotein can be sold as ready-to-use spray dried powder or as a colloidal solution. In order to preserve the colloidal solution after opening, 14 g/l sodium bisulfite is added. The colloidal solution contains 20% ± 2% dry matter.

[1] Provided no other nitrogen sources are present, the maximal protein content is N x 6,25

2.6 Method of Manufacture

Bakers yeast (*Saccharomyces cerevisiae*) naturally contains Mannoprotein. Apart from being used in bread, beer and wine, bakers yeast is used to produce a variety of food ingredients, including yeast extract. Bakers yeast extract will contain a significant part of the Mannoprotein, and can therefore be used as a starting material for the production of Mannoprotein.

The classical bakers yeast strain used by DSM contains about 16% Mannoprotein, and the extract derived from the bakers yeast contains between 8 and 12% Mannoprotein. DSM uses this food-grade yeast extract as a starting material for the production of the Mannoprotein described in this notification. However, in principle also other food grade yeast extract products can be used.

The whole production process of Mannoprotein from yeast extract as described below is performed according to current Good Manufacturing Practices:

The first step in the manufacturing process of the Mannoprotein is the micro-filtration of the yeast extract. The aim of this filtration step is to remove particles and high Molecular Weight proteineous material to enable the subsequent ultrafiltration step.

The micro-filtered, clear liquid is subsequently subjected to ultrafiltration (UF) in order to concentrate the high Molecular Weight Mannoproteins (> 20 kDa). The UF-concentrate is then treated with the enzyme phosphodiesterase from *Leptographium procerum* in order to hydrolyze residual yeast RNA. This is followed by a diafiltration step in order to remove low Molecular Weight molecules such as salts, amino acids, nucleotides and sugars.

After diafiltration, the diluted solution is again concentrated by UF to obtain a Mannoprotein containing UF-concentrate of 20% +/- 2% dry matter. In order to inactivate residual enzyme activity, the UF-concentrate is subjected to UHT treatment (130-150 °C) and subsequently cooled down to 20-40 °C. The UHT-treated concentrate is then polish filtered in order to remove insoluble proteineous material that was formed during the UHT treatment.

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After filtration, the clear solution is stabilized with 14 g/l sodium bisulfite to prevent microbial growth after opening of the package. The stabilized Mannoprotein is packed in 2.5 or 20 liter cans and stored below 10 °C.

A flow diagram of the manufacturing process is given in Annex 5.

2.7 Characteristic Properties

Mannoprotein is water soluble and insoluble in ethanol. When in solution, Mannoprotein precipitates by the addition of 1 volume of ethanol.

The Bakers Yeast Mannoprotein colloidal solution preparation described in this dossier is a light brown to brown colloidal solution. The content of polysaccharides expressed in equivalent of mannose is above 600 g/kg dry matter.

2.8 No Potential Human Toxicants From Raw Materials

The Mannoprotein is isolated from bakers yeast extract, where it is naturally present. Bakers yeast is known to be a non-pathogenic and non-toxicogenic microorganism. Moreover, the bakers yeast extract was affirmed by the FDA to be GRAS (21 C.F.R. § 184.1983).

Toxicological studies have shown that the phosphodiesterase enzyme preparation used as a processing aid in the manufacture of the Bakers Yeast Mannoprotein is safe for consumption (Steensma et al., 2004). Therefore, there is no scientific basis to assume the presence of any human toxicants.

The addition of sodium bisulfite to the colloidal Mannoprotein solution can be considered of no safety concern as well. The use of sodium bisulfite in a wide variety of foods and beverages is considered Generally Recognized as Safe (21 C.F.R. § 182. 3739). When dissolved in wine, about half of the weight of sodium bisulfite is transformed into sulfur dioxide (about one gram of sulfur dioxide is produced when two grams of sulfite are added to the wine). Sulfur dioxide is used a common ingredient in wine as it protects the color of the wine from oxidation and preserves its flavor after bottling. According to the U.S. regulations (27 C.F.R. § 24.246), the sulfur dioxide content of the finished wine shall not exceed the limitations prescribed in 27 C.F.R. § 4.22, being 350 mg/l.

The sodium bisulfite content in the Mannoprotein solution is 14 g/l, whereas the amount of Mannoprotein solution added to the wine will not exceed 2 ml/l wine. As a

consequence, the maximal amount of sodium bisulfite added to the wine will be 28 mg/l, resulting in 14 mg/l sulfur dioxide. This maximal amount can be considered negligible, especially when compared to the amounts that are maximally allowed in wine. According to the EU Miscellaneous Additive Directive (European Parliament and Council Directive 95/2/EC, 1995), a sulfur dioxide content below 10 mg/kg or 10 mg/l is not considered to be present.

2.9 Specifications for Food-Grade Material

The Bakers Yeast Mannoprotein described in this notification is manufactured to comply with the purity specifications laid down by the Organisation Internationale du Vin (OIV) in Resolution OENO 26/2004, see Annex 1.

The chemical and microbial purity specifications and the actual values of three different batches are summarized in the following Table:

	Unit	**Limit**	**Batch** (b)(4)	**Batch** [redacted]	**Batch** [redacted]
Total heavy metals	mg/kg	≤ 30	<10	<26	<29
Pb	mg/kg	≤ 5	<1	<1	<1.2
Hg	mg/kg	≤ 0.15	0.1	<0.1	<0.2
As	mg/kg	≤ 1	<0.1	<0.1	<0.2
Cd	mg/kg	≤ 0.5	<0.05	<0.05	<0.06
Total aerobic mesophile germs	CFU/g	≤ 104	<5	<5	<5
Molds	CFU/g	≤ 50	<10	<10	<10
Yeasts	CFU/g	≤ 100	<10	<10	<10
Coliforms	CFU/g	≤ 10	<10	<10	<10
Escherichia coli	CFU/25g	Absent	Absent	Absent	Absent
Staphylococcus aureus	CFU/g	Absent	Absent	Absent	Absent
Salmonella	CFU/25g	Absent	Absent	Absent	Absent
Lactic bacteria	CFU/g	≤ 104	<10	<10	<10

3. Information on Any Self-Limiting Levels of Use

The Mannoprotein will be used in all types of wines according to the Quantum Satis principle. The level of use will not exceed 400 mg dry Mannoprotein per liter of wine (e.g., 2 ml of the Bakers Yeast Mannoprotein colloidal solution preparation per liter of wine). As stated earlier, the amount of Bakers Yeast Mannoprotein added will, amongst other factors, relate to the amount of naturally occurring Mannoprotein and tartrate in the wine.

4. Basis for exemption from premarket approval

4.1 Scientific data to establish the safety of Bakers Yeast Mannoprotein

The Bakers Yeast Mannoprotein described in this notification is derived from a food-grade extract of bakers yeast (*Saccharomyces cerevisiae*). Therefore, the scientific data to establish the safety of the Bakers Yeast Mannoprotein are based on the following information:

i) Scientific literature establishing the safety of bakers yeast (*Saccharomyces cerevisiae*) in food and food ingredient production. The scientific literature is supported by the safe consumption of various food ingredients prepared from *Saccharomyces cerevisiae* as established by history of safe use, FDA food additive and GRAS regulations and the Alcohol and Tobacco Tax and Trade Bureau ("TTB") regulations pertaining to wine, including the safety of the yeast extract/cell walls containing Bakers Yeast Mannoprotein

ii) Safety studies establishing the safety of DSM's yeast extract and yeast Mannoprotein generally. In addition to the safety studies, the safety of the Bakers Yeast Mannoprotein is supported by the following:

- the natural occurrence and preexisting presence of Mannoprotein from *Saccharomyces cerevisiae* in wine
- the safety of Mannoprotein's addition to wine as supported by EU and Australia, New Zealand, and Argentina

- Substantial equivalence between the Bakers Yeast Mannoprotein and Mannoprotein present in other established safe yeast-derived products
- Intake data of Bakers Yeast Mannoprotein

4.1.1 Safety of Bakers Yeast *Saccharomyces cerevisiae* in Food and Food Ingredient Production

The published scientific literature concurs on the established safety of the use of bakers yeast (*Saccharomyces cerevisiae*) in food. As discussed in the book, Enzymes in Food Processing, bakers yeast (*Saccharomyces cerevisiae*) has a long history of safe use in food (Tucker et al., 2000). This microorganism has been used as early as the Stone Age (about 9000 BC) as starter cultures in the production of bread, beer and wine. An article written by Pretorius and published by Yeast in 2000 notes that between 1857 and 1863, Louis Pasteur demonstrated that the yeast *Saccharomyces cerevisiae* is the micro-organism responsible for the fermentation of bread, beer, wine and cider. He discovered that a number of varieties of *Saccharomyces cerevisiae* exist in nature which are more or less adapted to these different fermentations. This is possibly the reason why *Saccharomyces cerevisiae* is also known under various other names. The formally accepted taxonomic name of the species is *Saccharomyces cerevisiae* Meyen ex Hansen (1883), but taxonomic textbooks list many synonyms, such as *Saccharomyces bayanus*, *Saccharomyces pastorianus*, *Saccharomyces carlsbergensis*, *Saccharomyces uvarum*, *Saccharomyces sake*, and *Saccharomyces vini* (Kreger-van Rij, 1984; Barnett et al., 1990).

According to scientific experts, a nontoxigenic organism is defined as "one which does not produce injurious substances at levels that are detectable or demonstrably harmful under ordinary conditions of use or exposure" and a nonpathogenic organism as "one that is very unlikely to produce disease under ordinary circumstances." (Pariza and Foster, 1983).

The European Food Safety Agency (EFSA) came to the conclusion that bakers yeast fits this definition of nontoxigenicity and nonpathogenicity[2] and that it is also not a major food

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[2] As discussed later in this document, isolated cases of adverse effects (invasive infections in critically ill or immunocompromised populations associated with a probiotic preparation of *S.*

allergen. EFSA therefore gave bakers yeast a Qualified Presumption of Safety (QPS) status (EFSA, 2007). This means that in Europe, bakers yeast can be safely used in food and feed production and is freed from the need for further safety assessment.

In addition to the published literature, the FDA has approved dried yeast as an ingredient for food (21 C.F.R. §172.896). In addition, GRAS notifications have been submitted and accepted by FDA with no questions for the use of genetically modified yeasts as starter cultures for wine (GRAS GRN No. 120 and GRAS GRN No. 175). Indeed, a few food standards direct the addition of food grade bakers yeast (*Saccharomyces cerevisiae*) (21 C.F.R. § 160.105 Dried eggs, 21 C.F.R. § 160.145 Dried egg whites and 21 C.F.R. § 160.185 Dried egg yolks).

Moreover, it is well established that people safely consume bakers yeast daily through the consumption of bread and other baked products, and they ingest the related Brewers yeast through the drinking of yeast-containing beer. In addition, yeast cell preparations are commercialized as food supplements or medicines because of their nutritional characteristics (proteins, amino acids, vitamins, minerals and trace elements, see www.vitacost.com and Moyad, 2007 and 2008). Yeast is recommended for human health against ulcerative colitis, in combination with standard antibiotics against *Clostridium difficile* disease, and as a treatment against persistent diarrhea in children (EFSA, 2007). An article published in Microbes and Infection discusses the use of *Saccharomyces cerevisiae* as a bio-control agent against *Solanaceae* diseases (Czerucka and Rampal, 2002). Humans are thus daily exposed to bakers yeast (*Saccharomyces cerevisiae*).

Apart from the safe use of bakers yeast (*Saccharomyces cerevisiae*) in food production, the safe use of bakers yeast in food ingredient production supports the published literature's assertion that bakers yeast and its derivatives are safe for consumption. Since the 1930s, yeast extract products have been developed to enhance the flavor of a variety of foodstuffs like soups, sauces and ready-to-eat meals. For instance, bakers yeast extract (21 C.F.R. §184.1983) has been affirmed by the FDA as a GRAS flavoring agent and adjuvant. Moreover, autolyzed yeast extract is used as food spread and sold under the brand name Marmite (http://en.wikipedia.org/wiki/Marmite) since 1902 (http://www.unilever.co.uk/ourbrands/foods/marmite.asp).

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cerevisiae; isolated incidence of allergic reaction to freshly prepared bread) do not change the conclusion that *S. cerevisiae* is not pathogenic among healthy populations.

As further indication of its safety, bakers yeast (*Saccharomyces cerevisiae*) is also widely used for the production of macromolecular cellular components such as proteins including enzymes (see below), lipids and vitamins (Soni, 2007).

The FDA has approved various yeast-derived products for the use in food. These include Bakers yeast protein (21 C.F.R. §172.325), Yeast-malt sprout extract (21 C.F.R. §172.590) and Bakers yeast glycan (21 C.F.R. §172.898).

Moreover, GRAS notifications were submitted and accepted by FDA with no questions for invertase from *Saccharomyces cerevisiae* (GRAS GRN No. 88) and Bakers yeast beta-glucan (GRAS GRN No. 239).

In addition, autolyzed yeast and cell wall/membranes of autolyzed yeast are authorized materials for the treatment of wine in the U.S. (27 C.F.R. § 24.246) and in the European Union (Commission Regulation (EC) No 1410/2003).

The above examples show that many different types of products derived from bakers yeast (*Saccharomyces cerevisiae*) are suitable and safe for human consumption. Many of the above mentioned products contain – depending on the exact production process – Mannoprotein as a component. As a matter of fact, the FDA specifications of Bakers yeast glycan state that the carbohydrate is composed of glucan and mannan units in approximately a 2:1 ratio. See 21 C.F.R. § 172.898. The enzyme invertase is a Mannoprotein in itself (Dupin et al., 2000).

Finally, bakers yeast and derived products (yeast extract and invertase) from DSM have a long history of safe use. DSM has been in the business of manufacturing bakers yeast since 1870, when Mr van Marken started to produce bakers yeast using the co-called Vienna process. The factory, called KNG&SF (Koninklijke Nederlandse Gist- en Spiritus Fabrieken or Royal Dutch Yeast and Alcohol Factories), later became known as Gist-brocades until it was acquired by DSM in 1998. Gist-brocades has always been a major yeast producer with manufacturing sites in various countries in Europe. In the United States, Gist-brocades acquired the bakers yeast production sites from Anheuser Busch in 1988, which were sold to Lallemand in 1995.

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In the 1930s Gist-brocades developed production processes for yeast extracts. For the production of bakers yeast and the production of yeast extracts, the company developed a whole range of commercial yeast strains. The strains were selected from various commercial yeast strains available in the market place and were improved for various

applications by classical strain breeding techniques such as mutation and selection and crossing of strains, followed by selection of the off-spring for the wanted properties. The collection of commercial bakers yeast strains was taxonomically identified by yeast experts of the Dutch culture collection, the Centraalbureau voor Schimmelcultures (CBS). This is an independent, internationally recognized laboratory. The expert concluded that based on morphological and physiological analyses the strains were to be identified as *Saccharomyces cerevisiae* s.l. (see Annex 6). The addition s.l. (*sensu lato* or: in the broad sense) means that by the methods used the strains could not be differentiated from synonymous strains such as *Saccharomyces bayanus, Saccharomyces paradoxus* and *Saccharomyces pastorianus.*

The present commercial strains employed by DSM for the production of yeast extracts and related products such as the Mannoprotein described in this dossier all are derivatives of the strains mentioned in Annex 6.

4.1.2 Safety studies establishing the safety of DSM's yeast extract and yeast Mannoprotein generally

As shown in Section 4.1.1, bakers yeast has been safely consumed for centuries. Since Mannoprotein is part of the yeast cell, it can be scientifically concluded that humans have also been daily exposed to Mannoprotein for centuries.

The published scientific literature demonstrates that Mannoproteins are naturally produced during the alcohol fermentation with bakers yeast in the production of wine. The safe addition of Mannoproteins to wine has been affirmed by the EU, Australia/New-Zealand and Argentina. The Bakers Yeast Mannoprotein is substantially equivalent to Mannoproteins found in other safe food ingredients.

4.1.2.1 Relevant safety studies

Because the Bakers Yeast Mannoprotein described in this dossier is derived from yeast extract from DSM, which naturally contains between 8 and 12% Mannoprotein, safety studies performed with the yeast extract can be considered as relevant. Moreover, because the Bakers Yeast Mannoprotein proved to be substantially equivalent with

those from other sources (see below), safety studies performed on other Yeast Mannoprotein can also be considered as relevant.

4.1.2.1 Safety studies performed on yeast extract from DSM

Safety studies were performed on two yeast extract products from DSM with the commercial names of Gistex® standard powder and Maxarome® powder. Both products are derived from *Saccharomyces cerevisiae.*

Acute toxicity I

The acute oral toxicity of Gistex® standard powder (batch FM 8850-03) and Maxarome® powder (batch 8829-03) was tested in mice under GLP according to OECD guideline 401 (adopted February 1987) and the EEC directive in the Official Journal of the European Communities L251, volume 27 of September 19, 1984.

The test substances were given to groups of 5 male and 5 female mice in one single dose of 2000 mg test substance/kg body weight. The test substances were dissolved in de-mineralized water at a concentration of 20 g/100 ml. The test substances were given by gavage at 10.0 ml/kg body weight.

Results

No signs of toxicity were observed after treatment. All animals gained weight during the 14-day observation period, and no mortalities occurred. Macroscopic examination at the end of the observation period did not reveal any treatment-related gross alterations.

Conclusion

Since no mortalities occurred, it can be concluded that the LD50 of Gistex® and Maxarome® exceeds 2000 mg/kg body weight, both in male and female mice.

Acute toxicity II

Maxarome® (batch 5002), Gistex® (batch 44-3) and Maxarome® (batch 5002 with NaCl added to the same level as in Gistex®) were studied for acute toxicity in Wistar rats. Animals of 75-80 g received the test substance dissolved in distillated water at a level of 10 ml/kg body weight. The test substance was administered by the oral (gavage), intraperitoneal and intravenous route. After administration, the animals were observed during 5 days.

Results

The LD50 for the oral and intraperitoneal route was >8000 mg/kg for all three test substances. The LD50 for the intravenous route was 2000 mg/kg for Maxarome® and Gistex and 1410 mg/kg for Maxarome® with added salt.

Subacute toxicity I

5 male and 5 female Wistar rats per group received by gavage Maxarome® (batch 5002), Gistex® (batch 44-3) and Maxarome® (batch 5002 with NaCl added to the same level as in Gistex®) for 10 days at a dose of 2000 mg/kg. The control group received distilled water.

Results

All animals behaved normally and none of the animals died. Body weight gain was comparable among the groups. Significant differences in organ weight (heart, liver, kidneys) were not observed. Macroscopy did not reveal any treatment-related effects.

Subacute toxicity II

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Male and female Wistar rats were dosed Maxarome® paste by gavage at a level of 50, 175 and 650 mg/kg body weight. Maxarome® paste was dissolved in physiological salt solution. The first control group received 15.2 mg NaCl/kg body weight, the same salt level as Maxarome® paste, and the second control group received physiological salt solution. Dosing was at 10 ml/kg body weight. All groups consisted of 5 males and 5 females, except the

control group 2, which contained 15 males and 15 females. Dosing continued for 27 days.

Results

No animals died and no differences were observed between the groups concerning clinical signs. Body weight gain, clinical chemistry and hematology, urine analysis, absolute and relative organ weights and histopathology did not show any treatment-related effects.

Bacterial reverse mutation test

The mutagenicity potency of Gistex® standard powder (batch PHS2 A04) was studied under GLP and in compliance with the OECD guideline 471 (adopted 21 July 1997). The test was performed with four histidine-dependent *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and one tryptophan-dependent *Escherichia coli* strain WP2 *uvrA.*

One test was performed with all strains in both the presence and absence of S9-mix with five different concentrations of the test substance ranging from 62-5000 µg/plate. The test compound was dissolved in water. Negative (solvent) and positive controls were run simultaneously with the test substance.

Results

The test substance was not toxic to any strain, as was evidenced by the absence of a decrease in the mean number of revertant colonies. In both the absence and presence of S9-mix, the test substance did not cause a reproducible increase in the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative control. The positive controls gave the expected increase in the number of revertants in both the presence and absence of S9-mix.

Conclusion

It is concluded that the test substance was not mutagenic under the conditions employed in this study.

Micronucleus assay

Gistex® (batch FM 8850-03) and Maxarome® (batch 8829-03) were examined for their potential to induce micronuclei in bone marrow of mice under GLP and in compliance with EEC-protocol B.12 and OECD guideline 474 (adopted May 26, 1983).

15 Males and 15 females were treated once orally with 10 ml per kg body weight of a 20% (w/v) solution of Gistex®/Maxarome® in water. This amount is equivalent to 2000 mg per kg body weight, which represents the limit dose. Negative (water) and positive (mitomycin C) controls were run simultaneously. At 24, 48 and 72 hours after treatment, the animals were sacrificed and bone marrow was collected for analyses.

Results

Treatment with Gistex® or Maxarome® did not induce any signs of toxicity and mean body weights were not affected.

The incidences of micronucleated poly- and normochromatic erythrocytes (MPE and MNE) in mice treated with Gistex® or Maxarome® were fully comparable to those found in the concomitant negative controls. There were no indications for decreases in the number of poly- and normochromatic erythrocytes (PE and NE) per 1000 E at any of the three sacrifice times attributable to treatment with Gistex® or Maxarome®. The positive controls showed an increased incidence of MNE and MPE and a decrease in the number of PE per 1000 E.

In conclusion, Gistex® and Maxarome® did not show any mutagenic activity.

4.1.2.3 Safety studies performed on Yeast Mannoprotein described in literature

Safety studies performed on Mannostab™, another bakers yeast mannoprotein,[3] are described in the Australian approval (Food Standards Australia New Zealand, 2008).

An acute irritant/corrosive study on the skin was performed according to OECD guideline 404. Three male rabbits were subjected to 0.5 g of the Mannostab™ Mannoprotein on the skin by means of a semi-occlusive dressing and evaluated 1, 24, 48 and 72 hours after dressing removal. No macroscopic cutaneous reaction was observed.

The acute irritant/corrosive effect on the eyes was evaluated according to OECD guideline 405. The substance was applied neat (0.1 g) in one eye of 3 female rabbits and ocular reactions were evaluated 1, 24, 48 and 72 hours after instillation. Ocular reactions stayed very low and were totally reversible after 3 days. The Mannostab™ Mannoprotein is considered "weakly irritating for the eyes" according to the scale described in the Official Journal of the French Republic, dated July 10, 1992.

The Mannostab™ Mannoprotein was tested for the ability to sensitize the skin according to OECD guideline 406. Thirty-seven female guinea pigs were induced by dermal injection after which after several periods the release took place by topical application. Only limited reactions were observed, and these limited reactions had disappeared at the second release.

The performance of these rather unusual type of studies in relation to food additives was probably triggered by the fact that the Mannostab™ Mannoprotein is a powder formulation. It was concluded that the Mannostab™ Mannoprotein is not irritant/corrosive to the skin, is weakly irritating to the eyes, and is not a skin sensitizer.

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[3] Mannostab™ is produced by a different manufacturer. However, as discussed later in this GRAS notification, a comparison of NMR profiles establish the substantial equivalency of the Mannostab™ mannoprotein and the Bakers Yeast Mannoprotein that is the subject of this document.

In addition, published studies indicate that the Yeast Mannoprotein is digested like other dietary proteins and carbohydrates. From *in vivo* experiments with rats it was observed that 89% of the Mannoprotein was digested although it remained unclear if digestive enzymes were responsible for the hydrolysis or intestinal flora (Adrian et al., 1996). It was shown *in vitro*, by incubating Mannoprotein in a solution simulating intestinal fluid, that more than 80% of the Mannoprotein was degraded by pancreatic enzymes and especially by the proteolytic enzymes of the pancreas (Moine-Ledoux, 2008).

Yeast cell walls, which are mainly composed of Mannoprotein and beta-glucans (Lipke and Ovalle, 1998), were evaluated in a 4-week feeding study in rats (Nakamura and Shirasu, 2000). No significant changes on hematological and chemical mediators in serum and urine were found. Moreover, the cell walls did not affect the organ weights and there were no significant differences when compared to the control group. In addition, no statistically significant changes in body weight gain and feed consumption were found.

A literature search (see Annex 8) found are no reports to date addressing allergenic reactions or other negative effects due to oral intake of Mannoprotein via wine or other sources like bread, beer and yeast.

4.1.3. Safety of Naturally Occurring Mannoprotein from *Saccharomyces cerevisiae* in wine

As noted in the books, Enzymes in Food Processing (Tucker et al, 2000) and Enzymes in Food Technology (Whitehurst et al, 2002), Mannoprotein is naturally occurring in wine due to the alcohol fermentation with yeast in the wine manufacturing process. While yeast growth and autolysis take place during alcoholic fermentation, natural yeast Mannoprotein is released into the wine. As indicated by published scientific literature, the final wine product contains natural yeast Mannoprotein as a result of the yeast fermentation (Vidal et al., 2003; Gonçalves et al., 2002; Llauberes et al., 1987; Dupin et al., 2000).

The published scientific literature discusses the beneficial aspects of the presence of Mannoprotein in wine (Caridi, 2006; Caridi, 2007). Mannoprotein can interact with aroma compounds and thus potentially change the sensory

properties of wine (Lubbers et al., 1994). In tasting trials, wine with higher Mannoprotein content is often preferred because of the perceptible roundness (Dupin et al., 2000; Llauberes et al., 1987). In addition, Mannoprotein can enhance lactic acid bacteria growth and in that manner aid in the timely completion of malolactic fermentation. Finally, Mannoprotein can protect wine from tartrate precipitation (Gerbaud et al., 1997).

The scientific literature notes that wine may contain 200-500 mg natural Mannoprotein per liter of wine (Dupin et al., 2000; Llauberes et al., 1987). In those cases, where the natural Mannoprotein content is too low for stabilization (also depending on the tartrate concentration), extra Mannoprotein can be added to achieve effective stabilization of the wine.

Since Mannoprotein is naturally occurring in wine produced by yeast fermentation, and since the amount of Mannoprotein added will depend on the amount of natural Mannoprotein and tartrate present, a scientific assumption can be made that the addition of Bakers Yeast Mannoprotein to wine will not produce new or unexpected byproducts.

4.1.4 Substantial equivalence with Mannoprotein present in other yeast-derived products

Several expert groups, as well as FDA and FDA scientists, have discussed the concept of substantial equivalence to food safety assessment (OECD, 1993 and 2003; Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety, 1996). Essentially, all these groups conclude that if a food ingredient is substantially equivalent to an existing food ingredient known to be safe, then no further safety considerations other than those for the existing ingredient are necessary.

In order to compare the Bakers Yeast Mannoprotein described in this dossier with the ones present in other foods, NMR profiles of the Bakers Yeast Mannoprotein were compared with those present in the following products already established as safe:

- Autolyzed yeast extract food spread (Marmite)
- White wine (Chablis)
- Yeast extract from another producer
- Invertase from *Saccharomyces cerevisiae*
- Bakers Yeast Mannoprotein from another producer (Mannostab™)

From the results, which are presented in Annex 7, it can be concluded that the Mannoprotein from these sources are substantially equivalent with the Bakers Yeast Mannoprotein described in this dossier. Since the other sources of Mannoprotein are safe for use in foods (either as affirmed as GRAS by FDA or by independent findings of safety), and the Mannoprotein from these sources is substantially equivalent to Bakers Yeast Mannoprotein, one can scientifically conclude that Bakers Yeast Mannoprotein is also safe.

4.2 The safety of Mannoprotein as supported by EU, Australia, New Zealand, and Argentina

Mannoprotein from *Saccharomyces cerevisiae* was officially approved for the stabilization of wine in the European Union (Council Regulation (EC) No 2165/2005) and, more recently, in Australia and New Zealand (Food Standards Australia New Zealand, 2008) and in Argentina (Resolución C 19/2006).

4.3 Intake data of Bakers Yeast Mannoprotein

Intake of Yeast Mannoprotein has occurred for centuries through the consumption of beer, wine and bread. Moreover, Yeast Mannoprotein is consumed through yeast-derived products such as yeast extract. Compared to the intake via these products, the consumption of Bakers Yeast Mannoprotein when added to wine is negligible, as can be concluded from the calculation below:

Natural occurring levels of Yeast Mannoprotein in foods are:

Beer:	83-507 mg/l (Food Standards Australia New Zealand, 2008)
Wine:	200-500 mg/l (Dupin et al., 2000; Llauberes et al., 1987)
Bread/pastries:	3.2-8 g/kg[4]
Yeast extract:	80-120 g/kg

Based on these levels, the following Estimated Daily Intake (EDI) of natural occurring Yeast Mannoprotein can be calculated:

Food item	**Mean intake[a)] (g/per person/day)**	**90th percentile intake[b)] (g/per person/day)**	**Mannoprotein content (mg/l or mg/kg)**	**EDI of Mannoprotein (g/per person/day)**
Wine[c)]	25	50	200-500	0.03
Beer[d)]	409	818	83-507	0.41
Bread[e)]	106	212	3200-8000	1.70
Spreads[f)]	4	8	80000-120000	0.96
Supplements[g)]	3.15 (recommended intake)		16000	0.5
TOTAL				3.6

a) Combined results from UDSA's 1995 and 1995 continuing survey of food intakes by individuals and 1994 and 1995 diet and health knowledge survey (Wilson et al, 1997). In general, the mean intake is given. However, for wine and beer the highest intake is given, since the mean intake was much lower than the intake in a certain category.
b) 90th percentile is calculated as twice the mean intake (CFSAN, 2006)
c) Highest intake category: Women 50-59 years of age, overall mean = 10 g/day
d) Highest intake category: Men 20-29 years of age, overall mean = 90 g/day
e) Overall mean for following categories: 1) Yeast breads and rolls, 2) quick breads, pancakes and French toast and 3) Cakes Cookies, pastries and pies.
f) www.marmite.co.uk/love/nutrition
g) Recommendation on packaging label

As can be concluded from this calculation, the EDI of natural Yeast Mannoprotein through the consumption of bread is about a factor 50 higher than through the consumption of wine.

[4] According to the European Bakery Yeast Manufacturers Association Cofalec (www.Cofalec.com) bread contains 2-5% yeast (2-5 kg yeast/100 kg flour). Since yeast contains about 16% Mannoprotein, the Mannoprotein content of bread is 3.2-8 g/kg bread.

If the Bakers Yeast Mannoprotein described in this dossier is added in an amount of 50-400 mg/l wine, this will result in a maximal additional EDI of 0.02 g/per person/day, which is less than 2% of the EDI consumed via bread.

Due to the low amount of Yeast Mannoprotein present in the total diet via consumption of wine, there is no basis to believe that the presence of Bakers Yeast Mannoprotein will have a significant effect, if any, on processed foods or on the human body.

4.4 Information that may appear to be inconsistent with the GRAS determination

An extensive literature research on the safety of *Saccharomyces cerevisiae* has been performed by the European Food Safety Agency (EFSA, 2007). The following is an extract of the EFSA report:

> *Saccharomyces cerevisiae (also known as "baker's yeast" or "brewers yeast") is mostly considered to be an occasional digestive commensal. However, since the 1990's, there have been a growing number of reports about its implication as an aetiological agent of invasive infection in "fragile" populations. A particular feature of such infections is their association with a probiotic preparation of S. cerevisiae (subtype S. boulardii) for treatment of various diarrhoeal disorders (see below). The nature of S. cerevisiae (subtype S. boulardii) and its clinical applications are reviewed by (Buts and Bernasconi 2005[5]).*
>
> *In one review, 92 cases of Saccharomyces invasive infection were presented (Enache-Angoulvant and Hennequin 2005[6]). Predisposing factors were similar to those of invasive candidosis, with intravascular and antibiotic therapy being the most frequent. Blood was the most frequent site of isolation (78% or 72 patients). S. cerevisiae (subtype S. boulardii) accounted for 51.3% (47 cases) of fungaemias and was exclusively*

[5] Buts, J. P. and P. Bernasconi (2005). "Saccharomyces boulardii: basic science and clinical applications in gastroenterology." Gastroenterol Clin North Am **34**(3): 515-32

[6] Enache-Angoulvant, A. and C. Hennequin (2005). "Invasive Saccharomyces infection: a comprehensive review." Clin Infect Dis **41**(11): 1559-68

isolated from blood. Special caution should be taken regarding the use of S. cerevisiae (subtype S. boulardii) preparations (Fleet and Roostita, 2006[7]). There are number of recent reports and reviews regarding the safety of S. cerevisiae (subtype S. boulardii) preparations involved in:

- *A case of Saccharomyces cerevisiae acquired fungaemia (Cassone, Serra et al. 2003; Graf and Gavazzi 2007[8]). The authors concluded that probiotics should be used cautiously in certain high-risk populations.*

- *A review of the current literature reinforces the view that fungaemia and sepsis are rare complications of the administration of S. cerevisiae (subtype S. boulardii) in immunocompromised patients but confirms that the most important risk factor for S. cerevisiae fungaemia is the use of probiotics (Herbrecht and Nivoix 2005[9]; Munoz, Bouza et al. 2005[10]). This raises the question of the risk-benefit ratio of these agents in critically ill or immunocompromised patients who are likely to develop an infection after exposure to high amounts of a microorganism with a low virulence.*

The body of knowledge is considered as sufficient (long history of safe use) with only 92 cases of pathogenic cases involving S. cerevisiae reported in total (15 cases diagnosed before 1990); all patients had at least one condition facilitating the opportunistic development of S. cerevisiae. S. bayanus and S. pastorianus are used in wine and beer production. There are no foodborne infection issues for these species.

On basis of this research, EFSA classified *Saccharomyces cerevisiae* as a QPS (Qualified Presumption as Safe) organism, a non-pathogen and not a major food

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[7] Fleet, G. H., Roostita, R. (2006). The public health and probiotic significance of yeasts in foods and beverages. The Yeast Handbook. A. Querol, Fleet, G.H. Berlin, Springer

Cassone, M., P. Serra, et al. (2003). "Outbreak of Saccharomyces cerevisiae subtype boulardii fungemia in patients neighboring those treated with a probiotic preparation of the organism." J Clin Microbiol **41**(11): 5340-3

[9] Herbrecht, R. and Y. Nivoix (2005). "Saccharomyces cerevisiae fungemia: an adverse effect of Saccharomyces boulardii probiotic administration." Clin Infect Dis **40**(11): 1635-7

[10] Munoz, P., E. Bouza, et al. (2005). "Saccharomyces cerevisiae fungemia: an emerging infectious disease." Clin Infect Dis **40**(11): 1625-34.

allergen. As stated earlier, this means that it can be safely used in food and feed without additional safety testing.

As concluded by the EFSA, *Saccharomyces cerevisiae* is not a major food allergen. However, it is well known that when inhaled, proteins in general can cause sensitization and allergic reactions. Inhalation allergy towards *Saccharomyces cerevisiae* has been described by Baldo and Baker (1988). This type of allergy is predominantly observed in bakers.

Regarding oral allergy against *Saccharomyces cerevisiae* only one possible case is known (Pajno et al., 2005). An atopic 6-year old boy experienced generalized urticaria and asthma after eating pizza and bread fresh from the oven (within one hour). The reaction towards yeast was confirmed in a prick-by prick procedure. The reaction only occurred when the bread just had been prepared, whilst no symptoms occurred when the bread was eaten more than one hour after preparation. Since this phenomenon is uncommon with regard to allergens, the cause of the boy's allergic reaction remains inconclusive.

From literature research regarding the allergenicity or sensitization by Mannoproteins in general, several references describing antigenicity from Mannoproteins from the yeast *Candida albicans* were found (see Annex 8). *Candida albicans* is an invasive opportunistic fungal pathogen and this type of allergy thus requires direct blood contact. No references were found describing oral allergenicity due to the consumption of Bakers Yeast Mannoprotein.

Based on the above discussion, it can be scientifically concluded that it is unlikely that Bakers Yeast Mannoprotein will result in sensitization or allergies after consumption through wine.

5. Conclusions

- The published literature discusses a long history of safe human consumption of bakers yeast (*Saccharomyces cerevisiae*), primarily due to its use in baking and brewing. Yeasts are safely consumed as dietary and nutritional supplements. As further indication of the safety of bakers yeast, various yeast-derived products have been approved as food additives, affirmed as GRAS, or are the subject of

GRAS notifications accepted by FDA.

- Toxicological studies have shown that the starting material for the production of Bakers Yeast Mannoprotein, DSM yeast extract, is safe for consumption. Furthermore, yeast extract has been affirmed as safe by FDA regulations.

- Mannoprotein released from wine yeast during fermentation is naturally present in wine. Yeast Mannoprotein is digested as normal dietary proteins and carbohydrates and is nontoxic and nonpathogenic as established by scientific studies. Literature research on the Mannoprotein itself did not reveal any issues of toxicological concern. The EU, Australia, New Zealand, and Argentina authorize the use of Mannoprotein in wines. The Bakers Yeast Mannoprotein is substantially equivalent to the Mannoprotein in other food ingredients found to be safe. No new or unexpected compounds will be formed or added with the addition of Bakers Yeast Mannoprotein to wine.

- The estimated intake of Bakers Yeast Mannoprotein will cause a de minimus increase in the overall consumption of Mannoprotein, and there is no basis to believe this additional amount of Mannoprotein will have a significant effect, if any, on the safety profile of the finished food product.

- Toxicological studies have shown that the phosphodiesterase enzyme preparation used as a processing aid in the processing of Bakers Yeast Mannoprotein is safe for consumption.

- Product specifications indicate the Bakers Yeast Mannoprotein does not contain chemical or microbiological contaminants above relevant limits.

- When applying the decision tree that was developed by Pariza and Johnson (2001) to evaluate the safety of microbial enzymes, it was scientifically concluded that Bakers Yeast Mannoprotein is safe for human consumption (see Annex 9)

Based on a review of scientific literature, it is concluded that use of the Bakers Yeast Mannoprotein for the stabilization of wine is GRAS.

6. List of Annexes

1. Resolution OENO 26/2004 of the OIV
2. Method to determine the efficiency of Bakers Yeast Mannoprotein to inhibit tartrate precipitation
3. Application trials performed with Bakers Yeast Mannoprotein
4. NMR spectrum of Bakers Yeast Mannoprotein
5. Flow diagram of the production process of Bakers Yeast Mannoprotein
6. Taxonomy of bakers yeast strains of DSM
7. Proof of substantial equivalence between Bakers Yeast Mannoprotein and other Mannoprotein containing food ingredients
8. Results of a literature review on negative effects due to the intake of Mannoprotein
9. Pariza & Johnson decision tree for Bakers Yeast Mannoprotein

7. List of References

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1

OIV

RESOLUTION OENO 26/2004

YEAST MANNOPROTEINS

THE GENERAL ASSEMBLY,

In view of Article 2 paragraph iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

On the proposal of the Sub-Commission of Methods of Analysis and Appraisal of Wines,

DECIDES to introduce the following method in the Compendium of International Methods of Analysis of Wines and Musts:

YEAST MANNOPROTEINS

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Mannoproteins are extracted from *Saccharomyces cerevisiae* yeast cell walls by physico-chemical of enzymatic methods.

Mannoproteins are different structures depending on their molecular weight, their degree and type of glycosylation, and their load size. Depending on their extraction mode, they have different tartaric and/or protein stabilisation of wine.

2. LABELLING

The label must indicate the field of application (tartaric and/or protein stabilisation of wine), security and storage conditions in addition to the date of expiry.

For solution preparations, the concentration of mannoproteins, the content of sulphur dioxide must likewise be indicated.

3. CHARACTERISATION

3.1 - Mannoproteins are found in powder form, usually microgranulated, white or beige in colour, odorless, or in a colloidal solution , yellow in colour, translucid.

3.2 - Mannoproteins are water soluble and insoluble in ethanol. In solution form, they precipate when 1 volume of ethanol is added.

3.3 – Optical rotation

The specific optical rotation is measured at 589 nm (sodium D line) and is related to a 10 g/l mannoprotein solution with a length 1dm.

Certains mannoproteins with a $[\alpha]_D^{20°C}$ rotary power between 80 ° and 150° can be distinguished from the arabic gum with a rotary power below 50°.

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Other preparations can only be distinguished by the percent composition in sugar (see point 4.12)

4. TRIALS

4.1 Loss through desiccation

4.1.1 Powder mannoprotein:
Put 5 g of mannoproteins in a 70 mm diameter silica capsule. Place in 100-105 °C drying chamber for 5 hours. Weight loss must not be more than 15%.

4.1.2 Mannoproteins in solution:
Put 10 g of mannoproteins in a 70 mm diameter silica capsule. Put over 100 °C water bath for 4 hours in a 100-105 °C drying chamber for 3 heures.
The quantity of dry residue must be at least 10%.

The limits set below are related to dry products.

4.2 Ashes

Incinerate dry residue at 550-600 °C. Ash content must not be more than 8%.

4.3 Preparation of solution for trials

Prepare a 10 g/l mannoprotein solution in water.
In the case of mannoprotein solution, weigh amount corresponding to 5 g of dry residue, evaporate until almost dry and dissolve again to 10 g/l in water.

4.4 Heavy metals

Determine iron on the solution prepared for trials (4.3) according to the method described in Chapter II of the International Oenological Codex
The content expressed in lead must be less than 30 mg/kg.

4.5 Lead

Determine lead on the solution prepared for trials (4.3) according to the method described in Chapter II of the International Oenological Codex
The content expressed in lead must be less than 5 mg/kg.

4.6 Mercury

Determine mercury on the solution prepared for trials (4.3) according to the method described in Chapter II of the International Oenological Codex
without evaporating the solution.
The content of mercury must be less than 0.15 mg/kg.

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4.7 Arsenic
Determine arsenic according to the method described in Chapter II of the International Oenological Codex on a solution for trials (4.3)
The content of arsenic must be less than 1 mg/kg.

4.8 Cadmium
Determine cadium according to the method described in Chapter II of the International Oenological Codex on a solution for trials (4.3)
The content of cadium must be less than 0.5 mg/kg.

4.9 Total nitrogen
Introduce 5 g of mannoproteins in a 300 ml mineralisation flask with 15 ml of concentrated sulphuric acid (R) and 2 g of mineralisation catalyser (R). Continue the determination as indicated in Chapter II of the International Oenological Codex.
In the case of mannoprotein solution, weigh an amount corresponding to 5 g of dry residue, evaporate until almost dry then proceed as in the above.
The content of nitrogen must be between 5 and 75 g/kg

4.10 Microbiological analysis

4.10.1 Total aerobic mesophile flora
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Not more than 10 000 total aerobic mesophile germs in 1 g.

4.10.2 Coliforms
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Not more than 10 CFU/g of preparation.

4.10.3 *Staphylococcus aureus*
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Absence checked for *Staphylococcus aureus* on a 1 g sample.

4.10.4 Salmonella
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Absence checked for salmonella on a 25 g sample

4.10.5 Escherichia coli
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Absence checked for *Escherichia coli* on a 25 g sample.

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4.10.6 Lactic bacteria
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Not more than 10^4 CFU/g of perparation.

4.10.7 Mould
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Not more than 50 CFU/g of preparation.

4.10.8 Yeasts
Proceed with counting according to method described in Chapter II of the International Oenological Codex.
Not more than 10^2 CFU/g of preparation.

4.11 Polysaccharides

4.11.1 Principle:
Measure colour intensity using hot phenol solution in a sulfuric medium.

4.11.2 Products:
4.11.2.1 15 mg/l mannoprotein solution
Dissolve 150 mg of mannoproteins in 100 ml of distilled water, then dilute this solution 1/100 with distilled water.
4.11.2.2 50 g/l phenol solution
Dissolve 5 g of pure phenol in 100 ml of distilled water.

4.11.3 Protocol:
200 µl of phenol (4.11.2.2) then 1 ml of pure sulphuric acid (R) are added to 200 µl of solution to be determined (4.11.2.1). After immediately mixing, the tubes are heated at 100°C in a bath water for 5 minutes and then cooled to 0 °C.
After reaching room temperature, absorbance is measured at 490 nm. 100 mg/l mannose solution is the reference solution.
(Content of polysaccharides expressed in equivalent of mannose above 600 g/kg)

4.12 Centesimal composition of glucidic monomers

4.12.1 Principle:
Enzymatic determination of glucose and mannose after acid hydrolysis.
The determination of mannose is carried out following the determination of fructose and the addition of phosphomannose isomerase (PMI).

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4.12.2 Products:

4.12.2.1 Mannoprotein solution 5 g/l
Dissolve 500 mg of mannoproteins in 100 ml of distilled water.

4.12.2.2 Sulphuric acid solution 5 M
Place 28 ml of sulphuric acid in 100 ml of distilled water.

4.12.2.3 Potassium hydroxide solution 10 M
Dissolve 46 g of potassium hydroxide in 100 ml of distilled water.

4.12.2.4 Phosphomannose isomerase 616 U/ml.

4.12.3 Protocol:

Place 100 µl of solution to be determined (4.12.2.1) in airtight sealed tubes and add 1 ml of sulphuric acid (4.12.2.2). After mixing, the tubes are heated at 100 °C in a water bath for 30 minutes and then cooled to 0 °C. After room temperature is reached, 1 ml of potassium hydroxide is added to neutralise the medium.
The detrmination of glucose and mannose can be carried out according to the method described in the compendium. Mannoproteins must contain at least 70% of mannose compared to total polysaccharides determined in 4.11.

4.13 Mannoprotein efficency test with regards to tartaric precipitation

4.13.1 Principle:
Determination of dose of mannoproteins to delay cristallisation of potassium hydrogenotartrate in a hyrdoalcoholic solution

4.13.2 Produits:

Crysalised tartaric Ac: PM = 150.05
Ethanol at 95% volume
Potassium chloride: PM= 74,5
Potassium hydrogenotartrate: PM= 188

4.13.3 Protocol:

4.13.3.1 Mannoprotein solution 10 g/l
Dissolve 1 g of mannoproteins in 100 ml of distilled water.
4.13.3.2 Hydro-alcoholic matrix
In a 1 liter volumetric flask half filled with distilled water dissolve:

- Tartaric acid: 2.1 g
- Potassium chloride: 1.1 g
- Ethanol at 95 % volume: 110 ml

Homogenise and fill up with distilled water.

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4.13.4 Test:
Place increasing quantities of mannoprotein solution (4.13.3.1) in a 100ml volumetric flask 0 – 1 – 2 – 3 - 4 ml and the volume is brought up to 100 ml with hydro-alcoholic matrix (4.13.3.2). These quantities correspond to final quantities of 0 – 100 – 200 – 300 - 400 mg/l of mannoproteins.
Add potassium hydrogenotartrate in each 100 mg flask.
Heat at 40 °C in a water bath for 1 hour until the complete solubilization of potassium hydrogenotartrate.
Stack the flasks in a refrigerator at 4 °C.

Observation after 48 hours:
The reference flask containing 0 ml of mannoprotein solution (4.13.3.1)
Presents potssium hydrogenotartrate crystals.
The absence of crystals in flasks containing mannoproteins aids in appraising the effectiveness. In all cases, crystals must be absent in a solution containing 400 mg/l of mannoproteins.

4.14 Mannoprotein efficiency test regarding protein casses

4.14.3 Principle
Determination of mannoprotein dose needed to improve protein stabilisation of wine.

4.14.4 Product:
Bovine serum albumen (Fraction V) (BSA)

4.14.5 Protocol:

4.14.5.1 10 g/l bovine serum albumen solution
Dissolve 2 g of bovine serum albumen in 200 ml of distilled water.
4.14.3.2 20 g/l mannoprotein solution
Dissolve 2 g of mannoproteins in 100 ml of distilled water.

4.14.4 Test

Placer 1 ml of BSA solution (4.14.3.1) in two 100 ml volumetric flasks and bring up to 100 ml in each flask with dry white wine which presents no cloudiness with heating (or stabilised if necessary with an adequate dose of bentonite treatment), and homogenize.
Adjust 0 and 1 ml of mannoprotein solution (4.14.3.2) and homogenize. These quantities correspond to 0 and 200 mg/l final doses of mannoproteins.
Filter reference and treated solutions through a membrance with a 0.45 µm pore diameter. Pour the filtered solutions in two 50 ml flasks.

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Place the 2 50 ml flasks and heat at 80 °C in a water bath for 30 minutes. Let cool to room temperature for 45 minutes, measure turbidity of reference and treated solution.
The decrease in turbidity between the reference sample and the treated sample must be at least 50%.

4.15 Dosage in wine

Principle
The dosage of mannoproteins in wine can be carried out after precipitating ethanol (5 volumes), acid hydrolysis from the precipitate and determination of released mannose according to the method listed in the annex.

5. STORAGE CONDITIONS

Solid mannoproteins have a 2 year shelf life if they are stored away from humidity in a sealed pack in a temperate room.
Mannoproteins present in colloidal solutions ready to be used must be stored in a hermetically sealed container.

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Annex

Determination of mannose using enzymatic method

Principle

Mannose is phosphorylated like glucose and fructose:

$$\text{Mannose} + \text{ATP} \longrightarrow \text{M6P} + \text{ADP}$$

Following the determination of glucose and fructose, mannose-6-phosphate is transformed due to the action of phosphomannose isomerase (PMI) in fructose-6-phosphate.

$$\text{M-6-P} \xrightarrow{\text{PMI}} \text{F-6-P}$$

Fructose-6-phosphate formed again is transformed as before in glucose-6-phosphate which is dosed.

Protocol

Place 5 ml of wine in a centrifuge tube and add 25 ml of 95% ethanol, after mixing the tubes are put in a 4°C refrigerator for 12 hours. The precipate formed is recuperated by a centrifuge, washed 2 times by 10 ml of 95% ethanol. The hydrolysis of the precipitate is carried out as in 4.12.
This determination does not enable the differentiation of mannoproteins added and natural mannoproteins.

Additional reagent regarding the method of the Compendium of International Methods of Analysis of Wines and Musts

Solution 6: phosphomannose isomerase (616 U/ml).
the suspension is used without diluting.

Determination

After measuring A_3 following the methods of the Compendium of Methods of Analysis of Wines and Musts, add

	Reference	Determination
Solution 6	0.02 ml	0.02 ml

Mix; carry out the determination after 30 min; monitor the end of the reaction after 2 min. (A_4)
Determine the absorbances differences:
$A_4 - A_3$ corresponding to mannose for the reference and the determination

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Subtract the aborbance difference for the reference (ΔA_T) and for the determination (ΔA_D) and establish: $\Delta A_M = \Delta A_D - \Delta A_T$ for mannose.

Results

For mannose: Cg/l = 0.423x ΔA_M is obtained.

Remark: If the measurements were carried out with wave lengths 334 or 365 nm, we obtain:

For a 334 nm measurement:

For mannose: Cg/l = 0.430x ΔA_M

365 nm measurement

For mannose: Cg/l = 0.783x ΔA_M

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2

METHOD OF ANALYSIS		No: S2531 Version 1
Department: DFS/R&D/Analysis	Title: Determination of the activity of Claristar in wine, R&D	
Date of issue: 02-[illegible]	Product: n.a.	Product code: n.a.
Compiled by: P.P. Lankhorst Date: 27/1/2009		Validated method NO
Checked by Expert: Date: 27/1/2009	Approved by Team manager: P.P. Lankhorst Date: 27/1/2009	Approved by QA/QC-officer: M.M. Immerzeel Date: 22-02-2009

1 SAFETY AND ENVIRONMENT

Restrictions for working with chemicals and ML-I samples are mentioned in the work instructions concerning management, storage and use of chemicals, the handling of dangerous substances and standard rules for ML-I laboratories. These restrictions are also applicable for material that has been in contact with ML-I samples.

2 PRINCIPLE

Wine can be considered as a supersaturated solution of potassium bi tartrate (KHT). Mannoproteins (Claristar) have the property to delay crystallization of KHT to a large extent. The activity of the product Claristar is measured by comparing the time needed for crystallization at -4 °C in a blank wine with the time needed in the same wine after addition of Claristar. A wine is generally considered as stable towards KHT crystallization if the wine does not exhibit any crystals for at least 6 days at -4 °C.

3 APPARATUS AND CONDITIONS

3.1 Apparatus

Experiments are performed in a refrigerator or cooling incubator with a stable temperature at -4 °C. The temperature should be stable to within ± 1 °C. Preferably better.
Revco ULT-350-a-V12 is suitable
Binder KB53 and KB115 are suitable

3.2 Conditions

The temperature of the refrigerator should be set at -4 °C.

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4 MATERIALS

4.1 Chemicals

Tap water

Unstable white or rosé wine

1. The activity of mannoproteins should be determined in white or rosé wine obtained directly from a winery. The wine should not contain any added mannoproteins or metatartaric acid, CMC or other stabilizing agents. The wine should not be stabilized by cold stabilization or electrodialysis or any other techniques. The wine can be kept for even more than one year, provided that it is stored at or slightly above room temperature (20 -25). It should be noted, that the properties of the wine may change over time. Some KHT might crystallize, even at room temperature, proteins may hydrolyze, and polyfenols may react upon prolonged storage. Therefore, the activity of one and the same product may differ, when measured at different points in time.
2. The wine used for this purpose should be rather unstable, but not extremely unstable. The wine is fit for the purpose if the blank wine shows crystals overnight at -4 °C (app 16 hours). If the wine shows crystals within 5 hours at -4 °C it is probably too unstable.

4.2 References, standards and controls

One previous batch of Claristar with good activity is selected as a control sample

4.3 Reagents

5 PROCEDURE

5.1 Preparation

The wine is centrifuged in a 50 ml Greiner-tube in order to remove precipitates, microbial contamination or any seeds of KHT. The clear wine is transferred carefully into a clean beaker. App 5 ml is left with the sediment in the Greiner-tube. One should take care that no precipitates are transferred into the beaker. The beaker should be clean, it should not contain dust.

One-way reaction tubes are first labelled according to the test. Next, the tubes are cleaned by blowing a strong nitrogen stream or air into the tube, which is kept upside down. In this way all dirt and dust is removed from the test-tube. Then the tubes are filled with 5.00 ml of the blank wine. Care should be taken (gloves, masks) not to contaminate the tubes with dust and hairs. These particles may act as a seed for crystallization, and they can be mistaken for a crystal

A stock solution of mannoproteins is prepared in a concentration of 10 mg/ml. In the case of a liquid product of 20% dry matter this can be realized by diluting 0.500 ml in 10 ml volumetric flask with tap-water. In the case of a powder of 100% dry matter 10-20 mg are weighed exactly to within 1 decimal, for instance 16.1 mg, and by means of an eppendorf pipette, the corresponding amount of tap-water is added. In this case 1.61 ml. After homogenization, the mixture is added to the wine by means of an eppendorf pipette 25 µl, 50 µl and 100 µl to achieve final concentrations of 50, 100 and 200 mg/l respectively of dry matter Claristar in the wine. Finally the bottle is closed well.

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5.2 Pretreatment reference

n.a.

5.3 Pretreatment standard

n.a.

5.4 Pretreatment control

n.a.

5.5 Pretreatment samples

n.a.

5.6 Preparation measurement

n.a.

5.7 Measurement

Test-tubes are capped, and stored in a refrigerator, kept at a constant temperature of -4 °C. The growth of crystals is monitored visually every day (see Figure 1). Observations are written down in a table (see Table 1).

Table 1: visual observation of the appearance of crystals in wine at -4 °C

		20/6	23/6	24/6	25/6	26/6	27/6	30/6	1/7	2/7	3/7	4/7
		fr	mo	tu	we	th	fr	mo	tu	we	th	fr
day nr		start	3	4	5	6	7	10	11	12	13	14
product 1	50mg/l		-	-		+		+	+	+	+	+
	100mg/l		-	-	-	-	-	+ -	+	+	+	+
	200mg/l		-	-	-	-	-	-	-	-	-	+/-
product 2	50mg/l		-	-	+	+	+	+	+	+	+	+
	100mg/l		-	-	-	-	+/-	+	+	+	+	+
	200mg/l		-	-	-	-	-	-	-	+/-	+/-	+
blank			+	+	-	+	+	+	+	+	+	+

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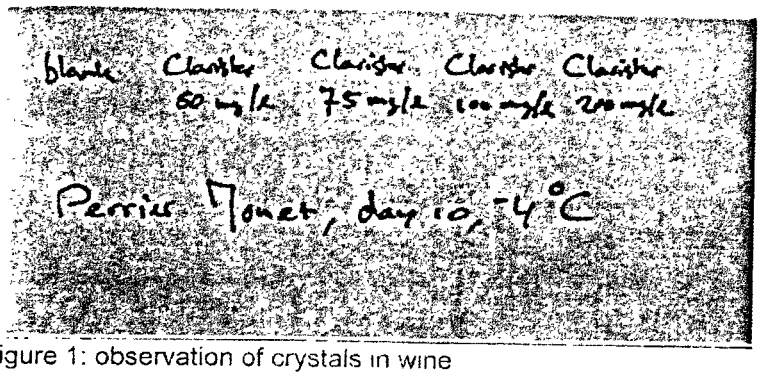

Figure 1: observation of crystals in wine

6 CALCULATION

Table 2: Activity of mannoproteins expressed as first day of observation of crystals.

	product 1	product 2
0 mg/l (blank)	<3	<3
50 mg/l	6	5
100 mg/l	11	10
200 mg/l	>14	14

7 ASSESSMENT

7.1 Requirements

N.a.

7.2 Actions

N.a.

7.3 Authorisation

N.a.

8 REFERENCES

N.A.

9 REMARKS

N.A.

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DSM

METHOD OF ANALYSIS	No: S2531 Version 1
Department: DFS/R&D/Analysis	Title: Determination of the activity of Claristar in wine, R&D

Product: n.a.

Product code: n.a.

HISTORY

Version	Description of the modification
1	First version
2	

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3

Application trials performed with Bakers Yeast Mannoprotein (Claristar™)

Subject:
Claristar™, A new ingredient for tartrate stabilisation

Source:
Publication on Claristar™: Copyright: DSM Food Specialties B.V. 07/2007

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Material and Methods:
Validation trials described in this article have been performed in collaboration with the "Station Experimentale d'Oenologie, INRA", Pech Rouge, with wines from the 2004 and 2005 vintages.

Abbreviations:
DIT: Degree de l'Instabilite de Tartrique (Degree of Tartaric Acid Instability)
KHT: Potassium Bi-Tartaric Acid
ISTC 50: Indice de Stabilité Tartrique Critique 50 (Index of Critical Tartaric Stability)[1]

Materials
Wine
- Blend of Grenache blanc and Viognier 2004 with a DIT of 18 % (Lot A) and 22.5 % (Lot B)
- Sauvignon blanc 2005 with a DIT of 22 %.

Mannoprotein
Claristar™ produced by DSM. This product can be used for the tartaric stabilisation of wine in compliance with the OIV International Oenological Codex and with European Council Regulation EC No 2165/2005 of 20-12-2005.

Methods
The wine was divided into two 10 L lots for the Grenache blanc / Viognier blend and 35 hl for the Sauvignon.
The first lot was an untreated control and the second was treated with Claristar™ at the dosage of 20 g/hl expressed in dry matter. All wines were previously fined with bentonite at 30 g/hl and filtered on Kieselguhr white earth.

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[1] ISTC50 is a quick method to check the tartaric stability of dry wines correlated to the stability at -4 °C. 50 mg of KHT is dissolved in 100 mL of wine, thus 25 mg potassium tartrate in 50 mL of wine. The temperature is maintained at -4 °C while stirring. When at -4 °C the time starts and every 10 minutes the conductivity is measured The quantity of KHT added may induce the crystallization and hence a drop in conductivity in case of unstable or less stable wines Drop < 120 min : wine instable , drop > 120 min. : wine stable

Application trials performed with Bakers Yeast Mannoprotein (Claristar™)

Methods for wine characterisation

The following analyses were performed on the control wine and after the addition of Claristar™.

- Potassium concentration (measured by photometry)
- Tartaric acid concentration (measured by HPLC Ionic Chromatography)
- Turbidity (measured by nephalometry)
- Conductivity (with a reference at 20 °C)
- pH
- Vmax (Filtration flow rate at 1 Bar on a 25 mm diameter membrane and a cut off of 0,65 µm)
- Coating index (Filtration flow rate at 2 Bar on a 25 mm diameter membrane and a cut of 0,65 µm)
- Reference for Vmax and Coating index supplied below[2]
- DIT: 4 g/l of standard KHT crystals were added to the wine at - 4 °C, with constant stirring at 500 rpm. (Model based on "contact" treatment used in wineries). Conductivity was monitored for 4 hours and data extrapolated until equilibrium. The instability was expressed as the conductivity decrease percentage. Most commercial wines have a DIT of 20-25 % at time of bottling.

Methods to measure wine stability

- Cold stabilisation: the wine was stored at - 4 °C. The visual appearance of the wine was checked daily until crystals were visible (0 no crystal, *suspicion of crystals, **visible crystals). A commercial wine is generally considered to be stable when no crystals are formed after 6 days.
- ISTC 50: 50 mg KHT was dissolved into 100 ml of wine. The wine was cooled down to - 4 °C with constant stirring at 500 rpm and conductivity monitored. The results were expressed in time (min) needed to get a conductivity decrease. The test is comparable to the cold stabilisation test at - 4 °C but gives a faster response. A commercial wine is generally considered to be stable when no crystals are formed after 180 min for red and rosé wines or after 120 min for white.

Results and Discussion

Effect on wine composition

Alcoholic degree, pH, turbidity, conductivity, tartaric acid concentration, potassium concentration, Vmax, coating index and DIT were measured on three different wines before and after the addition of Claristar™. The results are presented in Tables 1 and 2.

Claristar™ did not affect the wine composition nor the turbidity (Table 1).
The addition of Claristar™ impacted Vmax and coating index within acceptable limits for commercial bottling processes.

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[2] *Vmax and coating index: web reference: http://www.matevi-france.com/visualisation.asp?rub=7&ch=75&pg=130*

Application trials performed with Bakers Yeast Mannoprotein (Claristar™)

	Grenache - Viognier 2004 Lot A		Grenache - Viognier 2004 Lot B		Sauvignon 2005	
	Before	After	Before	After	Before	After
Alcoholic degree	14.08	13.96	12.59	12.61	-	-
pH	3.43	3.43	3.42	3.42	3.39	3.37
Turbidity (NTU)	3.8	3.9	2	3.6	4.5	6.7
Conductivity (µS cm 20 °C)	1638	1645	1436	1443	1708	1725
Tartric acid (g/l)	-	-	-	-	1.85	1.7
K+ (mg/l)	-	-	-	-	779	768
Mg++		-	-	-	1372	1044
Colloids (g/l)	-	-	-	-	3	6
DHT	16 %	13.6 %	22.5 %	20 %	42 %	20.4 %

Table 1: Wine composition before and after treatment with Claristar™.

Effect on wine flavour profile

Claristar™ was added at a dose of 20 g/hl (d.m.) to Sauvignon and Chardonnay. Each wine was tasted and compared to the control.

A triangular test (n = 42) concluded that no significant difference was detected in flavour profile for either wine (Risk ß 50 % of 0.1 % and <0.01 %).

Effect on wine stability

Stability tests at - 4 °C and ISTC 50 were completed on both wines before (control) and after the addition of Claristar™ (T0 = just after wine treatment, T12 = after 12 months storage at 18 °C). The analyses after 12 months were only performed on the Sauvignon 2005.

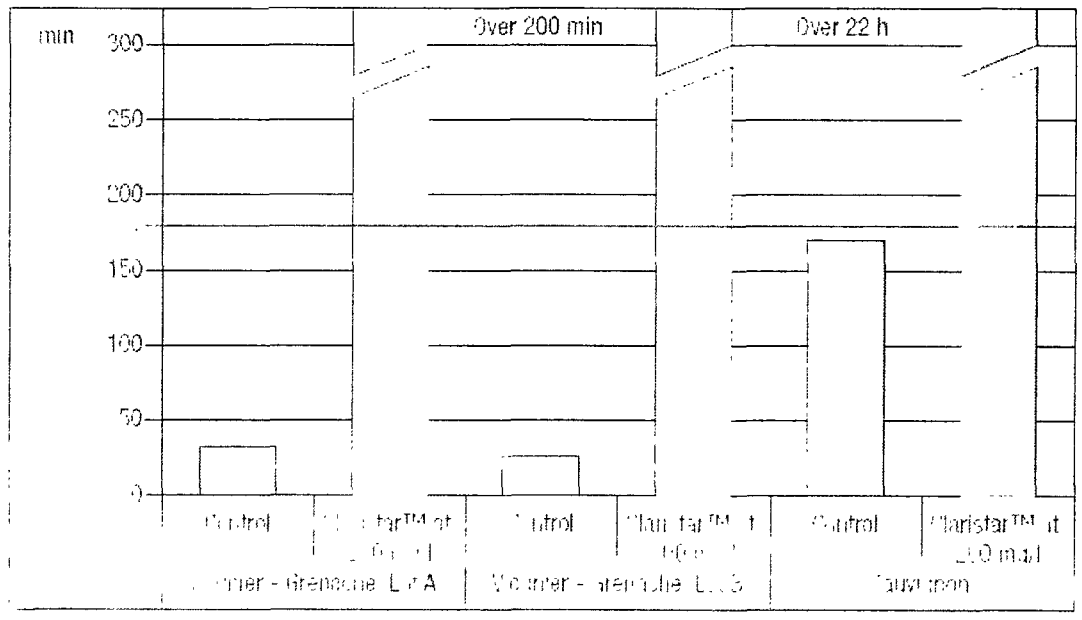

Figure 1: ISTC 50 (experiments conducted at INRA). Time when conductivity decrease (min) as a function of wine type.

Figure 1 shows the effect of Claristar™ on the ISTC 50 on Viognier / Grenache and Sauvignon. Results are expressed in time necessary to obtain a conductivity decrease. A wine is generally considered to be stable when the time for detectable drop in conductivity is greater than 180 min.

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Application trials performed with Bakers Yeast Mannoprotein (Claristar™)

Figure 1 shows that all the untreated wines were unstable (25 min for the Viognier-Grenache and 160 min for the Sauvignon). All the wines treated with Claristar™ were stable (times exceeding 180 min).

0: no crystal *: suspicion of cristal **: visible crystals	Grenache - Viognier Lot A		Grenache - Viognier Lot B		Sauvignon	
	Control	Claristar™ at 200 mg/l	Control	Claristar™ at 200 mg/l	Control	Claristar™ at 200 mg/l
Day 1	*	0	*	0	0	0
Day 2	**	0	**	0	*	0
Day 3	-	-	-	-	**	0
Day 4	-	-	-	-	**	0
Day 5	**	0	**	0	**	0
Day 6	**	0	**	0	**	0
Day 7	**	0	**	0	**	0
Day 8	**	0	**	0	**	0
Day 9	**	0	**	0	**	0
Day 10	**	0	**	0	-	-
Day 16	-	-	-	-	-	-
Day 29	**	0	**	0	**	0
Day 33	**	0	**	*	-	-
Day 34	**	0	**	**	-	-
Day 36	**	0	**	**	-	-
Day 37	**	0	**	**	-	-

Table 2: *effect of Claristar™ on the kinetics of crystal appearance.*

Table 2 shows the effectiveness of Claristar™ when monitored with the cold stabilisation test at - 4 °C.

Table 2 shows that crystals appeared in the control wines within one or two days. However, when Claristar™ had been added, the appearance of crystals was delayed to more than 29 days at -4 °C. Therefore, the addition of Claristar™ improved the stability of these highly unstable wines.

Effect on conductivity

Conductivity was measured over time for the Sauvignon 2005. Results are presented in Figure 2.

Application trials performed with Bakers Yeast Mannoprotein (Claristar™)

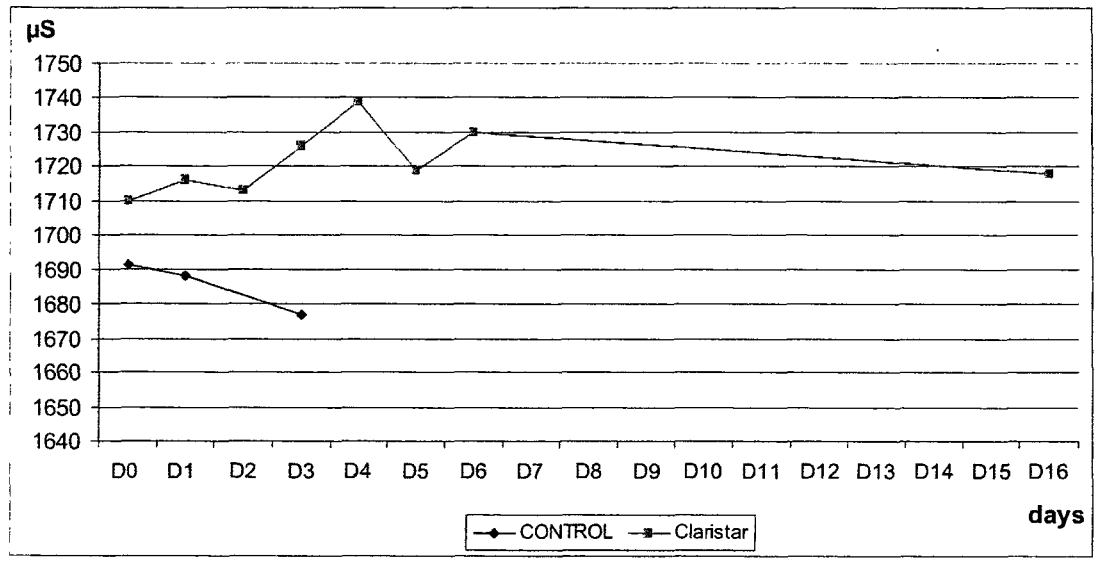

***Figure 2**: Conductivity as a function of time.*

Significant conductivity decrease was observed on day 3 in the untreated wine. No conductivity decrease was observed in the Claristar™ treated wine after 16 days at - 4 °C. This result is in line with the visual observations and confirms the protective effect of Claristar™.

		Control		Claristar™ at 200 mg/hl	
		Conductivity	Visual	Conductivity	Visual
ISTC 50 min		110		>300	
Conductivity µS/cm 20 °C	Day 0	1702	-	1713	-
	Day 1	1707	0	1735	0
	Day 2	1713	0	1737	0
	Day 3	1702	*	1761	0
	Day 4	1694	**	1716	0
	[illegible]	[illegible]	[illegible]	[illegible]	
	Day 6			1724	0
	Day 7			1724	0
No crystal / suspicion of crystal / some crystals	Day 8			1739	0
	Day 9			1739	0
	Day 16			1713	

***Table 3:** Conductivity and visual observation at -4 °C as a function of time and ISTC 50 after 12 months stored at 18 °C.*

Stability of wine over time

The stability of the Sauvignon was checked after 12 months storage at 18 °C. The results are presented in Table 3. Visual observation, conductivity measurements and ISTC 50 were repeated. The results indicate that even after 12 months storage the wine treated with Claristar™ remained stable (ISTC 50 > 300min and no crystals were observed after 16 days at - 4 °C). In conclusion, Claristar™ was highly effective in long term stability to KHT crystallisation

Application trials performed with Bakers Yeast Mannoprotein (Claristar™)

Conclusions

These studies summarise the compounding evidence behind the new Mannoprotein ingredient, Claristar™. This specific Mannoprotein fraction proved to be very efficient at preventing tartrate crystallisation across all the wines tested for a period of at least 18 months. Furthermore, Claristar™ did not impact wine composition nor taste.

Results to date confirm that Claristar™ is a highly effective and easy to use solution for the problem of tartrate precipitation.

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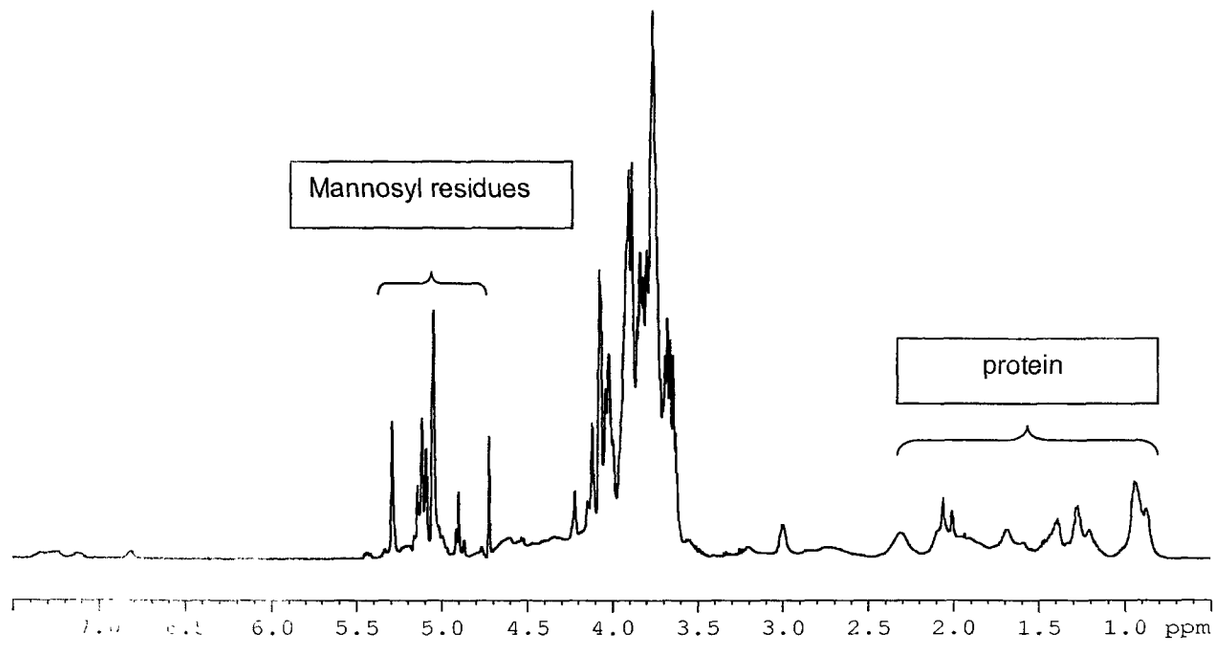

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Figure 1: 600 MHz ^{1}H NMR spectrum of Bakers Yeast Mannoprotein from DSM

Signals between 5.5 and 4.8 ppm represent the anomeric protons of all mannosyl residues of the Mannoprotein molecules.
Signals between 2.5 and 0.5 ppm represent the protons of the protein moieties of the Mannoprotien molecules.
The rather complex group of signals between 4.5 and 3.5 ppm is caused by the overlapping signals of all non-anomeric protons of the mannosyl residues in combination with all Hα protons of the protein moieties.

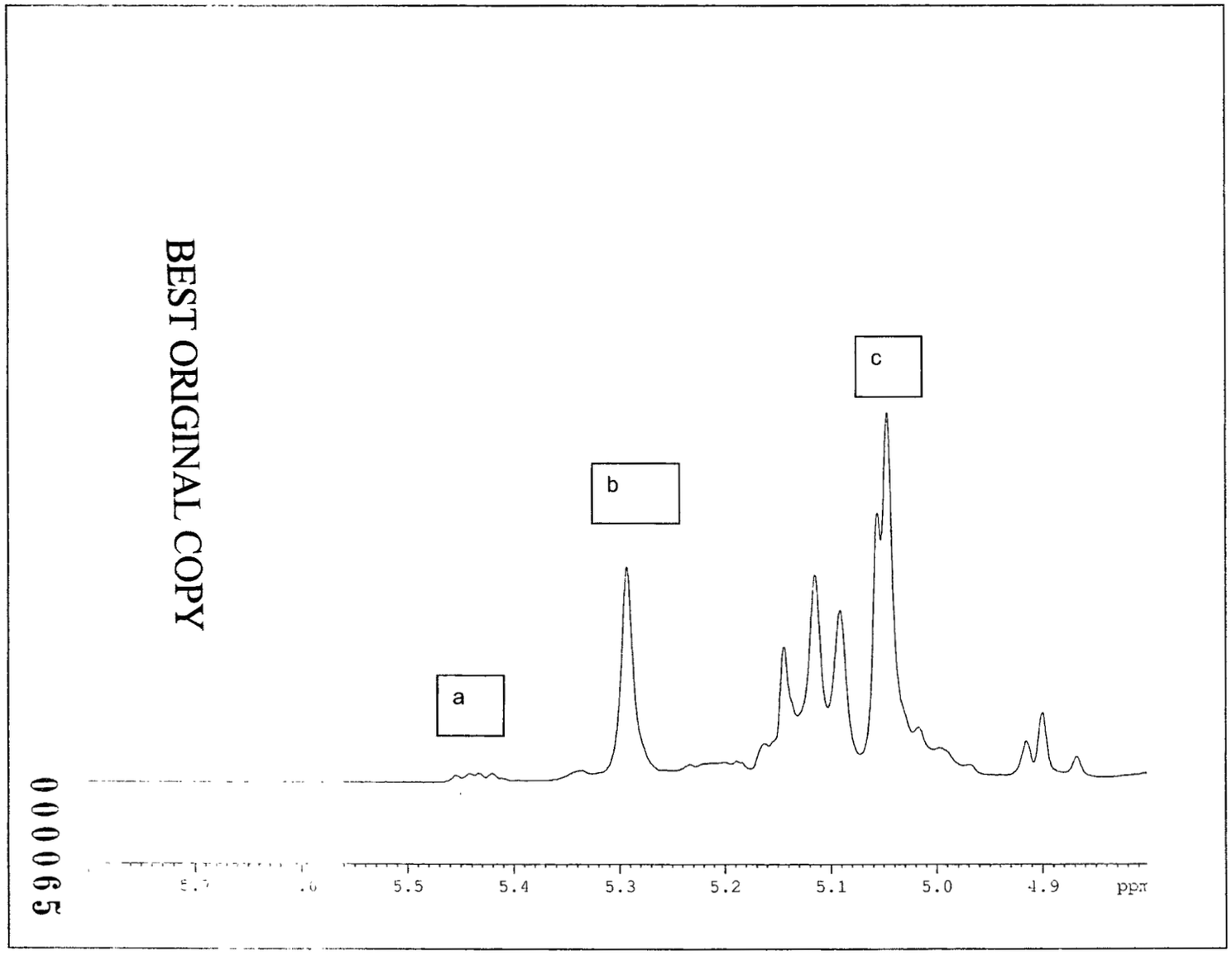

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Figure 2: Expansion of region between 5.5 and 4.8 ppm containing signals of anomeric protons

Partial assignment according to Vinogradov et al[1]: a: phosphorylated residues, b: branching sites (1→2) linkage attached to the (1→6) backbone, c: terminal residues of side-chains.

[1] Vinogradov, E., Petersen, B. and Bock, K., Carbohydrate Research **307** (1998), 177-183

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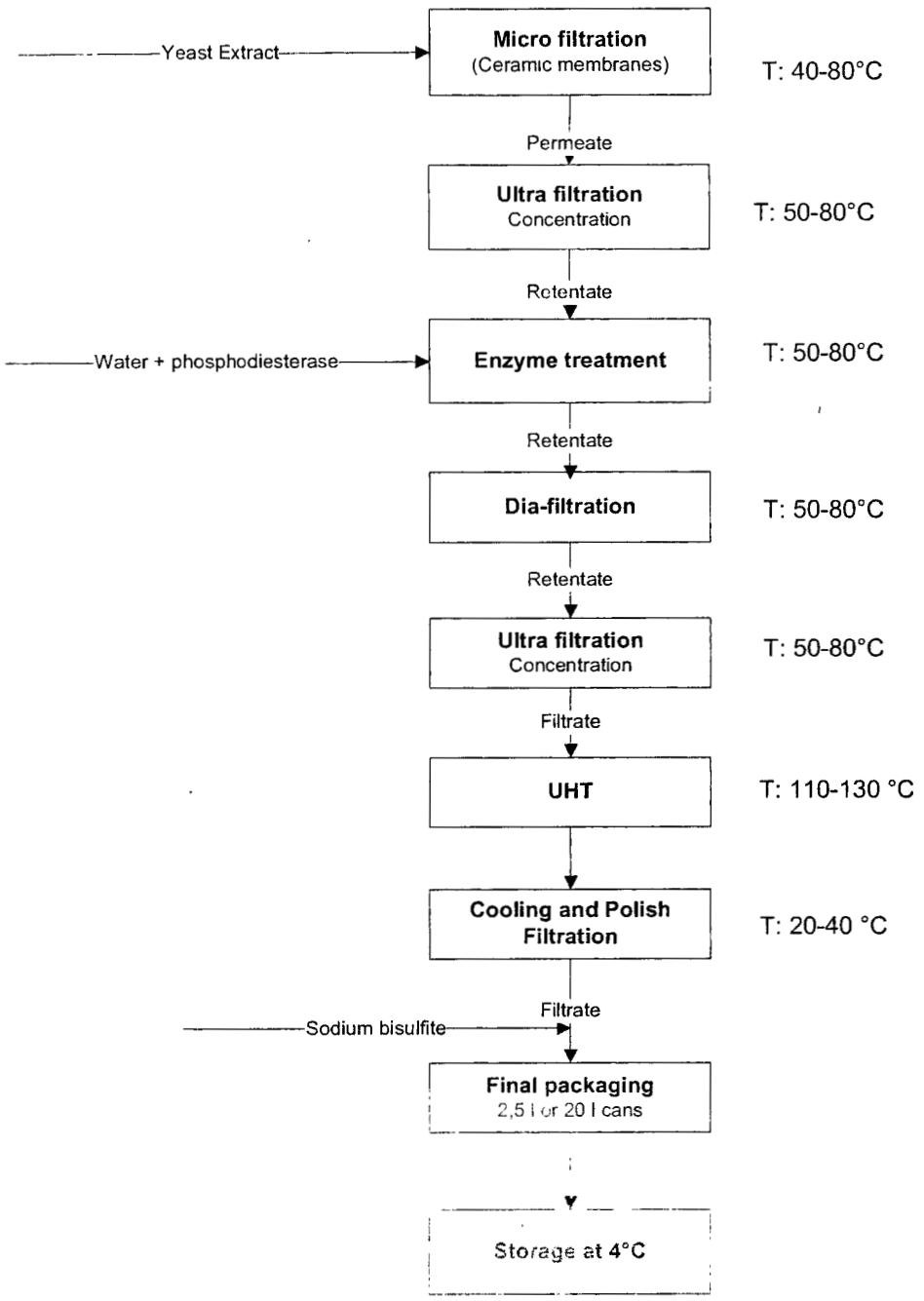
Bakers Yeast Mannoprotein Process



Note: UHT = Ultra Heat Treatment

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CENTRAALBUREAU VOOR SCHIMMELCULTURES

CERTIFICATE OF IDENTITY

To whom it may concern

We hereby certify that the strains

210Ng; 227Ng; 237Ng; 247Ng; 248Ng; 250Ng; 251Ng; 252Ng; 253Ng; 254Ng; 255Ng; 256Ng; 257Ng provided by

Gist Brocades
Wateringseweg 1,
P.O.Box 1,
2600 MA Delft
The Netherlands

has been examined by us and that they are identified as

Saccharomyces cerevisiae s.l.

The identification is based on morphological characters and physiological tests which are recommended and widely used for yeast identification. The examinations were carried out by Maudy Smith (senior scientist of the CBS Department of Yeast in Delft)

22 December 1995

Dr. R. A. Samson

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Substantial equivalence between the Bakers Yeast Mannoprotein (Claristar™) and Mannoprotein present in other yeast-derived food products

Introduction:

Several expert groups, as well as FDA and FDA scientists, have discussed the concept of substantial equivalence to food safety assessment (OECD, 1993 and 2003; Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety, 1996). Essentially, all these groups conclude that if a food ingredient is substantially equivalent to an existing food ingredient known to be safe, then no further safety considerations other than those for the existing ingredient are necessary.

Therefore, Bakers Yeast Mannoprotein (Claristar™) was compared with the Mannoprotein present in the following products already established as safe for human consumption:

- Autolyzed yeast extract food spread (Marmite)
- White wine (Chablis)
- Yeast extract from another producer
- Invertase from *Saccharomyces cerevisiae*
- Bakers Yeast Mannoprotein from another producer (Mannostab™)

In addition, the Bakers Yeast Mannoprotein was compared with the Mannoprotein from *Saccharomyces cerevisiae* described in literature. The study was limited to the comparison of the carbohydrate moiety (i.e. mannan) of the Mannoprotein, because it is generally the most abundant part of the molecule (60- >90%) and because the peptide moiety is difficult to detect in food matrices containing already a large quantity of peptides and proteins. The comparison was performed by means of NMR, because it is an excellent tool for identification of the Mannoprotein and the glycosidic linkages.

Materials and methods:

Isolation of Mannoprotein

- *Marmite (Unilever Bestfoods, UK) and Yeast extract from another producer:* Mannoprotein was isolated by means of ultrafiltration on an Amicon YM-3 (MW cut-off 3kD) membrane. The retentate was extensively washed with tap-water. In

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an attempt to remove other polysaccharides than Mannoprotein from the retentate, the isolate was incubated with amylase and glucanase, and the products of hydrolysis were removed by a second ultrafiltration on the same membrane. This treatment reduced the amylose content, but complete removal was not obtained.

- *White wine:* Mannoprotein was isolated by ultrafiltration of Chablis on an Amicon YM-1 (MW cut-off 1kD) membrane. The retentate was washed with water concentrated by means of the same ultrafiltration.

- *Invertase from Saccharomyces cerevisiae*: a sample was taken from the powdered product as such.

- *Bakers Yeast Mannoprotein from another producer (Mannostab™)*: a sample was taken from the powdered product as such.

Mannoprotein identification

Samples were dissolved in D_2O (27 °C) and placed in a 5mm tube. NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer.

Results:

NMR spectra as described in literature

Figure 1a shows the NMR spectrum in the region between 5.8 and 3.2 ppm of Mannoprotein from *Saccharomyces cerevisiae* as described by Vinogradov et al. (1998).

The carbohydrate moiety (mannan) derived from the Mannoprotein can be found in the region between 5.5 and 4.8 ppm, which can be regarded as a fingerprint of the molecule. In this region, various regions with peaks can be found which are labeled 'a', 'b' and 'c'. The small peaks at 5.4 ppm, labeled 'a', represent phosphorylated residues. The peak at 5.3 ppm, labeled 'b', represent branching sites, where mannosyl residues have a (1→2) linkage attached to the main (1→6) backbone. The peak slightly above 5.0 ppm, labeled 'c', represents the terminal residues of these side chains.

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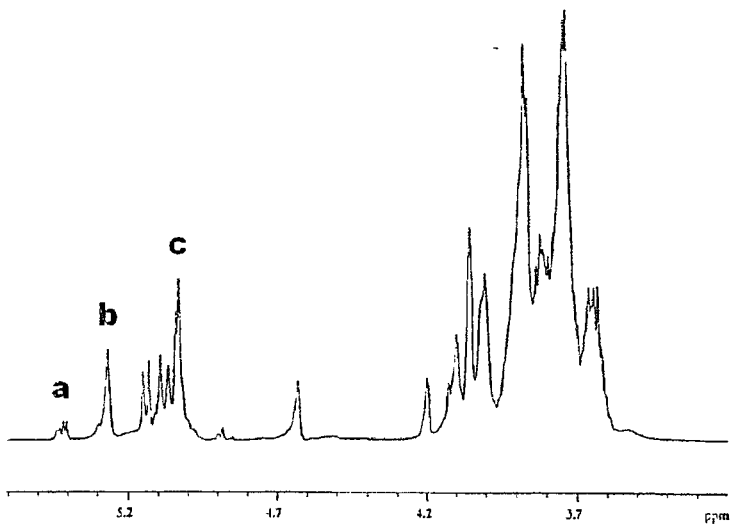

Fig. 1. 750 MHz ^{1}H NMR spectrum of mannan-1.

Figure 1a: Mannoprotein from Saccharomyces cerevisiae
(Vinogradov et al, 1998)

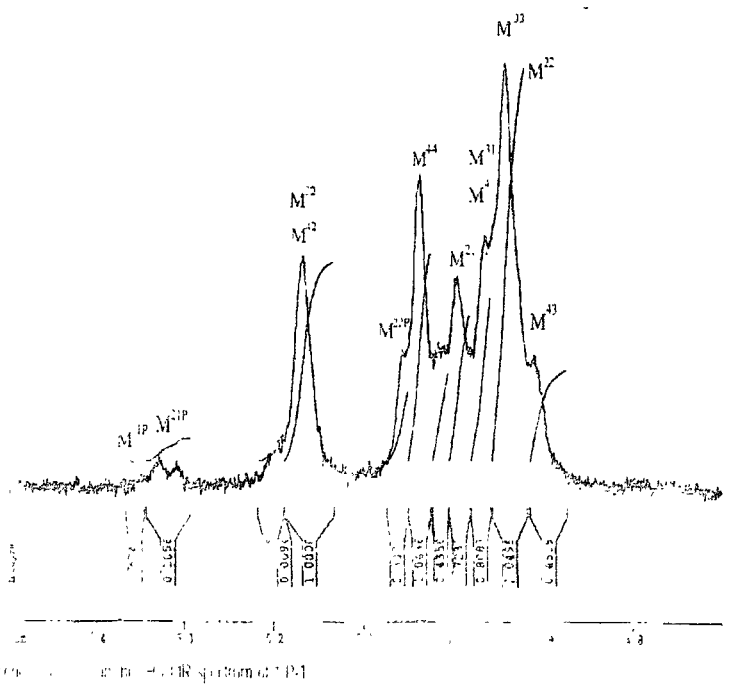

Figure 1b: Mannoprotein present in white wine
(Gonçalves et al (2)

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Figure 1b shows the NMR spectrum of Mannoprotein from white wine as described by Gonçalves et al. The Figure offers a closer look at the region between 5.5 and 4.8 ppm, where the Mannoprotein can be found. Again, three regions with peaks, previously indicated as (a), (b) and (c) in Figure 1a and accounting for the presence of the Mannan-moiety of the Mannoprotein can be observed. Therefore it can be concluded that the Mannoprotein isolated from *Saccharomyces cerevisiae* is equivalent to the Mannoprotein isolated from White Wine.

NMR spectra of Bakers Yeast Mannoprotein when compared to other sources

The NMR spectrum Bakers Yeast Mannoprotein (Claristar™) was compared with the Mannoprotein from other sources in the most relevant region between 5.8 and 4.8 ppm. The NMR spectra in this region of the Bakers Yeast Mannoprotein, Marmite, Chablis, yeast extract from another producer, invertase and Mannostab™ are presented in Figures 2a, 2b, 2c, 2d, 2e and 2f, respectively.

As can be concluded from the NMR spectrum, the carbohydrate moiety of Bakers Yeast Mannoprotein contains peaks in the same region as found in literature (see Figure 1a and 1b). Moreover, the same peaks are found in the NMR spectra of the abovementioned 5 other sources.

General conclusions:

The NMR spectrum of the carbohydrate moiety of the Bakers Yeast Mannoprotein (Claristar™) shows the same peaks as also found in the case of Mannoproteins from various other sources. It can therefore be concluded that the Bakers Yeast Mannoprotein is substantial equivalent when compared to Mannoproteins present in other yeast-derived food products already established as safe for human consumption.

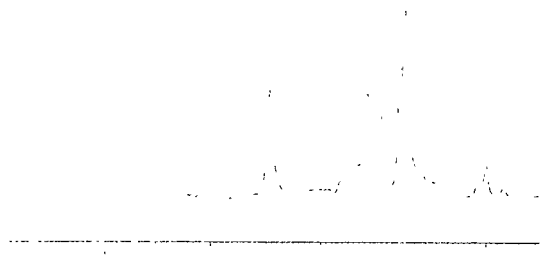

Figure 2a: Bakers Yeast Mannoprotein (Claristar™)

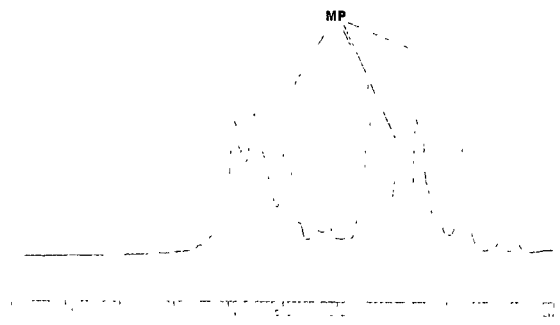

Figure 2b: Marmite

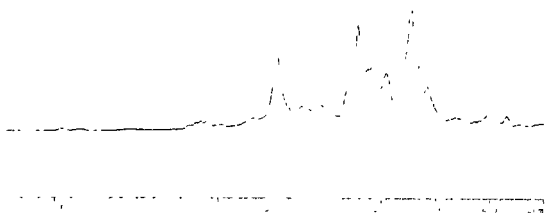

Figure 2c: isolate from Chablis

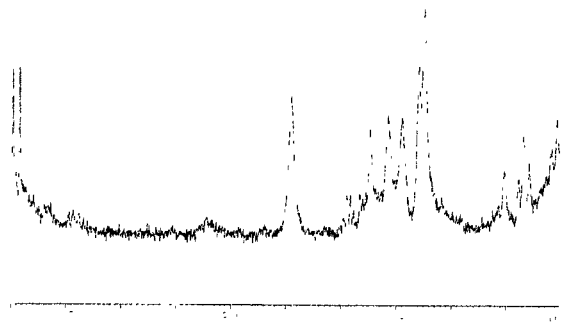

Figure 2g: yeast extract from other producer

Figure 2e. Invertase

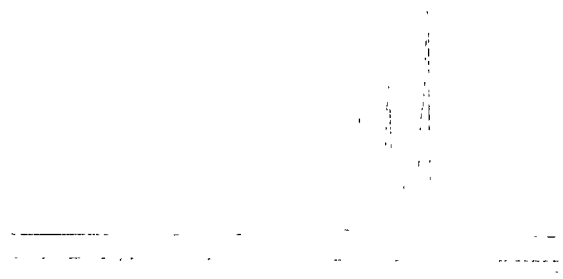

Figure 2f Mannostab™

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References:

Gonçalves, F., Heyraud, A., Norberta de Pinho, M. and Rinaudo, M., '*Characterization of white Wine Mannoprotein*' J. Agric. Food Chem **50** (2002), 6097-6101

Joint FAO/WHO Expert Consultation on Biotechnology and Food Safety Rome, Italy, 30 September to 4 October 1996

OECD (Organisation for Economic Cooperation and Development), 'Safety evaluation of foods produced by modern biotechnology: Concepts and principles', OECD, Paris, 1993

OECD (Organization for Economic Co-operation and Development), 'Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants', OECD, Paris, 2003

Vinogradov, E., Petersen, B. and Bock, K., 'Structural analysis of the intact polysaccharide mannan from Saccharomyces cerevisiae yeast using ^{1}H and ^{13}C NMR spectroscopy at 750 MHz', Carbohydrate Research **307** (1998), 177-183

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Results of a literature research on negative effects due to the intake of Mannoprotein

To find published research studies on negative effects due to the intake of Mannoprotein, a number of search queries were conducted in multiple databases.

Search strategy

The search strategies were centered on 5 topics:

- The use of Mannoproteins/yeast in food application
- Mannoproteins/yeast as food supplement
- Safety studies
- Negative effects of Mannoproteins
- Allergy/hypersensitivity to Mannoproteins.

The databases, all using STN, searched were:

- Biosis
- CAplus
- Embase
- FSTA
- Medline
- Toxcenter for toxicity data

The keywords used were:

- Additive
- Adverse
- Agricus
- Allergy
- Aversive
- Cell wall
- Characterization
- Cheese
- Cider
- Consumption
- Damage
- Detrimental
- Evaluation
- Food
- Fungus
- Fusarium
- Galactomanan
- Galactomannoprotein
- Geotrichum
- Harmful
- Hazard
- Heath
- Homo sapiens
- Hypersensitivity
- Lentidus

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Results of a literature research on negative effects due to the intake of Mannoprotein

- Lethal
- Man
- Mannoprotein
- Miso
- Mushroom
- Noxious
- Penicillium
- Poisonous
- Protein
- Rhizopus
- Safety
- Sake
- Sensitivity
- Side effect
- Shiitake
- Supplement
- Tempeh
- Toxic
- Yeast

Conjunctions to the above keywords, like toxic and toxicity, were also used.

Excluded terms were the keywords Candida and Cryptococcus, since a large number of studies are published on these pathogenic microorganisms, which have no relevancy to the topic. In one search also the keywords Marneffei and Fumigatus were excluded for the same reason. Selenium was excluded, resulting in the reduction of 11 publications, because these articles referred to yeast-based selenium food supplements.

Details of the searches

STN search transcript

STN index[1]
Expand Mannoproteins

Search 1: use of Mannoprotein in food and safety studies (2008-07-31)

File: Toxcenter
Search Mannoproteins OR Mannoproteines
Exclude Candida
Exclude Cryptococcus

File: CAplus
Search food AND Mannoproteins
Search health AND Mannoproteins

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[1] This initial search was used to get an impression in which database the highest number of hits on Mannoprotein would be found. The databases with the highest scores were searched in detail.

Results of a literature research on negative effects due to the intake of Mannoprotein

Search characterization AND Mannoproteins
Search yeast AND Mannoproteins
Search consumption AND Mannoproteins
Expand galactomanan
Search galactomannoprotein
Search supplement AND yeast AND Mannoprotein

Files: Biosis and FSTA
Search (Mannoprotein OR galactomannoprotein) AND yeast NOT Candida NOT Cryptococcus
Search consumption OR health OR food OR supplement
Combine two searches
Duplicate removal

Files: Medline and Embase
Search supplement (2a)[2] food
Search yeast (5a) previous search
Search mann? AND previous search
Duplicate removal
Search results combined with human OR homo sapiens OR man

Files: FSTA, Embase and Biosis
Search food additive
Search yeast cell wall
Combine
Search yeast (4a) food (2a) (additive OR supplement)
Duplicate removal
Combine with search evaluation
Combine with search protein
Combine with search NOT selenium
Combine with yeast NOT in title

Search 2: Mannoprotein in other sources (2008-09-16)

Files: FSTA, CAplus, Medline
Search Mannoproteins (L) (Penicillium OR cheese OR Agricus OR mushroom OR Rhizopus OR Tempeh OR Miso OR Geotrichum OR cider OR sake OR Lentidus OR Fusarium)

Files: Biosis
Search Sake AND Mannoprotein
Search Cider AND Mannoprotein
Search Tempeh AND Mannoprotein
Search cheese AND Mannoprotein
Search Shiitake AND Mannoprotein
Search Mannoprotein AND fungus
Search L12 NOT (Candida OR yeast OR Cryptococcus OR Marneffei OR Fumigatus)
Search L14 NOT Saccharomyces

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[2] Figures between brackets indicate the maximal words allowed between the keywords used

Results of a literature research on negative effects due to the intake of Mannoprotein

Search L15 NOT Fumigatus

Search 3: negative effects of Mannoproteins (2008-11-25)

Files: Medline, Embase, CAplus, FSTA and Biosis
Search Mannoprotein NOT Candida? NOT Cryptococcus
Search toxic? OR hazard? OR detriment? OR safety OR harmful OR lethal OR noxious OR poisonous OR adverse OR damag?
Searches combined with "AND"
Duplicate removal

Search 4: allergy/sensitivity to Mannoproteins (2009-01-27)

Files: Medline, Embase, CAplus, FSTA and Biosis
Search (Mannoprotein AND yeast) NOT Candida? NOT Cryptococcus
Search allerg? OR sensitiv? OR hypersensitive? OR aversiv? OR (side (w) effect?)
Searches combined with "AND"
Duplicate removal

Results and conclusions

The whole search resulted in 129 hits (see Enclosure). After evaluation of these references, 11 potential relevant publications were studied in more detail. These publications are highlighted in the search.

9 publications were used for the preparation of the dossier and have been inserted in the reference list of the dossier. 8 of these refer to the characterization, function and use of Mannoprotein in wines. One publication evaluates the safety of *Saccharomyces cerevisiae* in rats.

One reference concerned a patent referring to the use of Mannoprotein in cosmetics. Mannoproteins should have a positive effect on UV-light moderating effects. No literature was found indicating that potential negative effects of Mannoproteins in food exist.

Only one publication referred to allergy/sensitization in connection with Mannoprotein. Extracts from *Pityrosporum orbiculare*, a member of the residential skin microflora, containing high molecular weight polysaccharides or glycoproteins, presumably belonging to the mannan or mannorprotein fraction, were found to have - under certain circumstances - the possibility to induce allergenic reactions in patients with atopic dermatitis. No reference was made to any allergenic effects of these high molecular weight compounds after intake via food. Thus, it was concluded that in literature no indications have been found that Mannoprotein from *Saccharomyces cerevisiae* could induce sensitization or allergenicity through intake via food.

Enclosure: references found in the search

Results of a literature research on negative effects due to the intake of Mannoprotein

Enclosure: list of references found in the literature search
(highlighted: potential relevant publications which were studied in more detail)

Ref ID: 800
Journal Article (Electronic) Reference 1 of 129

Title:
Effects of yogurt supplemented with brewer's yeast cell wall on constipation and intestinal microflora in rats

Authors:
Nakamura,T.; Nishida,S.; Mizutani,M.; Iino,H.

Source:
Journal of Nutritional Science and Vitaminology, 2001, 47, 6, 367-372

Ref ID: 802
Journal Article (Electronic) Reference 2 of 129

Title:
Preservative stability and preventive effect of brewer's yeast cell wall on constipation and bowel movement in experimental constipation model

Authors:
Nakamura,T.; Mizutani,M.

Source:
Japanese Pharmacology and Therapeutics, 2000, 28, 8, 661-667

Ref ID: 711
Journal Article (Electronic) Reference 3 of 129

Title:
Deletion of Yeast CWP Genes Enhances Cell Permeability to Genotoxic Agents

Authors:
Zhang,Min; Liang,Yuping; Zhang,Xiaohua; Xu,Ying; Dai,Heping; Xiao,Wei

Source:
Toxicological Sciences, 2008, 103, 1, 68-76, CHINA

Ref ID: 1313
Journal Article (Electronic) Reference 4 of 129

Results of a literature research on negative effects due to the intake of Mannoprotein

Title:
Deletion of yeast CWP genes enhances cell permeability to genotoxic agents.

Authors:
Zhang,Min; Liang,Yuping; Zhang,Xiaohua; Xu,Ying; Dai,Heping; Xiao,Wei

Source:
Toxicological sciences : an official journal of the Society of Toxicology, 2008, 103, 1, 68-76. Electronic Publication: 2008-02-16, United States

Ref ID: 727
Journal Article (Electronic) Reference 5 of 129

Title:
Salt stress induces the expression of Schizosaccharomyces pombe och1+, which encodes an initiation-specific .alpha.-1,6-mannosyltransferase for N-linked outer chain synthesis of cell wall mannoproteins

Authors:
Yamamoto,Katsuyoshi; Okamoto,Michiyo; Yoko-o,Takehiko; Jigami,Yoshifumi

Source:
Biosci.Biotechnol.Biochem., 2003, 67, 4, 927-929, JAPAN

Ref ID: 739
Journal Article (Electronic) Reference 6 of 129

Title:
Isolation of mutants of Saccharomyces cerevisiae with a changed cell wall composition by screening on resistance to tannic acid

Authors:
Wauters,Tom; Verachtert,Hubert; Iserentant,Dirk

Source:
Food Technology and Biotechnology, 1999, 37, 4, 271-275, BELGIUM

Ref ID: 913
Journal Article (Electronic) Reference 7 of 129

Title:
CHARACTERIZATION OF FUCO MANNO PEPTIDE AND MANNO PROTEIN FROM ABSIDIA-CYLINDROSPORA.

Authors:
YAMADA H [Reprint author]; OHSHIMA Y; MIYAZAKI T

Results of a literature research on negative effects due to the intake of Mannoprotein

Source:
Carbohydr.Res., 110, 1, 113-126

Ref ID: 914
Journal Article (Electronic) Reference 8 of 129

Title:
HETEROGENEITY AND ANTIGENIC PROPERTIES OF MANNO HETERO GLYCAN FROM ABSIDIA-CYLINDROSPORA.

Authors:
YAMADA H [Reprint author]; OHSHIMA Y; MIYAZAKI Y

Source:
J.Gen.Microbiol., 128, 1, 189-198

Ref ID: 1328
Journal Article (Electronic) Reference 9 of 129

Title:
The progress of study on mechanism and application of yeast killer toxin.

Authors:
Zhang,Qing-Zhu (correspondence); Komiyama,Tadazumi; Komiyama,Tadazumi

Source:
China, ZYTOE8

Ref ID: 1329
Journal Article (Electronic) Reference 10 of 129

Title:
Zygocin, a secreted antifungal toxin of the yeast Zygosaccharomyces bailii, and its effect on sensitive fungal cells.

Authors:
Weiler,Frank; Schmitt Manfred,J.

Source:
FEMS yeast research, 3, 1, 69-76, Netherlands

Ref ID: 1340
Journal Article (Electronic) Reference 11 of 129

Results of a literature research on negative effects due to the intake of Mannoprotein

Title:
Influence of culture period on the allergenic composition of Pityrosporum orbiculare extracts.

Authors:
Zargari,A.; Doekes,G.; van Ieperen-van Dijk,A G.; Landberg,E.; Harfast,B.; Scheynius,A.

Source:
Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 25, 12, 1235-1245, ENGLAND: United Kingdom

Ref ID: 1314
Journal Article (Electronic) Reference 12 of 129

Title:
Surface enhanced Raman scattering analyses of individual silver nanoaggregates on living single yeast cell wall

Authors:
Sujith,Athiyanathil; Itoh,Tamitake; Abe,Hiroko; Anas,Abdul Aziz; Yoshida,Kenichi; Biju,Vasudevanpillai; Ishikawa,Mitsuru

Source:
Appl.Phys.Lett., 2008, 92, 10, 103901/1-103901/3, American Institute of Physics

Ref ID: 716
Conference Proceedings (Electronic) Reference 13 of 129

Title:
Brewing yeast flocculation: A model for onset and control

Authors:
Smart,K.A.; Lawrence,S.; Leclaire,J.; Davy,S.

Source:
Brewing and Distilling: A Common Heritage, IBD CD-ROM Proceedings,, 2006, UNITED KINGDOM, Hobart, Tasmania, Australia?

Ref ID: 721
Journal Article (Electronic) Reference 14 of 129

Title:
Preventing protein haze in bottled white wine

Authors:

Results of a literature research on negative effects due to the intake of Mannoprotein

Waters,E.J.; Alexanders,G.; Muhlack,R.; Pocock,K.F.; Colby,C.; O'Neill,B.K.; Hoj,P.B.; Jones,P.

Source:
Australian Journal of Grape and Wine Research, 2005, 11, 2, 215-225, AUSTRALIA

Ref ID: 724
Journal Article (Electronic) Reference 15 of 129

Title:
Importance of cell wall mannoproteins for septum formation in Saccharomyces cerevisiae

Authors:
Schmidt, Martin; Strenk, Meghan E.; Boyer, Michael P.; Fritsch, Bryan J.

Source:
Yeast (Chichester, England), 2005, 22, 9, 715-723, England: United Kingdom

Ref ID: 722
Conference Proceedings (Electronic) Reference 16 of 129

Title:
Differential regulation of yeast cell wall mannoproteins in response to stress during brewing yeast fermentation

Authors:
Smart,Katherine A.

Source:
Proceedings of the Congress - European Brewery Convention, 2003, 29, 44/1-44/9., UNITED KINGDOM, EBCPA6. ISSN: 0367-018X.

Ref ID: 789
Journal Article (Electronic) Reference 17 of 129

Title:
The polysaccharides of red wine: Total fractionation and characterization.

Authors:
Vidal,Stephane; Williams,Pascale; Doco,Thierry; Moutounet,Michel; Pellerin,Patrice

Source:
Carbohydr.Polym., 2003, 54, 4, 439-447

000086

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 736

Journal Article (Electronic) Reference 18 of 129

Title:
Preventive effects of polysaccharides on the protein haze in white wines

Authors:
Waters,Elisabeth; Dupin,Isabelle; Stockdale,Vanessa

Source:
Vignevini, 2000, 27, 7/8, 47-49, AUSTRALIA

Ref ID: 699

Journal Article (Electronic) Reference 19 of 129

Title:
Composition of tartrate precipitates deposited on stainless steel tanks during the cold stabilization of wines. Part I. White wines

Authors:
Vernhet,A.; Dupre,K.; Boulange-Petermann,L.; Cheynier,V.; Pellerin,P.; Moutounet,M.

Source:
American Journal of Enology and Viticulture, 1999, 50, 4, 391-397

Ref ID: 743

Journal Article (Electronic) Reference 20 of 129

Title:
Relative impact of major wine polysaccharides on the performances of an organic microfiltration membrane

Authors:
Vernhet,A.; Pellerin,P.; Belleville,M.-P.; Planque,J.; Moutounet,M.

Source:
Am.J.Enol.Vitic., 1999, 50, 1, 51-56, FRANCE

Ref ID: 757

Journal Article (Electronic) Reference 21 of 129

Title:
The retention mechanism of cell wall proteins in Saccharomyces cerevisiae. Wall-bound Cwp2p is .beta.-1,6-glucosylated

Results of a literature research on negative effects due to the intake of Mannoprotein

Authors:
van der Vaart,J.Marcel; van Schagen,Frank S.; Mooren,Arno T.A.; Chapman,John W.; Klis,Frans M.; Verrips,C.Theo

Source:
Biochimica et Biophysica Acta, 1996, 1291, 3, 206-214, NETHERLANDS

Ref ID: 791
Journal Article (Electronic) Reference 22 of 129

Title:
Charge properties of some grape and wine polysaccharide and polyphenolic fractions.

Authors:
Vernhet,A.; Pellerin,P.; Prieur,C.; Osmianski,J.; Moutounet,M.

Source:
Am.J.Enol.Vitic., 1996, 47, 1, 25-30

Ref ID: 764
Journal Article (Electronic) Reference 23 of 129

Title:
Identification of three mannoproteins in the cell wall of Saccharomyces cerevisiae

Authors:
van der Vaart,J.Marcel; Caro, L.H.; Chapman,John W.; Klis,Frans M.; Verrips,C.Theo

Source:
J.Bacteriol., 1995, 177, 11, 3104-3110, United States

Ref ID: 1055
Patent (Electronic) Reference 24 of 129

Title:
Cosmetics containing mannoproteins

Inventors:
Tanaka, Miwa; Yuki,Toshifumi

Source:
Jpn.Kokai Tokkyo Koho. JP 2007-65136 20070314. JP 2008222655 A, 9, Asahi Breweries, Ltd., Japan

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 1323

Journal Article (Electronic) Reference 25 of 129

Title:
Scw10p, a cell-wall glucanase/transglucosidase important for cell-wall stability in Saccharomyces cerevisiae.

Authors:
Sestak,Sergej; Hagen,Ilja; Tanner,Widmar; Strahl,Sabine

Source:
Microbiology, 150, Pt 10, 3197-3208, England: United Kingdom

Ref ID: 1324

Journal Article (Electronic) Reference 26 of 129

Title:
Increased mortality of Saccharomyces cerevisiae cell wall protein mutants.

Authors:
Teparic,Renata; Stuparevic,Igor; Mrsa,Vladimir

Source:
Microbiology, 150, Pt 10, 3145-3150, England: United Kingdom

Ref ID: 1338

Journal Article (Electronic) Reference 27 of 129

Title:
MNN6, a member of the KRE2/MNT1 family, is the gene for mannosylphosphate transfer in Saccharomyces cerevisiae.

Authors:
Wang,X H.; Nakayama,K.; Shimma,Y.; Tanaka,A.; Jigami,Y.

Source:
The Journal of biological chemistry, 272, 29, 18117-18124, United States

Ref ID: 1341

Journal Article (Electronic) Reference 28 of 129

Title:
Identification of SPT14/CWH6 as the yeast homologue of hPIG-A, a gene involved in the biosynthesis of GPI anchors.

Authors:

Results of a literature research on negative effects due to the intake of Mannoprotein

Vossen,Jack H.[Reprint author]; Ram,Arthur F.J.; Klis,Frans M.

Source:
Biochim.Biophys.Acta, 1243, 3, 549-551

Ref ID: 1345
Journal Article (Electronic) Reference 29 of 129

Title:
Blockage of cell wall receptors for yeast killer toxin KT28 with antimannoprotein antibodies.

Authors:
Schmitt,M J.; Radler,F.

Source:
Antimicrob.Agents Chemother., 34, 8, 1615-1618, United States

Ref ID: 1346
Journal Article (Electronic) Reference 30 of 129

Title:
Mannoprotein of the yeast cell wall as primary receptor for the killer toxin of Saccharomyces cerevisiae strain 28.

Authors:
Schmitt,M.; Radler,F.

Source:
J.Gen.Microbiol., 133, 12, 3347-3354, ENGLAND: United Kingdom

Ref ID: 1348
Journal Article (Electronic) Reference 31 of 129

Title:
Characterization of acetylated and acetolyzed glycoprotein high-mannose core oligosaccharides by fast-atom-bombardment mass spectrometry.

Authors:
Tsai,P K.; Dell,A.; Ballou,C E.

Source:
Proc.Natl.Acad.Sci.U.S.A., 83, 12, 4119-4123, United States

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 1350

Journal Article (Electronic) Reference 32 of 129

Title:
Characterization of a novel killer toxin encoded by a double-stranded linear DNA plasmid of Kluyveromyces lactis.

Authors:
Sugisaki,Y.; Gunge,N.; Sakaguchi,K.; Yamasaki,M.; Tamura,G.

Source:
European journal of biochemistry / FEBS, 141, 2, 241-245, GERMANY, WEST: Germany, Federal Republic of

Ref ID: 1056

Journal Article (Electronic) Reference 33 of 129

Title:
Effects of aging and heat treatment on whole yeast cells and yeast cell walls and on adsorption of ochratoxin A in a wine model system.

Authors:
Nunez,Y.P.; Pueyo,E.; Carrascosa,A.V.; Martinez-Rodriguez,A.J.

Source:
J.Food Prot., 2008, 71, 7, 1496-1499

Ref ID: 895

Journal Article (Electronic) Reference 34 of 129

Title:
4-ethylphenol and 4-ethylguaiacol in wines: Estimating non-microbial sourced contributions and toxicological considerations.

Authors:
Rayne,Sierra [Reprint Author]; Eggers,Nigel J.

Source:
Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes, 2007, 42, 8, 887-897

Ref ID: 672

Journal Article (Electronic) Reference 35 of 129

Results of a literature research on negative effects due to the intake of Mannoprotein

Title:
Characterization of Major Stable Proteins in Chardonnay Wine

Authors:
Okuda,T.; Fukui,M.; Takayanagi,t.; Yokotsuka,K.

Source:
Food Science and Technology Research, 2006, 12, 2, 131

Ref ID: 897
Journal Article (Electronic) Reference 36 of 129

Title:
Biosynthesis of glycoproteins in the human pathogenic fungus Sporothrix schenckii: Synthesis of dolichol phosphate mannose and mannoproteins by membrane-bound and solubilized mannosyl transferases.

Authors:
Ruiz-Baca,Estela; Villagomez-Castro,Julio C.; Leal-Morales,Carlos A.; Sabanero-Lopez,Myrna; Flores-Carreon,Arturo; Lopez-Romero,Everardo [Reprint Author]

Source:
Antonie Van Leeuwenhoek, 2005, 88, 3-4, 221-230

Ref ID: 734
Journal Article (Electronic) Reference 37 of 129

Title:
Evaluation of feeding study with brewer's yeast cell wall in rats

Authors:
Nakamura,Tomohiko; Shirasu,Yoshiharu

Source:
Japanese Pharmacology & Therapeutics, 2000, 28, 8, 669-679, JAPAN, JPTABU.

Ref ID: 737
Journal Article (Electronic) Reference 38 of 129

Title:
Sbe2p and Sbe22p, two homologous Golgi proteins involved in yeast cell wall formation

Authors:
Santos,Beatriz; Snyder,Michael

Source:

Results of a literature research on negative effects due to the intake of Mannoprotein

Mol.Biol.Cell, 2000, 11, 2, 435-452, UNITED STATES

Ref ID: 752

Journal Article (Electronic) Reference 39 of 129

Title:
Immobilization and ethanol stress induce the same molecular response at the level of the cell wall in growing yeast

Authors:
Parascandola,Palma; de Alteriis,Elisabetta; Sentandreu,Rafael; Zueco,Jesus

Source:
FEMS Microbiol.Lett., 1997, 150, 1, 121-126, ITALY

Ref ID: 1347

Journal Article (Electronic) Reference 40 of 129

Title:
Secretory pattern of a major integral mannoprotein of the yeast cell wall.

Authors:
Sanz,P.; Herrero,E.; Sentandreu,R.

Source:
Biochimica et Biophysica Acta - General Subjects, 1987, 924, 1, 193-203, Netherlands

Ref ID: 779

Journal Article (Electronic) Reference 41 of 129

Title:
Saccharomyces kluyveri mannoprotein mutants

Authors:
Pang,Dennis; Ballou,Lun; Zhang,Wei Jie; Ballou,Clinton E.

Source:
J.Biol.Chem., 1981, 256, 19, 10080-10083, UNITED STATES

Ref ID: 783

Journal Article (Electronic) Reference 42 of 129

Title:
Site of initial glycosylation of mannoproteins from Saccharomyces cerevisiae

Results of a literature research on negative effects due to the intake of Mannoprotein

Authors:
Ruiz-Herrera, J.; Sentandreu, R.

Source:
J.Bacteriol., 1975, 124, 1, 127-133, United States

Ref ID: 907
Journal Article (Electronic) Reference 43 of 129

Title:
Cryoprotection of Plagiomnium affine induced by various natural and artificial substances.

Authors:
Ruetten,Dorothea; Santarius,Kurt A.[Reprint author]

Source:
Canadian Journal of Botany, 71, 6, 793-798

Ref ID: 909
Journal Article (Electronic) Reference 44 of 129

Title:
Variations in the ability of galactose and mannose-specific lectins to bind to cell wall surfaces during growth of the insect pathogenic fungus Paecilomyces farinosus.

Authors:
Pendland,Jacquelyn C.[Reprint author]; Boucias,Drion G.

Source:
Eur.J.Cell Biol., 60, 2, 322-330

Ref ID: 911
Conference Proceedings (Electronic) Reference 45 of 129

Title:
INHIBITION OF LYMPHOCYTE PROLIFERATION BY CRYPTOCOCCAL MANNOPROTEIN.

Authors:
REUBEN J [Reprint,author]; LI,S.

Source:

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Ref ID: 1315

Journal Article (Electronic) Reference 46 of 129

Title:
Dissecting the role of dolichol in cell wall assembly in the yeast mutants impaired in early glycosylation reactions.

Authors:
Orlowski,Jacek; Machula,Katarzyna; Janik,Anna; Zdebska,Ewa; Palamarczyk,Grazyna

Source:
Yeast (Chichester, England), 24, 4, 239-252, England: United Kingdom

Ref ID: 1316

Journal Article (Electronic) Reference 47 of 129

Title:
Glycosylphosphatidylinositol-anchored proteins are required for the transport of detergent-resistant microdomain-associated membrane proteins Tat2p and Fur4p.

Authors:
Okamoto,Michiyo; Yoko-o,Takehiko; Umemura,Mariko; Nakayama,Ken-Ichi; Jigami,Yoshifumi

Source:
The Journal of biological chemistry, 281, 7, 4013-4023, United States

Ref ID: 1327

Journal Article (Electronic) Reference 48 of 129

Title:
A Saccharomyces cerevisiae genome-wide mutant screen for altered sensitivity to K1 killer toxin.

Authors:
Page,Nicolas; Gerard-Vincent,Manon; Menard,Patrice; Beaulieu,Maude; Azuma,Masayuki; Dijkgraaf Gerrit,J P.; Li,Huijuan; Marcoux,Jose; Nguyen,Thuy; Dowse,Tim; Sdicu,Anne-Marie; Bussey,Howard

Source:
Genetics, 163, 3, 875-894, United States

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Ref ID: 1331

Journal Article (Electronic) Reference 49 of 129

Title:
Rho1p mutations specific for regulation of .beta.(1.fwdarw.3)glucan synthesis and the order of assembly of the yeast cell wall.

Authors:
Roh,Dong-Hyun; Bowers,Blair; Riezman,Howard; Cabib,Enrico (correspondence)

Source:
United Kingdom, MOMIEE

Ref ID: 1333

Journal Article (Electronic) Reference 50 of 129

Title:
Sbe2p and sbe22p, two homologous Golgi proteins involved in yeast cell wall formation.

Authors:
Santos,B.; Snyder,M.

Source:
Mol.Biol.Cell, 11, 2, 435-452, United States

Ref ID: 1342

Journal Article (Electronic) Reference 51 of 129

Title:
A mutant approach and molecular strategy to study fungal cell walls.

Authors:
Pramanik,A.; Martinez,M.; McKoy,J F.; Ospina,R.; Daly,J.; Moore,C W.

Source:
France, Cellular and molecular biology (Noisy-le-Grand, France), (1995) Vol. 41 Suppl 1, pp. S73-81. Journal code: 9216789. ISSN: 0145-5680.

Ref ID: 719

Journal Article (Electronic) Reference 52 of 129

Title:
Fungal cell wall - challenge in the search for new antimycotics

Authors:
Mazan,Marian; Mazanova,Katarina; Farkas,Vladimir

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Source:
Chem.Listy, 2006, 100, 6, 433-439, SLOVAKIA

Ref ID: 720
Journal Article (Electronic) Reference 53 of 129

Title:
Genome-wide expression analysis of yeast response during exposure to 4.degree.C

Authors:
Murata,Yoshinori; Homma,Takayuki; Kitagawa,Emiko; Momose,Yuko; Sato,Masanori S.; Odani,Mine; Shimizu,Hisayo; Hasegawa-Mizusawa,Mika; Matsumoto,Rena

Source:
Extremophiles, 2006, 10, 2, 117-128, JAPAN

Ref ID: 797
Journal Article (Electronic) Reference 54 of 129

Title:
Effect of brewer's yeast cell wall administration on levels of blood lipids in hypercholesterolemic male adults

Authors:
Nakamura,T.; Shirasu,Y.; Tsuchida,T.; Shimasaki,H.

Source:
Pharmacometrics, 2001, 61, 4-5, 237-243

Ref ID: 798
Journal Article (Electronic) Reference 55 of 129

Title:
Effects of fermented milk supplemented with brewer's yeast cell wall on constipation and fecal microflora in healthy adults

Authors:
Nakamura,T.; Agata,K.; Nishida,S.; Shirasu,Y.; Iino,H.; Tanaka,T.; Amamoto,T.

Source:
Pharmacometrics, 2000, 59, 3-4, 47-55

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 799

Journal Article (Electronic) Reference 56 of 129

Title:
Influence of fermented milk (yogurt) containing brewer's yeast cell wall at relatively low doses and over-ingestion on defecation and bowel movement in humans

Authors:
Nakamura,T.; Nishida,S.; Iino,H.

Source:
Pharmacometrics, 2000, 59, 3-4, 57-63

Ref ID: 747

Journal Article (Electronic) Reference 57 of 129

Title:
Role of NaOH-extractable cell wall proteins Ccw5p, Ccw6p, Ccw7p and Ccw8p (members of the Pir protein family) in stability of the Saccharomyces cerevisiae cell wall

Authors:
Mrsa,V.; Tanner,W.

Source:
Yeast (Chichester, England), 1999, 15, 10A, 813-820, ENGLAND: United Kingdom

Ref ID: 755

Journal Article (Electronic) Reference 58 of 129

Title:
Identification of a mannoprotein present in the inner layer of the cell wall of Saccharomyces cerevisiae

Authors:
Moukadiri, I.; Armero, J.; Abad, A.: Sentandreu,Rafael; Zueco,Jesus

Source:
J.Bacteriol., 1997, 179, 7, 2154-2162, United States

Ref ID: 766

Journal Article (Electronic) Reference 59 of 129

Title:
Glucomannoproteins in the cell wall of Saccharomyces cerevisiae contain a novel type of carbohydrate side chain

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Authors:
Montijn,Roy C.; van Rinsum,Johanna; van Schagen,Frank A.; Klis,Frans M.

Source:
J.Biol.Chem., 1994, 269, 30, 19338-19342, NETHERLANDS

Ref ID: 1351
Journal Article (Electronic) Reference 60 of 129

Title:
Temperature-sensitive forms of large and small invertase in a mutant derived from a SUC1 strain of Saccharomyces cerevisiae

Authors:
Mizunaga,Takemitsu; Tkacz,Jan S.; Rodriguez,Luis; Hackel,Richard A.; Lampen,J.Oliver

Source:
Mol.Cell.Biol., 1981, 1, 5, 460-468

Ref ID: 902
Conference Proceedings (Electronic) Reference 61 of 129

Title:
New developments in the synthesis of fluorinated analogues of glutamic acid and glutamine.

Authors:
Meffre.,Patrick; Dave,,Rajesh; Badet,,Bernard

Source:

Ref ID: 906
Journal Article (Electronic) Reference 62 of 129

Title:
Chemical Structure of the Galactomannan Moiety in the Cell Wall Glycoproteins of Aspergillus oryzae.

Authors:
Nakajima,Tasuku [Reprint author]; Ichishima,Eiji

Source:
J.Ferment.Bioeng., 78, 6, 472-475

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Ref ID: 1080

Journal Article (Electronic) Reference 63 of 129

Title:
[A mannoprotein with a glycosyl-phosphatidyl-inositol substituent, responsible for tartrate stabilization of wines. Recent industrial developments.]

Authors:
Moine-Ledoux,V.; Dubourdieu,D.

Source:

Ref ID: 1093

Journal Article (Electronic) Reference 64 of 129

Title:
[Antibodies against nerve tissue proteins S100, GFAP, and MP65 and pregnant women with early toxemia]. Antitela k belkam nervnoi tkani S100, GFAP, MP65 u beremennykh s rannim toksikozom.

Authors:
Morozov,S G.; Poletaev,A B.; Murashko,L E.; D'iakova,E P.; Ponomareva,I V.; Gnedenko,B B.; Sukhikh,G T.

Source:
Voprosy medit sinskoi khimii, 44, 6, 576-583, RUSSIA: Russian Federation

Ref ID: 896

Journal Article (Electronic) Reference 65 of 129

Title:
Comparison of cell wall ultrastructures of Aspergillus nidulans in presence and absence of a mnpAp mannoprotein.

Authors:
Jeong,Hyo-Yong; Whang,Sung Soo; Chae,Keon-Sang [Reprint Author]

Source:
Integrative Biosciences, 2006, 10, 3, 131-135

Ref ID: 900

Journal Article (Electronic) Reference 66 of 129

Results of a literature research on negative effects due to the intake of Mannoprotein

Title:
A glucanase-driven fractionation allows redefinition of Schizosaccharomyces pombe cell wall composition and structure: assignment of diglucan.

Authors:
Magnelli,Paula E.; Cipollo,John F.; Robbins,Phillips W.[Reprint Author]

Source:
Anal.Biochem., 2005, 336, 2, 202-212

Ref ID: 901
Journal Article (Electronic) Reference 67 of 129

Title:
Presence of a mannoprotein, MnpAp, in the hyphal cell wall of Aspergillus nidulans.

Authors:
Jeong,Hyo-Young; Chae,Keon-Sang; Whang,Sung Soo [Reprint Author]

Source:
Mycologia, 2004, 96, 1, 52-56

Ref ID: 893
Journal Article (Electronic) Reference 68 of 129

Title:
Structure, occurrence and roles of carbohydrates with the hexo-D-manno configuration

Authors:
Matheson,Norman K.

Source:
Studies in Natural Products Chemistry, 2002, 26(Bioactive Natural Products, (Part G)), 1113-1173, Elsevier Science B.V., SNPCE2

Ref ID: 904
Journal Article (Electronic) Reference 69 of 129

Title:
Cell wall proteins of the ectomycorrhizal basidiomycete Pisolithus tinctorius: Identification, function, and expression in symbiosis.

Authors:
Martin,Francis [Reprint author]; Laurent,Pascal; de Carvalho,Dulcineia; Voiblet,Catherine; Balestrini,Raffaella; Bonfante,Paola; Tagu,Denis

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Source:
Fungal Genetics and Biology, 1999, 27, 2-3, 161-174

Ref ID: 753
Journal Article (Electronic) Reference 70 of 129

Title:
Demonstration and interpretation of the yeast lee ability to adsorb certain volatile thiols contained in wine

Authors:
Lavigne,Valerie; Dubourdieu,D.

Source:
Journal International des Sciences de la Vigne et du Vin, 1996, 30, 4, 201-206, FRANCE

Ref ID: 671
Journal Article Reference 71 of 129

Title:
Influence of mannoproteins from yeast on the aroma intensity of a model wine.

Authors:
Lubbers, S.; Voilley, A.; Feuillat, M.; Charpentier, C.

Source:
Lebensm. Wiss. Technol., 1994, 27, 108-114

Ref ID: 792
Journal Article Reference 72 of 129

Title:
Exocellular polysaccharides from Saccharomyces in wines.

Authors:
Llauberes, R. M.; Dubourdieu, D.; Villetaz, J. C.

Source:
Journal of the Sciences of Food and Agriculture, 1987, 41, 267-286

Ref ID: 905
Journal Article (Electronic) Reference 73 of 129

Results of a literature research on negative effects due to the intake of Mannoprotein

Title:
Fungal gene expression during ectomycorrhiza formation.

Authors:
Martin,F.[Reprint author]; Laurent,P.; De Carvalho,D.; Burgess,T.; Murphy,P.; Nehls,U.; Tagu,D.

Source:
Canadian Journal of Botany, 73, SUPPL 1, S541-S547

Ref ID: 1317
Journal Article (Electronic) Reference 74 of 129

Title:
FvVE1 regulates filamentous growth, the ratio of microconidia to macroconidia and cell wall formation in Fusarium verticillioides.

Authors:
Li,Shaojie; Myung,Kyung; Guse,Diana; Donkin,Brett; Proctor Robert,H.; Grayburn,W Scott; Calvo Ana,M.

Source:
Mol.Microbiol., 62, 5, 1418-1432, England: United Kingdom

Ref ID: 1318
Journal Article (Electronic) Reference 75 of 129

Title:
Yeast genes involved in response to lactic acid and acetic acid: acidic conditions caused by the organic acids in Saccharomyces cerevisiae cultures induce expression of intracellular metal metabolism genes regulated by Aft1p.

Authors:
Kawahata,Miho; Masaki,Kazuo; Fujii,Tsutomu; Iefuji,Haruyuki

Source:
FEMS yeast research, 6, 6, 924-936, Netherlands

Ref ID: 1321
Journal Article (Electronic) Reference 76 of 129

Title:
The ER-Golgi v-SNARE Bet1p is required for cross-linking alpha-agglutinin to the cell wall in yeast.

Authors:

Results of a literature research on negative effects due to the intake of Mannoprotein

Kipnis,Pearl; Thomas,Naomi; Ovalle,Rafael; Lipke Peter,N.

Source:
Microbiology, 150, Pt 10, 3219-3228, England: United Kingdom

Ref ID: 1336
Journal Article (Electronic) Reference 77 of 129

Title:
N-glycosylation is involved in the sensitivity of Saccharomyces cerevisiae to HM-1 killer toxin secreted from Hansenula mrakii IFO 0895.

Authors:
Kimura,T.[Reprint author]; Komiyama,T.; Furuichi,Y.; Iimura,Y.; Karita,S.; Sakka,K.; Ohmiya,K.

Source:
Appl.Microbiol.Biotechnol., 51, 2, 176-184

Ref ID: 785
Journal Article (Electronic) Reference 78 of 129

Title:
Polysaccharide profile and content during the vinification and aging of Tempranillo red wines.

Authors:
Guadalupe,Zenaida; Ayestaran,Belen

Source:
J.Agric.Food Chem., 2007, 55, 26, 10720-10728

Ref ID: 788
Journal Article (Electronic) Reference 79 of 129

Title:
Comparison of components released by fermented or active dried yeasts after aging on lees in a model wine.

Authors:
Guilloux-Benatier,Michele; Chassagne,David

Source:
J.Agric.Food Chem., 2003, 51, 3, 746-751

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 694

Journal Article (Electronic) Reference 80 of 129

Title:
Characterization of white wine mannoproteins

Authors:
Gonçalves,F.; Heyraud,A.; De Pinho,M.N.; Rinaudo,M.

Source:
Journal of Agricultural and Food Chemistry, 2002, 50, 21, 6097-6101

Ref ID: 731

Journal Article (Electronic) Reference 81 of 129

Title:
Cooperative functions of the mannoprotein-encoding genes in the biogenesis and maintenance of the cell wall in Saccharomyces cerevisiae

Authors:
Horie,T.; Isono,K.

Source:
Yeast, 2001, 18, 16, 1493-1503, England: United Kingdom

Ref ID: 748

Journal Article (Electronic) Reference 82 of 129

Title:
Influence of fatty acids on the growth of wine microorganisms Saccharomyces cerevisiae and Oenococcus oeni

Authors:
Guilloux-Benatier,M.; Le Fur,Y.; Feuillat,M.

Source:
J.Ind.Microbiol.Biotechnol., 1998, 20, 3/4, 144-149, FRANCE

Ref ID: 1105

Journal Article (Electronic) Reference 83 of 129

Title:
Study of the phenomenon of agglomeration in the yeast Saccharomyces cerevisiae

Authors:

Results of a literature research on negative effects due to the intake of Mannoprotein

Guinard,Jean Xavier; Lewis,Michael J.

Source:
J.Inst.Brewing, 1993, 99, 6, 487-503

Ref ID: 912
Journal Article (Electronic) Reference 84 of 129

Title:
MANNOPROTEINS FROM THE CELL WALL OF KLUYVEROMYCES-LACTIS.

Authors:
HERRERO E [Reprint author]; BOYD A

Source:
FEMS Microbiol.Lett., 36, 2-3, 207-212

Ref ID: 915
Journal Article (Electronic) Reference 85 of 129

Title:
CHARACTERIZATION OF PHOSPHORYLATED OLIGO MANNOSIDES FROM HANSENULA-WINGEI MANNO PROTEIN.

Authors:
HASHIMOTO C [Reprint author]; COHEN R E; BALLOU C E

Source:
Biochemistry (N.Y.), 19, 25, 5932-5938

Ref ID: 1094
Journal Article (Electronic) Reference 86 of 129

Title:
Influence of fatty acids on the growth of wine microorganisms Saccharomyces cerevisiae and Oenococcus oeni.

Authors:
Guilloux-Benatier,M.(correspondence); Feuillat.M.; Le Fur.Y.; Guilloux-Benatier,M.(correspondence); Guilloux-Benatier,M.(correspondence)

Source:
United Kingdom, JIMBFL

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 1325

Journal Article (Electronic) Reference 87 of 129

Title:
Sed1p and Srl1p are required to compensate for cell wall instability in Saccharomyces cerevisiae mutants defective in multiple GPI-anchored mannoproteins.

Authors:
Hagen,Ilja; Ecker,Margit; Lagorce,Arnaud; Francois Jean,M.; Sestak,Sergej; Rachel,Reinhard; Grossmann,Guido; Hauser Nicole,C.; Hoheisel Jorg,D.; Tanner,Widmar; Strahl,Sabine

Source:
Mol.Microbiol., 52, 5, 1413-1425, England: United Kingdom

Ref ID: 1334

Journal Article (Electronic) Reference 88 of 129

Title:
Fungal cell wall phosphomannans facilitate the toxic activity of a plant PR-5 protein.

Authors:
Ibeas,J I.; Lee,H.; Damsz,B.; Prasad,D T.; Pardo,J M.; Hasegawa,P M.; Bressan,R A.; Narasimhan,M L.

Source:
Plant J., 23, 3, 375-383, ENGLAND: United Kingdom

Ref ID: 733

Journal Article (Electronic) Reference 89 of 129

Title:
Update on antifungals targeted to the cell wall: focus on .beta.-1,3-glucan synthase inhibitors

Authors:
Georgopapadakou,Nafsika H.

Source:
Expert Opin.Investig.Drugs, 2001. 10, 2, 269-280, UNITED STATES

Ref ID: 749

Journal Article (Electronic) Reference 90 of 129

Results of a literature research on negative effects due to the intake of Mannoprotein

Title:
Influence of wine polysaccharides and polyphenols on the crystallization of potassium hydrogen tartrate

Authors:
Gerbaud,V.; Gabas,Nadine; Blouin,J.; Pellerin,P.; Moutounet,M.

Source:
Journal International des Sciences de la Vigne et du Vin, 1997, 31, 2, 65-83, FRANCE

Ref ID: 910
Journal Article (Electronic) Reference 91 of 129

Title:
BIOSYNTHESIS OF MANNOPROTEINS BY CELL-FREE EXTRACTS FROM PHYCOMYCES-BLAKESLEEANUS.

Authors:
FLORES-CARREON A [Reprint author]; SENTANDREU R

Source:
Exp.Mycol., 14, 2, 190-194

Ref ID: 794
Journal Article (Electronic) Reference 92 of 129

Title:
Yeast Macromolecules: Origin, Composition, and Enological Interest

Authors:
Feuillat,Michel

Source:
Am. J. Enol. Vitic., 2003, 54, 3, 211-213

Ref ID: 899
Journal Article (Electronic) Reference 93 of 129

Title:
Transcript patterns associated with ectomycorrhiza development in Eucalyptus globulus and Pisolithus microcarpus.

Authors:
Duplessis,Sebastien; Courty,Pierre-Emmanuel; Tagu,Denis; Martin,Francis [Reprint Author]

Results of a literature research on negative effects due to the intake of Mannoprotein

Source:
New Phytol., 2005, 165, 2, 599-611

Ref ID: 669
Journal Article (Electronic) Reference 94 of 129

Title:
The wine proteins

Authors:
Ferreira,Ricardo B.; Piçarra-Pereira,Maria A.; Monteiro,Sara; Loureiro,Virgílio B.; Teixeira,Artur R.

Source:
Trends in Food Science & Technology, 2001, 12, 7, 230-239

Ref ID: 667
Journal Article (Electronic) Reference 95 of 129

Title:
Saccharomyces cerevisiae mannoproteins that protect wine from protein haze: Their release during fermentation and lees contact and a proposal for their mechanism of action

Authors:
Dupin,I.V.S.; McKinnon,B.M.; Ryan,C.; Boulay,M.; Markides,A.J.; Jones,G.P.; Williams,P.J.; Waters,E.J.

Source:
Journal of agricultural and food chemistry, 2000, 48, 8, 3098-3105, Amer chemical soc

Ref ID: 767
Journal Article (Electronic) Reference 96 of 129

Title:
Flocculation of Kluyveromyces marxianus is induced by a temperature upshift

Authors:
Fernandes,P.A.; Sousa,M.; Moradas-Ferreira,P.

Source:
Yeast, 1993, 9. 8, 859-866, PORTUGAL

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 1098
Journal Article (Electronic) Reference 97 of 129

Title:
[Aroma as a quality parameter. Effects of yeast autolysis on aroma.]

Authors:
Feuillat,M.

Source:

Ref ID: 664
Journal Article (Electronic) Reference 98 of 129

Title:
Effect of flash release and pectinolytic enzyme treatments on wine polysaccharide composition

Authors:
Doco,T.; Williams,P.; Cheynier,V.

Source:
J. Agric. Food Chem., 2007, 55, 16, 6643-6649, Amer chemical soc

Ref ID: 668
Journal Article (Electronic) Reference 99 of 129

Title:
Dietary Fiber in Wine

Authors:
Diaz-Rubio,E.; Saura-Calixto,F.

Source:
AM. J. Enol. Vit., 2006, 57, 1, 69-72,

Ref ID: 723
Journal Article (Electronic) Reference 100 of 129

Title:
Characterisation of CwpA, a putative glycosylphosphatidylinositol-anchored cell wall mannoprotein in the filamentous fungus Aspergillus niger

Authors:
Damveld,Robbert A.; Arentshorst,Mark; VanKuyk,Patricia A.; Klis,Frans M.; Van Den

Results of a literature research on negative effects due to the intake of Mannoprotein

Hondel,Cees A.M.J.J.; Ram,Arthur F.J.

Source:
Fungal Genetics and Biology, 2005, 42, 10, 873-885, NETHERLANDS

Ref ID: 665
Journal Article (Electronic) Reference 101 of 129

Title:
Immunodetection of proteins from grapes and yeast in a white wine

Authors:
Dambrouck,T.; Marchal,R.; Marchal-Delahaut,L.; Parmentier,M.; Maujean,A.; Jeandet,P.

Source:
Journal of agricultural and food chemistry, 2003, 51, 9, 2727-2732, Amer chemical soc

Ref ID: 1332
Journal Article (Electronic) Reference 102 of 129

Title:
A genomic approach for the identification and classification of genes involved in cell wall formation and its regulation in Saccharomyces cerevisiae.

Authors:
de Groot,Piet W.J.; Ruiz,Cristina; Vasquez de Aldana,Carlos R.; Duenas,Encarnacion; Cid,Victor J.; Del Rey,Francisco; Rodriguez-Pena,Jose M.; Perez,Pilar; Andel,Annemiek; Caubin,Julio; Arroyo,Javier; Garcia,Juan C.; Gil,Concha; Molina,Maria; Garcia,Luis J.; Nombela,Cesar; Klis,Frans M.[Reprint author]

Source:
Comp.Funct.Genomics, 2001, 2, 3, 124-142

Ref ID: 1085
Journal Article (Electronic) Reference 103 of 129

Title:
Polysaccharides of wine

Authors:
Doco,T.; Williams,Pascale; Moutounet,M.; Pellerin,P.

Source:
Bulletin de l'O.I.V., 2000, 73, 837-838, 785-792, Office International de la Vigne et du Vin

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 670

Journal Article (Electronic) Reference 104 of 129

Title:
White Wine Protein Analysis by Capillary Zone Electrophoresis

Authors:
Dizy,Marta; Bisson,Linda F.

Source:
Am. J. Enol. Vitic., 1999, 50, 1, 120-127

Ref ID: 1344

Journal Article (Electronic) Reference 105 of 129

Title:
Biochemical and genetic studies on the function of, and relationship between, the PGI1- and CDC30-encoded phosphoglucose isomerases in Saccharomyces cerevisiae.

Authors:
Dickinson,J.R.

Source:
J.Gen.Microbiol., 1991, 137, 4, 765-770

Ref ID: 770

Journal Article (Electronic) Reference 106 of 129

Title:
The glucanase-soluble mannoproteins limit cell wall porosity in Saccharomyces cerevisiae

Authors:
de Nobel J G; Klis F M; Priem J; Munnik T; van den Ende H

Source:
Yeast (Chichester, England), 1990, 6, 6, 491-499, ENGLAND: United Kingdom

Ref ID: 790

Patent (Electronic) Reference 107 of 129

Title:
Biological substance for the physico-chemical stabilization of wines.

Inventors:

Results of a literature research on negative effects due to the intake of Mannoprotein

Dubourdieu, Denis; Moine, Virginie

Source:
US 6139891 20001031

Ref ID: 1097
Journal Article (Electronic) Reference 108 of 129

Title:
[Role of yeast mannoproteins in tartrate stability of wines.]

Authors:
Dubourdieu,D.; Moine,V.

Source:

Ref ID: 1102
Journal Article (Electronic) Reference 109 of 129

Title:
Electrophoretic mobility of external invertase from free and gel-immobilized yeast cells.

Authors:
De Alteriis,E.; Zueco,J.; Rico,H.; Sentandreu,R.; Parascandola,P.[Reprint author]

Source:
Res.Microbiol., 146, 3, 217-225

Ref ID: 1337
Journal Article (Electronic) Reference 110 of 129

Title:
Specific cell wall proteins confer resistance to nisin upon yeast cells.

Authors:
Dielbandhoesing,S.K.; Zhang,H.; Van Der Vaart,J.M.; Verrips,C.T.;
Brul,S.(correspondence); Caro,L.H.P.; Klis,F.M.; Verrips,C.T.; Brul,S.(correspondence)

Source:
United States, AEMIDF

Ref ID: 713
Journal Article (Electronic) Reference 111 of 129

Results of a literature research on negative effects due to the intake of Mannoprotein

Title:
New perspectives in safety and quality enhancement of wine through selection of yeasts based on the parietal adsorption activity

Authors:
Caridi,Andrea

Source:
Int.J.Food Microbiol., 2007, 120, 1-2, 167-172, ITALY

Ref ID: 793
Journal Article (Electronic) Reference 112 of 129

Title:
A Fourier transform infrared spectroscopy study of wine polysaccharides

Authors:
Boulet,J.C.; Williams,P.; Doco,T.

Source:
Carbohydrate Polymers, 2007, 69, 1, 79-85

Ref ID: 695
Journal Article (Electronic) Reference 113 of 129

Title:
Enological functions of parietal yeast mannoproteins

Authors:
Caridi,A.

Source:
Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 2006, 89, 3-4, 417-422

Ref ID: 718
Journal Article (Electronic) Reference 114 of 129

Title:
Ochratoxin A removal during winemaking

Authors:
Caridi,Andrea; Galvano,Fabio; Tafuri,Alessio; Ritieni,Alberto

Source:

Results of a literature research on negative effects due to the intake of Mannoprotein

Enzyme Microb.Technol., 2006, 40, 1, 122-126, ITALY

Ref ID: 696

Journal Article (Electronic) Reference 115 of 129

Title:
Release of macromolecules by Saccharomyces cerevisiae during ageing of French flor sherry wine "Vin jaune"

Authors:
Charpentier,C.; Dos Santos,A.M.; Feuillat,M.

Source:
International Journal of Food Microbiology, 2004, 96, 3, 253-262

Ref ID: 1320

Journal Article (Electronic) Reference 116 of 129

Title:
Saccharomyces cerevisiae Glutaredoxin 5-deficient Cells Subjected to Continuous Oxidizing Conditions Are Affected in the Expression of Specific Sets of Genes

Authors:
Belli,Gemma; Molina,Maria Micaela; Garcia-Martinez,Jose; Perez-Ortin,Jose E.; Herrero,Enrique

Source:
J.Biol.Chem., 2004, 279, 13, 12386-12395, American Society for Biochemistry and Molecular Biology

Ref ID: 894

Journal Article (Electronic) Reference 117 of 129

Title:
The incorporation of mannoproteins in the cell wall of S. cerevisiae and filamentous Ascomycetes.

Authors:
Brul,S.; King,A.; Van Der Vaart,J.M.; Chapman,J.; Klis,F.; Verrips.C.T.

Source:
Antonie Van Leeuwenhoek, 72, 3, 229-237

Ref ID: 1319

Results of a literature research on negative effects due to the intake of Mannoprotein

Journal Article (Electronic) Reference 118 of 129

Title:
The RIM101 pathway contributes to yeast cell wall assembly and its function becomes essential in the absence of mitogen-activated protein kinase Slt2p.

Authors:
Castrejon,F.; Gomez,A.; Sanz,M.; Duran,A.; Roncero,C.

Source:
Eukaryotic cell, 5, 3, 507-517, United States

Ref ID: 1322

Journal Article (Electronic) Reference 119 of 129

Title:
Yeast Kre1p is GPI-anchored and involved in both cell wall assembly and architecture.

Authors:
Breinig,Frank; Schleinkofer,Karin; Schmitt Manfred,J.

Source:
Microbiology, 150, Pt 10, 3209-3218, England: United Kingdom

Ref ID: 1339

Journal Article (Electronic) Reference 120 of 129

Title:
The incorporation of mannoproteins in the cell wall of S. cerevisiae and filamentous Ascomycetes.

Authors:
Brul,S.; King,A.; van der Vaart,J M.; Chapman,J.; Klis,F.; Verrips,C T.

Source:
Antonie Van Leeuwenhoek, 72, 3, 229-237, Netherlands

Ref ID: 1349

Journal Article (Electronic) Reference 121 of 129

Title:
Swainsonine and castanospermine blockade of mannose glycoprotein uptake by macrophages. Apparent inhibition of receptor-mediated endocytosis by endogenous ligands.

Authors:

Results of a literature research on negative effects due to the intake of Mannoprotein

Chung,K N.; Shepherd,V L.; Stahl,P D.

Source:
The Journal of biological chemistry, 259, 23, 14637-14641, United States

Ref ID: 714
Journal Article (Electronic) Reference 122 of 129

Title:
Induction of DAN/TIR yeast cell wall mannoprotein genes in response to high hydrostatic pressure and low temperature

Authors:
Abe,Fumiyoshi

Source:
FEBS Lett., 2007, 581, 25, 4993-4998, JAPAN

Ref ID: 698
Journal Article (Electronic) Reference 123 of 129

Title:
Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process

Authors:
Ayestarán,B.; Guadalupe,Z.; León,D.

Source:
Analytica Chimica Acta, 2004, 513, 1, 29-39

Ref ID: 1343
Journal Article (Electronic) Reference 124 of 129

Title:
Bleomycin affects cell wall anchorage of mannoproteins in Saccharomyces cerevisiae

Authors:
Beaudouin,Robert; Lim,Seung T.; Steide,Jean Alex; Powell,Marie; Koy,Judith M.; Pramanik,A.J.; Johnson,Elma: Moore,Carol Wood: Lipke.Peter N.

Source:
Antimicrob.Agents Chemother., 1993, 37, 6, 1264-1269

Results of a literature research on negative effects due to the intake of Mannoprotein

Ref ID: 1352

Journal Article (Electronic) Reference 125 of 129

Title:
Temperature-sensitive glucosamine auxotroph of Saccharomyces cerevisiae

Authors:
Ballou,Lun; Grove,J.Russell; Roon,Robert J.; Wiggs,Janey; Ballou,Clinton E.

Source:
Mol.Cell.Biol., 1981, 1, 1, 9-12

Ref ID: 780

Journal Article (Electronic) Reference 126 of 129

Title:
Saccharomyces cerevisiae mutants that make mannoproteins with a truncated carbohydrate outer chain

Authors:
Ballou,Lun; Cohen,Robert E.; Ballou,Clinton E.

Source:
J.Biol.Chem., 1980, 255, 12, 5986-5991, UNITED STATES

Ref ID: 786

Book, Section (Electronic) Reference 127 of 129

Section Title:
Production of antioxidants, aromas, colours, flavours, and vitamins by yeasts.

Authors:
Abbas, Charles,A.

Source:
SPRINGER-VERLAG BERLIN, HEIDELBERGER PLATZ 3, D-14197 BERLIN, GERMANY. Series: Yeast Handbook (Series), ISBN: 3-540-28388-9(H).

Ref ID: 1326

Journal Article (Electronic) Reference 128 of 129

Title:
Quantification of major grape polysaccharides (Tempranillo v.) released by maceration enzymes during the fermentation process.

Authors:

Results of a literature research on negative effects due to the intake of Mannoprotein

Ayestaran,B.(correspondence); Guadalupe,Z.; Leon,D.

Source:
Netherlands, ACACAM

Ref ID: 796

Journal Article (Electronic) Reference 129 of 129

Title:
Food additives. Bakers yeast extract, affirmation of GRAS status as direct human food ingredient; bakers yeast glycan and bakers yeast protein.

Authors:

Source:
Federal Register, 1974, 39, 185, 34186-34188

9

Pariza&Johnson decision tree for Bakers Yeast Mannoprotein (test article)

The "Decision Tree for evaluation of the relative safety of food ingredients derived from genetically modified organisms" was published in 1991. This publication from the International Food Biotechnology Council (IFBC) was an extension of an earlier publication by Pariza and Foster in 1983[1]. The 1991 IFBC Decision Tree was recently updated by Pariza and Johnson and is published in the April 2001 issue of Regulatory Toxicology and Pharmacology[2]. It is specifically designed for food enzymes derived from microorganisms.

Although Bakers Yeast Mannoprotein is not a food enzyme, the test article was evaluated according the Decision Tree published in 2001 because the Mannoprotein is derived from a microorganism, namely the yeast *Saccharomyces cerevisiae*, and is used in food. The scientific analysis is described below.

1. Is the production strain genetically modified?
   YES
   The strains of *Saccharomyces cerevisiae*, used for the production of Mannoprotein, were improved by classical strains breeding methods for their suitability as producers of yeast extracts.
   If yes, go to 2. ~~If no, go to 6.~~

2. Is the production strain modified using rDNA techniques?
   NO
   The strains of *Saccharomyces cerevisiae* were obtained by using traditional strain breeding techniques only.
   ~~If yes, go to 3~~. If no, go to 5.

3. Issues related to the introduced DNA are addressed in 3a-3e.
   3a. Do the expressed product(s) which are encoded by the introduced DNA have a history of safe use in food?
   NA
   ~~If yes, go to 3c. If no, go to 3b~~
   3b. Is the NOAEL for the test article in appropriate short-term oral studies sufficiently high to ensure safety?
   NA
   ~~If yes, go to 3c. If no, go to 12.~~
   3c. Is the test article free of transferable antibiotic resistance gene DNA?
   NA
   ~~If yes, go to 3e. If no, go to 3d~~.
   3d. Does (Do) the resistance gene(s) code for resistance to a drug substance used in treatment of disease agents in man or animals?
   NA
   ~~If yes, go to 12. If no, go to 3e~~.

---

[1] Pariza M.W. and Foster E.M., J. Food Protection **46** (1983). 453-468
[2] Pariza, M.W. and Johnson, E.A., Regulat. Toxicol. Pharmacol. **33** (2001), 173–186

3e. Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products?
NA
~~If yes, go to 4. If no, go to 12.~~

4. Is the introduced DNA randomly integrated into the chromosome?
NA
~~If yes, go to 5. If no, go to 6.~~

5. Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects, which may result in the synthesis of toxins or other unsafe metabolites, will not arise due to the genetic modification method that was employed?
YES
The production strain belongs to the species *Saccharomyces cerevisiae* and has been selected from a commercial baker's yeast strain with a history of safe use. Yeast extract from baker's yeast is GRAS and the yeast *Saccharomyces cerevisiae* and closely related species have the QPS (Qualified Presumption of Safety) status in Europe. *Saccharomyces cerevisiae* has never been implicated in the literature as having the potential to produce toxic secondary metabolites, including mycotoxins.
If yes, go to 6. ~~If no, go to 7~~.

6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?
YES
The strain of *Saccharomyces cerevisiae* is obtained by traditional strain breeding techniques from previous commercial strains. *Saccharomyces cerevisiae* enjoys a long history of safe use in food for thousands of years[3]. FDA reviewed the submission from the Enzyme Technical Association (ETA) that concluded that invertase from *Saccharomyces cerevisiae* is GRAS[4]. FDA has also affirmed that extract from bakers yeast is GRAS[5]. Thus, *Saccharomyces cerevisiae* has been established as a safe lineage.

If yes, the test article is ACCEPTED. ~~If no, go to 7.~~

7. Is the organism nonpathogenic?
NA
~~If yes, go to 8. If no, go to 12.~~

8. Is the test article free of antibiotics?
NA
~~If yes, go to 9. If no, go to 12.~~

---

[3] Pretorius, I.S., Yeast **16** (2000), 675-729; Tucker, G.A. and Woods, L.F.J., '*Enzymes in Food Processing*', (1995, 2nd Ed.) pages 99-100, 190, 232
[4] *See* FDA letter to ETA (April 3, 2002) for GRAS. GRN No. 88, *available at* http://www.cfsan.fda.gov/~rdb/opa-g088.html
[5] *See* 21 C.F.R. § 184.1983

Pariza&Johnson decision tree for Bakers Yeast Mannoprotein (test article)

9. Is the test article free of oral toxins known to be produced by other members of the same species?
   NA
   ~~If yes, go to 11. If no, go to 10.~~

10. Are the amounts of such toxins in the test article below levels of concern?
    NA
    ~~If yes, go to 11. If no, go to 12.~~

11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety?
    NA
    ~~If yes, the test article is ACCEPTED. If no, go to 12.~~

12. An undesirable trait or substance may be present and the test article is not acceptable for food use. If the genetic potential for producing the undesirable trait or substance can be permanently inactivated or deleted, the test article may be passed through the decision tree again.
    NA

Conclusion: Test Article is ACCEPTED.

SUBMISSION END

AM

Mcmahon, Carrie

From: Hsieh, Grace [Grace.Hsieh@klgates.com]
Sent: Monday, June 22, 2009 12:18 PM
To: Mcmahon, Carrie
Cc: Yingling, Gary L.
Subject: Response to Questions regarding Mannoprotein GRAS Notification (GRN 284)
Attachments: DC-#1340901-v1-DSM_-_Letter_to_McMahon_re_GRAS_Notification_(439397-1_06_22_2009_12_01_37_PM).PDF

Dear Dr. McMahon,

Please find attached a PDF of a letter responding to your email of June 9, 2009 regarding the Mannoprotein GRAS Notification (GRN 284). We will also be sending by Federal Express a hardcopy for your review. Please do not hesitate to contact Gary Yingling at (202) 778-9124 if you have any questions.

<<DC-#1340901-v1-DSM_-_Letter_to_McMahon_re_GRAS_Notification_(439397-1_06_22_2009_12_01_37_PM).PDF>>
Kind Regards,

Grace

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June 22, 2009

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Via E-Mail (Carrie.McMahon@fda.hhs.gov)
Via FedEx

Carrie H. McMahon, Ph.D. (HFS-255)
Consumer Safety Officer
FDA/CFSAN
Division of Biotechnology and GRAS Notice Review
University Station Bldg.
4300 River Road, Room 2039
College Park, MD 20740

Re: GRAS Notification (GRN 000284) for the use of bakers yeast mannoprotein as a stabilizing agent to prevent tartaric acid precipitation in wines

Dear Dr. McMahon:

This letter responds to the agency's email of June 9th, 2009 in which the agency asks some questions regarding the above mentioned GRAS notification. Revised pages to the original GRAS notification are embedded in this Word document and will be enclosed with a hardcopy of this letter.

Question 1
In Section 2.4 (Structural Formula), the text reads "The carbohydrate content of this Mannoprotein is at least 70 %, of which at least 60 % is composed of mannose." However, the table in Section 2.5 (Quantitative Composition) indicates that the carbohydrates limit is equal to or greater than 60 %, of which the fraction of mannose is equal to or greater than 70 %. Please explain.

Answer to question 1
The FDA correctly noticed that there is a divergence between the values mentioned in the text and in the table. By mistake, the values in the text have been mixed up. The text should therefore read: The carbohydrate content of this Mannoprotein is at least **60%,** of which at least **70%** is composed of mannose. The values mentioned in the table are correct.

Please find enclosed the corrected page 9.

Corrected page 9.doc

Question 2

In Sections 2.5 (Quantitative Composition) and 2.8 (No Potential Human Toxicants From Raw Materials), the text describe two forms of bakers yeast mannoprotein – a spray-dried powder and a colloidal solution – and indicate that the colloidal solution only is stabilized with sodium bisulfite. However, the text in Section 2.6 (Method of Manufacture) and the diagram in Annex 5 (Bakers Yeast Mannoprotein Process) do not distinguish between the two forms when describing the method of manufacture and the addition of sodium bisulfite. For clarity, please provide a revised text and diagram indicating the differences in manufacture of the two forms of bakers yeast mannoprotein.

Answer to question 2

Please find enclosed the corrected page 10, as well as the corrected Annex 5.

Corrected page 10.doc

Corrected Annex 5.doc

Question 3

In Section 2.9 (Specifications for Food-Grade Material), you provide chemical and microbial purity specifications and values of three different batches of bakers yeast mannoprotein. FDA notes that the value for Mercury in Batch 0821A (< 0.2) exceeds your specification of ≤ 0.15 for Mercury in food-grade material. Please explain.
*The text indicates that the table contains **actual** values of three different batches. However, these values are qualified with the "less than" symbol. Please explain.*

Answer to question 3

After re-checking the values of batch 0821A, DSM unfortunately noticed that the wrong data were copied from a worksheet. DSM has corrected these values now. Also, DSM has added the values of a fourth batch.

Please find enclosed the corrected page 12.

Corrected page 12.doc

As can be seen in the table, none of the values are above the limits established by the Organisation Internationale du Vin (OIV).

DSM also removed the word "actual" in the text. The wording merely meant to indicate that actual specification tests were conducted. Where the "less than" symbol is used, the values in the table reflect the detection limit of the tests. It is quite common to use the "less than" symbol in cases where nothing can be measured because the actual value is lower than the detection limit of the test.

In the case of the "Total heavy metals," the situation is a little bit more complicated. There, the "less than" symbol does not refer to the limit of one specific test because the "limit" is the sum of the detection limits for the 10 relevant metals (Ag, As, Bi, Cd, Cu, Hg, Mo, Pb, Sb, Sn). Moreover, even though the actual values for all these metals were below their detection limits (i.e., "less than"), the "Total heavy metals" sum varies because the value – which is measured in the liquid – is corrected for the dry matter. As the dry matter varies between 18-22% from batch to batch, the resulting values vary correspondingly. As a result, the variations mentioned in the table are not due to the variations in the measurements of the heavy metals themselves, but to the variations of the dry matter.

Question 4

In Section 4.1.2.1 (Safety Studies Performed on Yeast Extract from DSM), the text does not indicate the number of animals included in the Acute toxicity text II. Please provide the number of animals.

Answer to question 4

The acute toxicity test and the sub-acute toxicity test were performed in parallel. In both cases, 5 female and 5 male rats were used. Please find enclosed the corrected page 19.

Corrected page 19.doc

We hope this letter and the revised pages of the Mannoprotein GRAS notification are responsive to FDA's questions. Please do not hesitate to contact me at (202) 778-9124 if you have additional questions.

K&L|GATES

Sincerely,

(b)(6)

Gary L. Yingling

cc: DSM Food Specialties

Enclosures

Mannoprotein is found in yeasts where it forms, together with glucan, the main component of the cell walls (Lipke and Ovalle, 1998). Apart from the cell wall, yeast cells also contain some periplasmic Mannoproteins, such as invertase (Dupin et al., 2000; Whitehurst, 2002).

The Mannoprotein described in this notification is derived from an extract of bakers yeast (*Saccharomyces cerevisiae*).

The carbohydrate content of this Mannoprotein is at least 60%, of which at least 70% is composed of mannose. The Bakers Yeast Mannoprotein and the glycosidic linkages have been identified by NMR (see Annex 4).

2.5 Quantitative Composition

The chemical composition on a basis of 100% dry matter Bakers Yeast Mannoprotein is presented in the Table below:

	Method	Unit	Limit	Batch (b)(4)	Batch (b)(4	Batch (b)(4)	Batch (b)(4)
Ashes	550 – 600 °C	%	≤ 8	5.3	4.6	4.9	4.5
Nitrogen[1]	Kjeldahl or equivalent	%	0.5 – 7.5	5.9	5.7	6.3	4.6
Carbohydrates	Phenol/sulfuric acid	%	≥ 60	65	60	67	63
Fraction of mannose in carbohydrates	HPLC after TFA hydrolysis	%	≥ 70	95	96	n.d.	n.d.

The Bakers Yeast Mannoprotein can be sold as ready-to-use spray dried powder or as a colloidal solution. In order to preserve the colloidal solution after opening, 14 g/l sodium bisulfite is added. The colloidal solution contains 20% ± 2% dry matter.

[1] Provided no other nitrogen sources are present, the maximal protein content is N x 6,25

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2.6 Method of Manufacture

Bakers yeast (*Saccharomyces cerevisiae*) naturally contains Mannoprotein. Apart from being used in bread, beer and wine, bakers yeast is used to produce a variety of food ingredients, including yeast extract. Bakers yeast extract will contain a significant part of the Mannoprotein, and can therefore be used as a starting material for the production of Mannoprotein.

The classical bakers yeast strain used by DSM contains about 16% Mannoprotein, and the extract derived from the bakers yeast contains between 8 and 12% Mannoprotein. DSM uses this food-grade yeast extract as a starting material for the production of the Mannoprotein described in this notification. However, in principle also other food grade yeast extract products can be used.

The whole production process of Mannoprotein from yeast extract as described below is performed according to current Good Manufacturing Practices:

The first step in the manufacturing process of the Mannoprotein is the micro-filtration of the yeast extract. The aim of this filtration step is to remove particles and high Molecular Weight proteineous material to enable the subsequent ultrafiltration step.

The micro-filtered, clear liquid is subsequently subjected to ultrafiltration (UF) in order to concentrate the high Molecular Weight Mannoproteins (> 20 kDa). The UF-concentrate is then treated with the enzyme phosphodiesterase from *Leptographium procerum* in order to hydrolyze residual yeast RNA. This is followed by a diafiltration step in order to remove low Molecular Weight molecules such as salts, amino acids, nucleotides and sugars.

After diafiltration, the diluted solution is again concentrated by UF to obtain a Mannoprotein containing UF-concentrate of 20% +/- 2% dry matter. In order to inactivate residual enzyme activity, the UF-concentrate is subjected to UHT treatment (130-150 °C) and subsequently cooled down to 20-40 °C. The UHT-treated concentrate is then polish filtered in order to remove insoluble proteineous material that was formed during the UHT treatment.

After filtration, the clear solution is either 1) spray dried, packed as powder and stored at 4°C; or 2) stabilized with 14 g/l sodium bisulfite to prevent microbial growth after opening of the package (i.e., the product remains a colloidal solution). The stabilized Mannoprotein colloidal solution is packed in 2.5 or 20 liter cans and stored at 4 °C.

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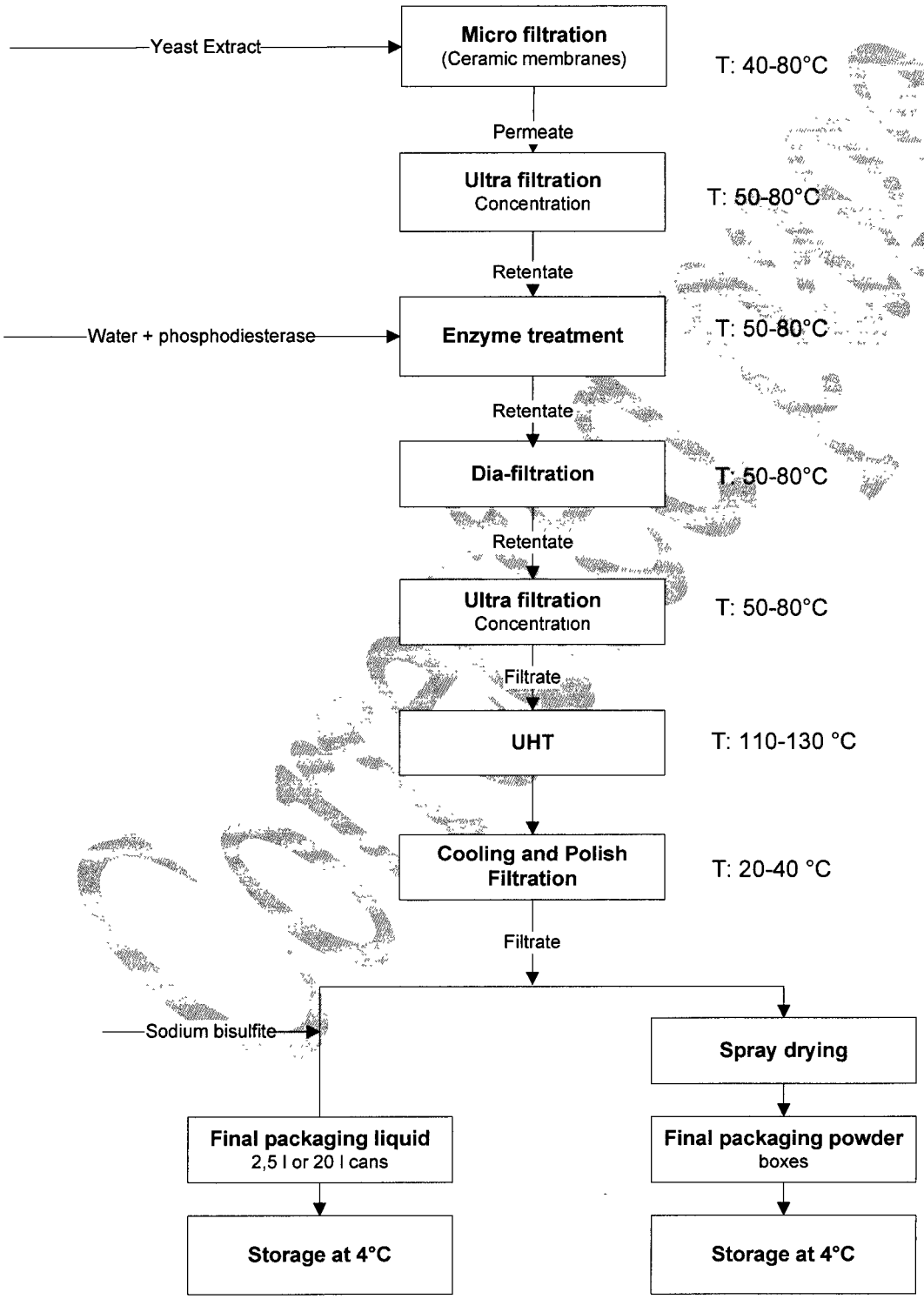
Bakers Yeast Mannoprotein Process



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consequence, the maximal amount of sodium bisulfite added to the wine will be 28 mg/l, resulting in 14 mg/l sulfur dioxide. This maximal amount can be considered negligible, especially when compared to the amounts that are maximally allowed in wine. According to the EU Miscellaneous Additive Directive (European Parliament and Council Directive 95/2/EC, 1995), a sulfur dioxide content below 10 mg/kg or 10 mg/l is not considered to be present.

2.9 Specifications for Food-Grade Material

The Bakers Yeast Mannoprotein described in this notification is manufactured to comply with the purity specifications laid down by the Organisation Internationale du Vin (OIV) in Resolution OENO 26/2004, see Annex 1.

The chemical and microbial purity specifications and the values measured in four different batches are summarized in the following Table:

	Unit	Limit	Batch (b)(4)	Batch (b)(4)	Batch (b)(4)	Batch (b)(4)
Total heavy metals	mg/kg	≤ 30	<10	<26	<29	<25
Pb	mg/kg	≤ 5	<1	<1	<1	<1
Hg	mg/kg	≤ 0.15	<0.1	<0.1	<0.1	<0.1
As	mg/kg	≤ 1	<0.1	<0.1	<0.1	<0.1
Cd	mg/kg	≤ 0.5	<0.05	<0.05	<0.05	<0.05
Total aerobic mesophile germs	CFU/g	≤ 10^4	<5	<5	<5	<5
Molds	CFU/g	≤ 50	<10	<10	<10	<10
Yeasts	CFU/g	≤ 100	<10	<10	<10	<10
Coliforms	CFU/g	≤ 10	<10	<10	<10	<10
Escherichia coli	CFU/25g	Absent	Absent	Absent	Absent	Absent
Staphylococcus aureus	CFU/g	Absent	Absent	Absent	Absent	Absent
Salmonella	CFU/25g	Absent	Absent	Absent	Absent	Absent
Lactic bacteria	CFU/g	≤ 10^4	<10	<10	<10	<10

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Acute toxicity II

Maxarome® (batch 5002), Gistex® (batch 44-3) and Maxarome® (batch 5002 with NaCl added to the same level as in Gistex®) were studied for acute toxicity in 5 male and 5 female Wistar rats. Animals of 75-80 g received the test substance dissolved in distillated water at a level of 10 ml/kg body weight. The test substance was administered by the oral (gavage), intraperitoneal and intravenous route. After administration, the animals were observed during 5 days.

Results

The LD50 for the oral and intraperitoneal route was >8000 mg/kg for all three test substances. The LD50 for the intravenous route was 2000 mg/kg for Maxarome® and Gistex and 1410 mg/kg for Maxarome® with added salt.

Subacute toxicity I

5 male and 5 female Wistar rats received by gavage Maxarome® (batch 5002), Gistex® (batch 44-3) and Maxarome® (batch 5002 with NaCl added to the same level as in Gistex®) for 10 days at a dose of 2000 mg/kg. The control group received distilled water.

Results

All animals behaved normally and none of the animals died. Body weight gain was comparable among the groups. Significant differences in organ weight (heart, liver, kidneys) were not observed. Macroscopy did not reveal any treatment-related effects.

Subacute toxicity II

Male and female Wistar rats were dosed Maxarome® paste by gavage at a level of 50, 175 and 650 mg/kg body weight. Maxarome® paste was dissolved in physiological salt solution. The first control group received 15.2 mg NaCl/kg body weight, the same salt level as Maxarome® paste, and the second control group received physiological salt solution. Dosing was at 10 ml/kg body weight. All groups consisted of 5 males and 5 females, except the

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