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Yeast Extracts containing Mannoproteins

(Tentative)

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YEAST EXTRACTS CONTAINING MANNOPROTEINS (TENTATIVE)

New specifications prepared at the 84th JECFA (2017) and published in FAO JECFA Monographs 20 (2017). No ADI was established at the 84th JECFA (2017) as the use of this substance is not of health concern when used for oenological uses at maximum use levels up to 400 mg/L for the stabilization of wine.

***Information required on**:*

- *composition of yeast extracts containing mannoproteins as well as the processes used in their manufacture;*
- *analytical data from five batches of each commercial product, including information related to impurities; and*
- *data on concentrations of yeast mannoproteins in wine in which yeast extracts containing mannoproteins have been used.*

*The Committee requests that this information be submitted by **December 2018**.*

SYNONYMS INS.No. 455

DEFINITION Yeast extracts containing mannoprotein represents a large family of natural compounds from yeast (*Saccharomyces cerevisiae*) in which polysaccharide chains are bound to proteins and peptides by covalent and non-covalent bonds (i.e., ionic interactions). The structures and molecular weights of mannoprotein vary, depending on the degree and type of glycosylation. 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. Several of the side chains may have phosphodiester linkages to other mannosyl residues. Yeast mannoproteins are extracted from purified yeast cell walls by enzymatic extraction using glucan 1,3-β-glucosidase (EC 3.2.1.58) or by thermal treatment extraction. The enzyme hydrolyses the yeast cell wall allowing the mannoproteins to be solubilized. The thermal treatment breaks the links with β-glucans. The mannoproteins thus solubilized by either treatment are then separated from the insoluble cell wall material, concentrated and micro- or ultra-filtered. Mannoproteins have molecular weights ranging from 20 kDa to more than 450 kDa.

Assay Total polysaccharides: Not less than 60% on dried basis

Mannose: Not less than 70% of the total polysaccharides

	Nitrogen content: 0.5-7.5% on the dried basis
DESCRIPTION	White or beige, odourless powder, or yellow, translucent colloidal solution
FUNCTIONAL USES	Stabilizer
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Soluble in water and insoluble in ethanol
PURITY	
Loss on drying (Vol.4)	Powder form: Not more than 15% (105°, 5h)
Specific rotation (Vol. 4)	$[\alpha]_D^{20}$: between +80 and +150°, Test solution: 1.0 g of dried sample in 100 mL of water, using an optical cell with 100-mm path length.
Total Ash (Vol. 4)	Not more than 8%, on dried basis
Lead (Vol. 4)	Not more than 2 mg/kg Determine using method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Microbiological criteria (Vol. 4)	Aerobic plate count: Not more than 10,000 CFU/g Coliforms: Not more than 10 CFU/g *Staphylococcus aureus*: Negative in 1 g *E. coli*: Negative in 25 g Salmonella: Negative in 25 g Moulds: Not more than 50 CFU/g Yeasts: Not more than 100 CFU/g

METHOD OF ASSAY

Total polysaccharides

Reagents:

- Mannose, >99 % pure
- Sulfuric acid, Concentrated
- Phenol solution (50 mg/mL): Dissolve 5 g of phenol in 100 mL of deionized water

Preparation of mannose standard solution (0.1 mg/mL): Accurately weigh 100 mg of mannose, dissolve in deionized water and make up to 100 mL in a volumetric flask. Pipette 5 mL of solution into a 50 mL volumetric flask and make up to volume with deionized water (0.1 mg/mL).

Preparation of sample solution (15 mg/L): Accurately weigh 150 mg (W) of sample, dissolve in deionized water and make up to 100 mL in a volumetric flask. Pipette 1 mL of solution into a 100 mL volumetric flask and make up to volume with deionized water (15 mg/L).

Procedure:
Add 200 µl of phenol solution and 1 mL of concentrated sulfuric acid to 200 µl of the sample solution and mix immediately. Prepare a reference solution by adding 200 µl of phenol solution and 1 mL of concentrated sulphuric acid to 200 µl of mannose standard solution and mix immediately. Heat both solutions to 100° in a water bath for 5 min, remove tubes and quickly cool to 0° in ice. Take out the tubes from ice and allow tubes to reach room temperature. Measure the absorbance values at 490 nm in a spectrophotometer against a blank solution prepared similarly omitting the standard.

Calculation

Calculate the amount of total polysaccharides according to:

$$Total\ polysaccharides = \frac{A_{Sample} \times 10^7}{A_{Std} \times W \times (100 - \%M)}$$

Where:

- Total polysaccharides are given in %w/w on dried basis
- A_{Sample} is the absorbance of the sample solution
- A_{Std} is the absorbance of the standard solution (0.1 mg/mL)
- W is the weight of sample, mg
- %M is the loss on drying, %

Mannose

Instrumentation and reagents:

- Spectrophotometer: 340 nm
- Stop-watch
- Triethanolamine hydrochloride ($C_6H_5NO_3$ · HCl): >99 % pure
- Magnesium sulfate ($MgSO_4$ · $7H_2O$): AR grade
- Sodium hydroxide: AR grade
- Disodium nicotinamide adenine dinucleotide phosphate: AR grade
- Adenosine-5'-triphosphate (ATP): AR grade
- Sodium hydrogen carbonate: AR grade
- Hexokinase solution: 2 mg of protein/mL or 280 U/mL
- Glucose-6-phosphate(G-6-P)-dehydrogenase solution: 1 mg of protein/mL
- Phosphoglucose-isomerase (PGI) solution: 2 mg of protein/mL or 700 U/mL
- Phosphomannose isomerase solution: 616 U/mL
- Sulfuric acid: 5 M
- Potassium hydroxide: 10 M

Buffer solution (0.3 M triethanolamine, 0.004 M Mg^{2+}, pH 7.6):
Dissolve 11.2 g of triethanolamine hydrochloride and 0.2 g magnesium sulfate in 150 mL deionized water, adjust the pH 7.6 with about 4 mL of 5 mol/L sodium hydroxide solution and make up to 200 mL.

Nicotinamide adenine dinucleotide phosphate (NADP) solution (10 mg/mL, about 0.012M):
Dissolve 50 mg disodium nicotinamide adenine dinucleotide phosphate in 5 mL of deionized water.

Adenosine-5'-triphosphate (ATP) solution (50 mg/mL, about 0.08 M):
Dissolve 250 mg disodium adenosine-5'-triphosphate and 250 mg sodium hydrogen carbonate in 5 mL of deionized water.

Hexokinase/glucose-6-phosphate(G-6-P)-dehydrogenase solution:
Mix 0.5 mL hexokinase solution with 0.5 mL G-6-P-dehydrogenase solution.

Preparation of sample solution (5 mg/mL):
Accurately weigh 500 mg (W) of sample, dissolve in 100 mL of deionized water. Place 100 µl of the sample solution in airtight sealed tubes and add 1 mL of 5M sulphuric acid solution. Cap the tubes, heat at 100° in a water bath for 30 min, remove tubes and quickly cool to 0° in ice. Take out the tubes from ice and allow tubes to reach room temperature. Neutralise the acid by adding 1 mL of 10 M potassium hydroxide solution to each tube.

Procedure:
Set the spectrophotometer at 340 nm wavelength. Using matched cells, zero the instrument (according to the manufacturer's instructions),

Prepare the reference cell with 1 cm path length with 2.50 mL buffer solution (@20°), 0.10 mL NADP solution, 0.10 mL ATP solution and 0.20 mL deionised water

Prepare the sample cell like the reference cell by replacing the deionised water with 0.20 mL of sample solution

Start the stop-watch and mix the solution in the cell. Add 0.02 mL of G-6-P-dehydrogenase solution to both cells after three minutes and mix. Add 0.02 mL of PGI Solution to both cells after 17 min and mix. Read the absorbance of the solution in reference as well as sample cells, after 10 min. After two more minutes, read the absorbance (A_1) of the solution to ensure that the reaction has stopped (indicated by no increase in absorbance).

Add 0.02 mL each of phosphomannose isomerase solution (616 U/mL) and mix. Read the absorbance after 30 min. Check absorbance (A_2) after two more minutes to ensure that the reaction has stopped (indicated by no increase in absorbance).

Note: The time needed for the completion of enzyme activity may vary from one batch to another. The above value is given only for guidance and it is recommended that it be determined for each batch.

Calculation:

Calculate the differences in absorbance between A_1 and A_2 for the reference cell (ΔA_T) and the sample cell (ΔA_D), and then obtain $\Delta A_M = \Delta A_D - \Delta A_T$

Calculate mannose %w/w (on dried basis) by the following expression:

$$Mannose = \frac{0.423 \times \Delta A_M \times 10^6 \times 21}{W \times (100 - \%M)}$$

Where:
- Mannose is expressed as % w/w on dried basis
- W is the weight of sample, mg
- %M is the loss on drying, %

Calculate the % Mannose in total polysaccharides as follows:

$$\% \text{ Mannose in total polysaccharides } = \frac{\%\text{Mannose on dried basis} \times 100}{\% \, Polysaccharide \, on \, dried \, basis}$$

Nitrogen Content

Weigh accurately 1.0 g of yeast mannoprotein, and proceed as directed under Nitrogen determination (Kjeldahl Method, Method 1) in Volume 4 (under “General Methods, Inorganic components”).