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## **MANNOPROTEINS FROM YEASTS CELL WALLS**

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## MANNOPROTEINS FROM YEAST CELL WALLS

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### SYNONYMS

INS.No. 455

### DEFINITION

Mannoproteins from Yeast Cell Walls are a large family of natural compounds from *Saccharomyces cerevisiae* in which polysaccharides are connected to proteins and peptides by covalent and non-covalent bonds. The structures and molecular weights of mannoproteins vary, depending on the degree and type of glycosylation. The polysaccharide chains consist almost exclusively of mannose units linked together by  $\alpha$ -links, with a long  $\alpha$ -1 $\rightarrow$ 6 linked backbone containing short  $\alpha$ -1 $\rightarrow$ 2- and  $\alpha$ -1 $\rightarrow$ 3 linked side chains. Several of the side chains may have phosphodiester linkages to other mannosyl residues. Mannoproteins are extracted from purified yeast cell walls by enzymatic extraction using glucan 1,3-  $\beta$ -glucosidase (EC 3.2.1.58) or by thermal treatment. The enzyme hydrolyses the yeast cell wall allowing the mannoproteins to be solubilized. The thermal treatment breaks the links with  $\beta$ -glucans. The mannoproteins thus solubilized by either treatment are then separated from the insoluble cell wall material and concentrated by micro- or ultra-filtration. Mannoproteins have molecular weights ranging from below 20 kDa to more than 450 kDa.

### Assay

Total polysaccharides: Not less than 60% expressed as mannose on the 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

Wine stabilizer

### CHARACTERISTICS

### IDENTIFICATION

#### Solubility

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").

**TESTS****PURITY TESTS****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.

## 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 a 0.1 mg/ml solution of mannose in water and mix immediately. Heat both solutions to 100° in a water bath for 5 min then cool to reach room temperature and measure the absorbance values at 490 nm in a spectrophotometer against a blank solution prepared similarly omitting the standard.

Total polysaccharides, %w/w (Expressed as mannose on the dried basis) =  $\frac{A_{\text{Sample}}}{A_{\text{STD}} \times (100 - \%M)}$

Where:  $A_{\text{Sample}}$  is the absorbance of the sample solution

$A_{\text{Std}}$  is the absorbance of the standard solution  
(0.1 mg/ml)

W is the weight of sample, g

**Mannose**

Instrumentation and reagents:

Spectrophotometer: 340 nm

Stop-watch

Triethanolamine: >99 % pure

Magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ): 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): 2 mg of protein/mL or 700 U/ml

Phosphomannose isomerase: 616 U/ml

Sulfuric acid: 5 M

Potassium hydroxide: 10 M

Buffer solution (0.3 M triethanolamine, 0.004 M  $\text{Mg}^{2+}$ , 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): Dissolve 50 mg disodium nicotinamide adenine dinucleotide phosphate in 5 ml of deionized water.

Adenosine-5'-triphosphate (ATP) solution (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 (5g/l):

Dissolve 0.500 g (W) of sample in 100 ml of deionized water.

Place 100  $\mu\text{l}$  of the sample solution in airtight sealed tubes and add 1 ml of 5M sulphuric acid solution. Cap the tubes, heat at  $100^\circ$  in a water bath for 30 min, remove tubes and quickly cool to  $0^\circ$  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).

Into two cells with 1 cm path length, place the following:

	Reference cell	Sample cell
Buffer solution (@ $20^\circ$ )	2.50 ml	2.50 ml
NADP solution	0.10 ml	0.10 ml
ATP solution	0.10 ml	0.10 ml
Sample to be measured	--	0.20 ml
Deionized water	0.20 ml	--

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 I 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  $A_2$  ( $A_2 - A_1$  corresponds to mannose) for the reference cell ( $\Delta A_T$ ) and the sample cell ( $\Delta A_D$ ), and then obtain  $\Delta A_M = \Delta A_D - \Delta A_T$

Calculate mannose by the following expression.

$$\text{Cg/l} = 0.423 \times \Delta A_M$$

$$\text{Mannose, \% w/w (on the dried basis)} = \frac{0.423 \times \Delta A_M \times 100}{W \times (100 - \%M)}$$

Where: W is the weight of sample, g  
%M is the loss on drying, g

% Mannose in total polysaccharides =

$$\frac{\% \text{Mannose on DB} \times 100}{\% \text{Polysaccharides DB}}$$

(DB = 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").