COEI-1-MANPRO Yeast mannoproteins

1. Object, origin and field of application

Mannoproteins are extracted from *Saccharomyces spp.* or non-Saccharomyces 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.

Their water solubility is determined by comparing the total dry matter (DM) with the insoluble DM that remains after a hot wash, according to the method described in Annex 2. The percentage of insoluble DM should be less than or equal to 1% $\pm$ 0.5

4. Trials

4.1. Loss through desiccation

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%.

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.

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.

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.

4.11.2.3. 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).

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.

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 100 mg potassium hydrogenotartrate in each 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**

4.14.1. Principle

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

4.14.2. Product:

Bovine serum albumen (Fraction V) (BSA)

4.14.3. Protocol:

4.14.3.1. 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.

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

4.15.1. 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 must be 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.

Annex 1 : Determination of mannose using enzymatic method

1. 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.

2. 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.

3. 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.

4. Determination

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

Solution 6	Reference	Determination
	0.02ml	0.02ml

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

Subtract the aborbance difference for the reference (ΔA_T) and for the determination (ΔA_p) and establish:

$\Delta A_M = \Delta A_D - \Delta A_T$ for mannose

5. 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:

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

For a 365 nm measurement

$$\text{For mannose:Cg/l} = 0.783 \times \Delta A_m$$

Annex 2 : Determination of the percentage of insoluble dry water

1. Principle

The analysis consists of comparing the total dry matter (DM) of the yeast mannoprotein preparation with the insoluble DM that remains after a hot wash.

2. Material and reagents

2.1. 4200 rpm (6000 g) Centrifuge and accessorie
2.2. Scale with 0.1 mg precision
2.3. Ovent at 105°C +/- 1°C

3. Procedure

Obtaining the insoluble part of the yeast mannoprotein preparation

- Place approx. 10 g of yeast mannoprotein preparation, previously placed in an oven at 105 °C until constant weight, in a tared centrifuge bucket. Note the exact weight, which will be referred to as M1.
- Suspend in hot water (70-80 °C).
- Stir.
- Centrifuge for 10 min at 4200 rpm (6000g).
- Discard the supernatant, stir again into hot water and centrifuge for 10 min at 4200 rpm.
- Carry out the procedure a third time.
- Place the tared centrifuge bucket containing the centrifugation pellet in an oven at 105 °C until constant weight. Note the weight. M2 is the weight of the residue of yeast mannoproteins preparation that make up the insoluble DM.

4. Calculations

Insoluble dry matter percentage (Insoluble DM%) = (M2/M1) x 100