COEI-1-YEHULL Cellular yeast hulls (Yeast walls)

1. Object, origin and field of application

The cellular yeast hulls are obtained from *Saccharomyces spp. or* non-Saccharomyces yeasts. The preparation mode must respect the surface area and consequently the adsorption capacity.

Cellular yeast hulls are found in fine powder or microgranulate forms, non-hygroscopic, cream coloured and slightly odorous. They do not leave harmful residues in grape musts and in wines. During the process, there is no addition of antibiotics or compounds other than those needed for the yeast to grow.

Cellular yeast hulls are packed under conditions which prevent oxidation.

They are used to prevent and deal with stuck fermentations. They have the property of fixing certain fatty acids (octanoic and decanoic) which disturb membrane permeability of yeasts.

When the cellular yeast hulls come from genetically engineered yeasts, these must be subject to the prior authorisation of the relevant authorities.

There is an addition limit on the usage of cellular yeast hulls.

2. Labelling

The label must include:

- The name of the genus and species
- The instructions for use
- Any additives
- The purity, batch number, expiry date and storage conditions under well-defined temperature, humidity and ventilation conditions
- An indication whether the cellular hulls come from genetically engineered yeasts, and the modified character if this is the case.

3. Composition of cellular yeast hulls (values)

Dry matter	≥ 94% m/m according to the method described in Annex 2
Carbohydrates	> 40% m/m

Carbohydrates:

The total glucans and mannans content must be more than 60% of the total carbohydrates according to the method described in Annex 1.

Solubility	< 10% m/v

4. Additives and ingredients

According to legislation.

5. Limits and trial methods

5.1. Lead

Proceed with an analysis according to the method described in Chapter II of the International Oenological Codex.

The content must be less than 2 mg/Kg.

5.2. Mercury

Proceed with an analysis according to the method described in Chapter II of the International Oenological Codex.

The content must be less than 1 mg/Kg.

5.3. Arsenic

Proceed with an analysis according to the method described in Chapter II of the International Oenological Codex.

The content must be less than 3 mg/Kg.

5.4. Cadmium

Proceed with an analysis according to the method described in Chapter II of the International Oenological Codex.

The content must be less than 1 mg/Kg.

6. Microbiological analyses

6.1. Revivable yeast

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: Less than 100 CFU per g.

6.2. Lactic bacteria

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: Less than 10^3 CFU per g.

6.3. Acetic bacteria

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: Less than 10^3 CFU per g.

6.4. Mould

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: less than 10^3 CFU per g.

6.5. Salmonella

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: Absence checked on 25 g sample.

6.6. Escherichia coli

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: Absence checked on 1 g sample.

6.7. Staphylococci

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: Absence checked on 1 g sample.

6.8. Coliforms

Proceed with counting according to the method described in Chapter II of the International Oenological Codex.

Content: Less than 100 CFU/g

7. Hygiene

Yeast hulls are produced in compliance with good food manufacturing practices.

They must not have a rancid odour and should not give an abnormal flavour to the wine (yeast flavour).

8. Activity

The stimulatory effect of yeast hulls is based on their capacity to adsorb certain toxic substances for yeasts, which they produce during the growth period. Decanoic acid is

the greatest growth inhibitor.

Technological activity (TA) expressed in grams (g) of product can thus be evaluated by absorption of decanoic acid.

A gram of cellular yeast hulls added to 100 mL alcohol solution of 10% vol., pH 3.5, containing 2 mg/L decanoic acid should adsorb, after 24 hours of contact at 18□22 °C, 50% of this acid.

Monitoring can be carried out by the determination of decanoic acid by chromatography in the gaseous phase with detection by flame ionization (GC/FID) in accordance with the following procedures provided as example:

- chromatography apparatus,
- polar capillary column, for example a FFAP type column, 50 m in length and 0.2 mm in interior diameter,
- melted silica support,
- programmed temperature of 60 °C to 180 °C, or 4 °C/min,
- injected volume of 1 µL of hydro-alcoholic solution (10 % vol.) to 2 mg/L decanoic acid treated with yeast hulls,
- heptanoic acid internal standard of 2 mg/L after adding,
- reference solution: hydro-alcoholic solution (10% vol.) to 2 mg/L decanoic acid.

9. Storage

The cellular yeast hulls must always be stored in airtight bags in a temperate environment.

Annex 1:Determination of glucans and mannans in cellular yeast hulls

The cellular yeast hulls are subjected to a pre-solubilisation with concentrated H_2SO_4 prior to hydrolysis with H_2SO_4 at 128 °C in an oven. This total hydrolysis of the glucans and mannans generates proportional quantities of glucose and mannose that are determined by Ionic Chromatography.

To eliminate the glycogen, the method must be preceded by a pre-washing of the sample with a 0.5 mole/L NaOH solution for 1 hour at room temperature, followed by a centrifugation and another washing with water).

1. Materials and equipment

- 100 mL capped flask (Duran or Schott Glass)
- Tube
- Polyethersulfone flter with average pore diameter of 0.45 µm
- Oven
- H_2SO_4 72%
- Ionic chromatography system with pulsed amperometry detector containing a gold electrode
- Vortex mixer
- NaOH 32%
- 100 mL and 50 mL volumetric flasks
- Distilled water
- HPLC grade Water
- Ionic chromatography column (Metrosep Carb1 Metrohm or equivalent)

2. Method

2.1. Preparation of standards

- Weigh 50 mg of glucose (note the exact weight Wglu) and 50 mg of mannose (note the exact weight Wman)
- Go to step 2.3.

2.2. Sample preparation

- Weigh 50 mg of cellular yeast hulls (note the exact weight Wy)
- Go to step 2.3.

2.3. Pre solubilisation

- Add 3.3 mL of H_2SO_4 72%
- Mix the sealed tube with a Vortex mixer
- Leave for one hour at ambient temperature and stir every 10 min

2.4. Acid Hydrolysis

- Pour the contents of the tube into a 100 mL flask
- Add 40 mL of distilled water
- Close the flask
- Put the capped flask in an oven at 128°c and incubate for 3 hours
- Take out the flask and cool it
- Neutralize with 8.112 mL of NaOH 32%
- Decant the contents of the flask into a 100 mL volumetric flask
- Adjust to 100 mL with distilled water
- Filter the solution through an Acrodisc IC filter

2.5. Chromatography

2.5.1. Preparation of standards

- Take 2.5 mL of the hydrolysed glucose and mannose solutions obtained in 2.4.
- Transfer to a 50 mL volumetric flask
- Adjust with distilled water
- Put in a chromatography vial for the autosampler

2. Sample preparation

- Take 7.5 mL of hydrolysed material obtained in 2.4.
- Transfer to a 50 mL volumetric flask
- Adjust with distilled water
- Put in a chromatography vial for the autosampler

3. Preparation of the mobile phase

- Measure one litre of HPLC grade water.
- Filter using a 0.45 µm membrane
- Degas under vacuum for 1 h 30 min
- Measure 7.57 mL of NaOH 51% into the flask intended for the mobile phase

- *Be careful to use only a polypropylene flask for the mobile phase*
- Add the 1 litre of degassed water
- Stir using a magnetic stirrer

4. Calibration solutions for chromatography

- Prepare, using HPLC grade water, solutions of glucose and mannose at 10 mg/L, 30 mg/L and 40 mg/L
- Use them for calibrating the chromatography

5. Chromatographic Conditions

- Condition the column using the mobile phase at a flow rate of 1 mL/min for 2 hours.
- Inject 20 µ L of :
- The three calibration solutions (§2.5.4.)
- The standard solution
- The standard solution
- Calibrate the system with the calibration solution. Trace the calibration curves Area = f (concentration)

The chromatography equipment will give the concentration in mg/L for:

the standard solution:

Concentration of Mannose in mg/L:CmanSt (mg/L)

Concentration of Glucose in mg/L:CgluSt (mg/L)

the sample solution :

Concentration of Mannose in mg/L:CmanY (mg/L)

Concentration of Glucose in mg/L:CgluY (mg/L)

3. Calculation

3.1. Yield calculation

Calculate the recovery yield for the standard mannose and glucose solutions as follows:

$$Yman = CmanSt\ (mg/L)/\ Wman\ (mg) \times 10 \times (2.5/50)$$

$$Yglu = CgluSt\ (mg/L)/\ Wglu\ (mg) \times 10 \times (2.5/50)$$

Wman and Wglu are the measured weights of mannose and glucose in mg (See §2.1.)

4. Concentration of mannans and glucans in cellular yeast hulls

Concentration of mannans in g% m/m:

$$Cmannans = 0.9 \times [CmanY \times (50/7.5))/(Wy\ (mg) \times 10)] \times (1/Yman)$$

Concentration of glucans in g% m/m:

$$Cglucans = 0.9 \times [CgluY \times (50/7.5))/(Wy\ (mg) \times 10)] \times (1/Yglu)$$

Wy: weight of cellular yeast hulls (see § 2.2.)

Yman and Yglu: yields of mannose and glucose (see § 3.1.)

Annex 2: Determination of the percentage of insoluble dry matter

1. Principle

The analysis consists in comparing the total dry matter (DM) of the cellular yeast hulls with the dry matter remaining (insoluble DM) after a hot wash.

2. Material and reagents

- 4200 rpm centrifuge and accessories
- Scales at 1/10 mg
- Weighing cabinet for DM (FST 350)
- Oven at 105 °C +/- 1 °C

3. Method

Obtaining the insoluble part of the cellular yeast hulls

In a calibrated centrifuge crucible, place around 10 g of cellular yeast hulls dried beforehand to constant weight in an oven at 105 °C. Note the exact weight, which is: M1.

Stir into very hot water (70 - 80 °C).

Mix well.

Centrifuge for 10 mins at 4200 rpm.

Discard the supernatant, mix into very hot water and centrifuge for 10 mins at 4200 rpm.

Perform the operation a third time.

Place the calibrated centrifuge crucible containing the centrifugation pellet in an oven at 105 °C to constant weight and weigh it. M2 is the weight of the washed and dried hulls which make up the insoluble DM

4. Calculations

Percentage of insoluble dry matter

$$\%\text{insoluble DM} = (M2/M1) \times 100$$