COEI-1-AUTLYS Yeasts autolysates

1. Object, origin and scope of application

Yeast autolysates are used as nutrients during the rehydration of dry active yeasts intended for alcoholic fermentation, and also as nutrients during alcoholic fermentation. Yeast autolysates are derived from *Saccharomyces spp.* or non-Saccharomyces yeast biomass. They are obtained from yeast biomass through autolysis, in some cases combined with heat treatment and/or modification of the pH. Autolysis is defined as the self-digestion of proteins and other cell tissues by the enzymes contained within the yeast cells.

The micro-organism production techniques are those conventionally used for yeast biomasses. There is no addition of antibiotics or compounds during the process other than those required for yeast growth. If the autolysates come from genetically modified yeasts, the yeasts must be submitted for prior authorisation by the competent authorities.

2. Labelling

The label must indicate:

- the name of the genus and the species of the yeast autolysate,
- the organic nitrogen content,
- the amino acid content,
- any additives,
- instructions for use,
- the batch number, the expiry date, and the storage conditions in terms of well-defined temperature, humidity and ventilation conditions,
- if applicable, the indication that the autolysates were obtained from genetically modified yeasts, and the modified characteristic.

3. Characteristics

In solid form they are available as powder, flakes or granules, light yellow to brown in colour, with an odour characteristic of yeast. In liquid form, they are available in tan to brown colour.

Yeast autolysates are highly water-soluble. The soluble part is less than 80% of the dry

matter. The soluble part of the dry matter present in the liquid autolysate must also be less than 80%.

4. Limits and test methods

4.1. Nitrogen

4.1.1. The total nitrogen content, expressed as element N, must be less than 12% of the dry matter according to the method of analysis described in Chapter II of the International Oenological Codex.

4.1.2. The ammoniacal nitrogen content, expressed as element N, must be less than 0.5% of the dry matter. It is determined by the following method.

Place 1 g of dry matter in 100 mL of 0.5 M KCl and stir for 20-30 min. Introduce the 100 mL into the steam distillation apparatus described in Chapter II of the International Oenological Codex for the determination of total nitrogen, add 50 mL of 30% sodium hydroxide (R) and distil by collecting 250 mL in a conic flask containing 5 mL of 4% boric acid (R), 10 mL of water and 2-3 drops of methyl red-methylene blue mixed indicator (R). Titrate the distillate with 0.1 M hydrochloric acid until the indicator turns pink-purple.

1 mL of hydrochloric acid solution corresponds to 1.4 mg of nitrogen N.

Where n is the number of mL poured: 100 g of yeast autolysates contain $0.14n$ g of ammoniacal nitrogen, expressed as element N.

4.1.3. Organic nitrogen equals total nitrogen minus ammoniacal nitrogen.

4.1.4. The amino acid content, in glycine equivalent, must be between 10% and 20% of dry matter, according to the DNFB method described in the appendix, or, if expressed as element N, must be between 1.9% and 3.7% of the dry matter.

4.2. Humidity

Measured by the loss in weight of 5 g of product, dried at 105°C until the weight is constant (approximately 3 hours)

The maximum humidity of the solid forms must be less than 7%.

Heavy metal limits concern the dry matter of the dry and liquid forms.

4.3. Lead

Determination according to the method indicated in Chapter II of the International Oenological Codex

The lead content must be less than 2 mg/kg of dry matter.

4.4. Mercury

Determination according to the method indicated in Chapter II of the International

Oenological Codex

The mercury content must be less than 1 mg/kg of dry matter.

4.5. Arsenic

Determination according to the method indicated in Chapter II of the International Oenological Codex

The arsenic content must be less than 3 mg/kg of dry matter.

4.6. Cadmium

Determination according to the method indicated in Chapter II of the International Oenological Codex

The cadmium content must be less than 1 mg/kg of dry matter.

4.7. Viable yeasts

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

The viable yeast count must be less than or equal to 10^2 CFU/g or per mL for the liquid form.

4.8. Moulds

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

The mould count must be less than 10^3 CFU/g or per mL for the liquid form.

4.9. Lactic bacteria

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

The lactic bacteria count must be less than 10^3 CFU/g or per mL for the liquid form.

4.10. Acetic acid bacteria

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

The acetic acid bacteria count must be less than 10^3 CFU/g or per mL for the liquid form.

4.11. Salmonella

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

Absence must be checked on a sample of 25 g, or mL for the liquid form.

4.12. Escherichia coli

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

Absence must be checked on a sample of 1 g, or mL for the liquid form.

4.13. Staphylococci

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

Absence must be checked on a sample of 1 g, or mL for the liquid form.

4.14. Coliforms

Enumerate according to the method indicated in Chapter II of the International Oenological Codex

The coliform count must be less than 10^2 CFU/g or per mL for the liquid form.

5. Additives

They must comply with the currently applicable regulations.

6. Storage

Yeasts autolysates must always be stored in sealed bags sheltered from the air. Store in a cool, dry place.

In all cases, refer to the manufacturer's instructions.

Appendix 1: Dinitrofluorobenzene method

1. Introduction

This method is used to quickly determine the amino nitrogen in a biological solution compared with a standard range produced with a solution of glycine.

2. Scope

Oenological products of plant or animal origin

3. Definition

Dinitrofluorobenzene (DNFB) reacts with free NH_2 functions contained in the amino acids to give a bright yellow compound determined by colorimetry at 420 nm. The reaction takes place at pH> 9.3.

4. Reagents and Products

Reagents:

- Borax or sodium tetraborate,
- Dinitrofluorobenzene,
- 10 M Hydrochloric acid,
- Glycine.

5. Equipment

- haemolysis tubes,
- micropipettes,
- Visible spectrophotometer,
- Water bath at 60°C.

6. Sampling

- Prepare a solution of 5% sodium tetraborate in pure water,
- Prepare a solution with DNFB: introduce 130 µl of DNFB in 10 mL of 95% ethanol,
- Prepare a solution of hydrochloric acid 2M,
- Produce a standard range from a stock solution of glycine with 2 g/l (M = 75.07 g) e.g. 0.50 mg/l, 100 mg/l, 200 mg/l, 500 mg/l,
- Prepare a solution with 2 g/l of the product to be titrated.

7. Procedure

In a test tube, insert:

- 380 µl of 5% Borax,
- 20 µl of the sample to be titrated,
- 20 µl of the DNFB solution,
- perform in identical fashion with the glycine range,

- Stir and place in water bath at 60°C for 30 min,
- Add 3 mL of HCL 2M,
- Stir and read the specific absorbance at 420 nm for the sample,
- Produce a calibration curve with the Glycine range.

8. Results

Plot the value of absorbance at 420 nm for the sample on the calibration curve. The results are expressed in g/l of Glycine.