COEI-1-INAYEA Inactivated yeasts

1. Object, origin and scope of application

Inactivated yeasts are used as nutrients for yeasts at the beginning of and during alcoholic fermentation, and also to encourage the rehydration of active dry yeasts. They can help to reduce the level of Ochratoxin A in the steps for wine maturing and clarification operations.[1]

They are produced by the biomass of *Saccharomyces spp.* or non-Saccharomyces, inactivated by heat and/or by modification of the pH. They may have undergone the beginnings of a natural autolysis through the action of the endogenous enzymes. Production techniques are those conventionally used for the biomass of yeasts. In the process, there is no addition of antibiotics or of compounds other than those needed to grow the yeast.

When inactivated yeasts come from genetically modified yeasts, they must be authorised for use beforehand by the relevant authorities.

2. Labelling

The label must indicate:

- the name of the genus and the species of inactivated yeasts,
- the organic nitrogen content,
- any additives,
- instructions for use,
- the batch number as well as the expiry date, and the storage conditions in terms of well-defined temperature, humidity, and ventilation conditions,
- the indication that the inactivated yeasts are derived from yeasts obtained by genetic modification and the modified characteristic if such is the case.

3. Characteristics

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

Inactivated yeasts are partially soluble in water, the insoluble part being greater than or equal to 60% m/m of the dry matter.

4. Limits and test methods

4.1 - Nitrogen content

4.1.1. The total nitrogen content, expressed as element N, is less than 10% 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 and is determined according to the following method of analysis.

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 inactive dry yeasts contain 0.14*n* g of ammoniacal nitrogen, expressed as element N.

4.1.3. The organic nitrogen content is obtained through the difference between the total nitrogen content and the ammoniacal nitrogen content.

4.1.4. The free and soluble amino-acids and small peptides content must be lower than 10% of dry matter in glycine equivalent, according to the DNFB method described in the appendix, or, if expressed as element N, must be 1.9% of the dry matter.

4.2. Humidity

This is 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 must be less than 7%.

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.

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 of dry matter.

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 of dry matter.

4.10. Acetic bacteria

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

The acetic bacteria count must be less than 10^3 CFU / g of dry matter.

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 of dry matter.

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 of dry matter.

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 of dry matter.

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 of dry matter.

5. Additives

They must comply with the currently applicable regulations.

6. Storage

Inactivated yeasts 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 of application

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 a pH > 9.3.

4. Reagents and Products

Reagents:

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

5. Apparatus

- Haemolysis tubes,
- Micropipettes,
- Spectrophotometer for measurements in the visible range,
- Water bath at 60°C.

6. Sampling

Prepare a solution of 5% sodium tetraborate in pure water,
Prepare a DNFB solution: introduce 130 µL of DNFB in 10 mL of 95% vol. Ethanol,
Prepare a 2M hydrochloric acid solution,
Produce a standard range from a 2 g/L stock solution of glycine (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 determined.

7. Procedure

In a haemolysis tube, insert:
380 µL of 5% Borax,
20 µL of the sample to be determined,
20 µL of the DNFB solution,
Perform in identical fashion for the glycine range,

Stir and place in the water bath at 60°C for 30 min,
Add 3 mL of 2M HCl,
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.

[1] Code of sound vitivinicultural practices in order to minimise levels of Ochratoxin A in vine-based products