COEI-1-UREASE Urease

E.C.3.5.1.5

CAS N°: 9002-13-5

General specifications

The specifications must be in compliance with general specifications for enzymatic preparations as provided for in the International Oenological Codex.

1. Object, origin and field of application

The purpose of an enzyme is to break down urea into ammonia and carbon dioxide. Urease is produced from *Lactobacillus fermentum.* It belongs to the urease group collectively called “urease acids”. They are activated at low pH levels.

L. fermentum is grown in a synthetic environment. After fermentation, the culture is filtered, washed in water and the cells are killed in 50% vol alcohol. The suspension is freeze dried or dried by pulverisation.

The preparation consists of a powder made up of whole dead cells containing enzymes.

Urease contains no substances, nor micro-organisms nor collateral enzymatic activities which are:

- harmful to health,
- harmful to the products treated,
- lead to the formation of undesirable products
- produces or facilitates fraud

2. Labelling

The concentration of the product must be indicated on the label in addition to security and storage conditions and the to the expiration date.

3. Enzymatic activity

The claimed enzymatic specific activity is posted at 3.5 U/mg. Note that one unit is defined as the quantity of enzymes which release one micromole molecule of ammonia hydroxide from 5 g/l dose of urea, per minute at pH level 4 in a citrate

buffer 0.1 M medium, at 37 ^{0}C.

This activity is the only isolation.

4. Characteristics

Urease can be found in the crystal powder form, white, odourless, with a mild taste

5. Supports, diluents, preservation agent

The only substance added for conditioning is dextrin.

6. Trials

6.1. Sulphuric ashes

Determine sulphuric ashes according to the method in Chapter II in the International Oenological Codex. The rate of sulphuric ashes in urease must not be over 8%.

6.2. Solution for trials:

Dissolve 5 g of urease in 100 ml of water.

6.3. Heavy metals

A 10 ml of solution for trials (6.2), add 2 ml of buffer solution pH 3.5 (R), 1.2 ml of thioacetamide (R) reagent. There should be no precipitation. If brown colouring occurs, it should be less than demonstrated in the trial prepared as indicated in Chapter II of the International Oenological Codex.

The contents of heavy metals expressed in lead, must be less than 30 mg/kg.

6.4. Arsenic

Measure arsenic according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of arsenic must be less than 2 mg/kg.

6.5. Lead

Measure lead according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of lead must be less than 5 mg/kg.

6.6. Mercury

Measure mercury according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of mercury must be less than 0.5 mg/kg.

6.7. Cadmium

Measure cadmium according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of cadmium must be less than 0.5 mg/kg.

7. Biological contaminants

Carry out a counting according the method described in Chapter II of the International Oenological Codex

7.1. Total bacteria under 5 x 104 CFU/g

7.2. Coliformesteneur under 30 CFU/g of preparation

7.3. *Escherichia coli* absence checked on 25 g sample

7.4. St. aureus absence checked on 1 g sample

7.5. Salmonella absence checked on 25 g sample.

No mutagenic or bacterial activity should be detectable

It is also admitted that no *Lactobacillus* strain should produce antibiotics.

8. Application to wine

Urease must be carefully incorporated and mixed in wine to be aged more than 1 year if it contains more than 3 mg/l of urea. The dose to be used will be 25 mg/l to 75 mg/l, according to tests carried out beforehand. This procedure is carried out in less than 4 weeks at a temperature above 15°C and when there is less than 1 mg/l fluoride ions.

After a noticeable decrease in urea, for example less than 1 mg/l, all enzymatic activity is eliminated by filtering the wine. (diameter of pores under 1 µm).

9. Storage conditions

Urease can be stored for several months at a low temperature (+ 5 °C). There is a 50% loss in activity annually.