COEI-1-BGLUCA Beta-Glucanases from Trichoderma Sp.

(E.C. 3-2-1-58)

(C.A.S. No. 9073-49-8)

Glucan 1,3-beta-glucosidase

(exo-1,3-beta-glucosidase; beta-1,3-glucan exo-hydrolase; exo-1,3-beta-glucanase; endo-1,3-beta-glucanase)

and glucan 1,6-beta-glucosidase

General specifications

The specifications must comply with general specifications for enzymatic preparations that appear in the International Oenological Codex.

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

The degradation of beta-glucans present in wines, in particular those from grapes affected by *Botrytis cinerea* or yeast glucans. These molecules of a very high molecular weight hydrolyse the beta-1,3 and beta-1,6 bonds of 1,3 (1,6)-beta-D-glucans with glucose production.

Secondary activities: hemicellulases, cellulases.

The beta-1,3-D-glucanases are produced from *Trichoderma harzianum* .and/or *Trichoderma ressei*

The preparation of the enzyme is without any harmful consequences as is production and purification.

Beta-glucanases do not contain any substances, micro-organisms nor collateral enzymatic activities that can:

- be harmful to health,
- be harmful to the quality of the products treated,
- lead to the formation of undesirable products or flavour problems.

There are regulatory limits for the use of beta-glucanases in wine.

2. Labelling

The concentration of the product must be indicated on the label, as well as the safety conditions, storage conditions and the expiry date.

3. Characteristics

In general, it is greyish to light brown amorphous powder or light brown to dark brown liquids or granules.

4. Solubility

Soluble in water and practically insoluble in ethanol.

5. Enzymatic activity

Activity is the quantity of enzyme necessary for liberating in standardised conditions (see activity measured according to a method to be described), a quantity of reducing sugars corresponding to 1 µmole of glucose per minute.

Remark: the enzyme produced according to paragraph 6 simultaneously has beta-1,3-glucanase and beta-1,6-glucanase activities which gives it the sought oenological properties.

6. Source of enzyme and production means

The beta (-1,3-1,6) glucanases are produced by submerged culture of a selected non pathogenic, non toxic strain of *Trichoderma harzianum* and/or *Resei* that is not genetically modified, in pure culture.

7. Diluents, preservatives and additives

The preparation of beta-glucanase is generally in the form of granules. These products are prepared with food diluents or food additives such as maltodextrin, sodium citrate, citric acid, starch or glucose.

8. Test trials

8.1. Loss at desiccation: Less than 10%. (does not apply to liquid preparations)

8.2. Ashes/Sulphuric ashes

Determine the sulphuric cinders according to the method in Chapter II of the International Oenological Codex.

The rate of sulphuric ashes of beta-glucanases should not be more than 2% of dry

matter.

8.3. Preparation of the test solution

Dissolve 5 g of beta-glucanases in 100 ml of water.

8.4. Heavy metals

Add 2 ml of buffer solution pH 3.5 (R) and 1.2 ml of thioacetamide reagent (R) to 10 ml of the test trial solution (8.3). No precipitate should form. If a brown colouration appears, it should be

lighter than the control prepared as indicated in Chapter II of the International Oenological Codex.

The heavy metal content expressed in lead should be less than 30 mg/kg.

8.5. Arsenic

In 2 ml of test trial solution (8.3), search by the method indicated in

- Chapter II of the International Oenological Codex.

Arsenic content should be less than 3 mg/kg.

8.6. Lead

Using the test trial solution (8.3) determine the lead according to the method described.

Lead content should be less than 5 mg/kg.

8.7. Mercury

Using the test trial solution (8.3) determine the mercury according to the method described in Chapter II of the International Oenological Codex.

Mercury content should be less than 0.5 mg/kg.

8.8. Cadmium

Using the test trial solution (8.3) determine the cadmium according to the method described in chapter II of the International Oenological Codex.

Cadmium content should be less than 0.5 mg/kg.

8.9. Biological contaminants

Total microorganisms	less than $5\ 10^4$ CFU/g of preparation
Total bacteria	less than 10^3 CFU/g of preparation
Total coliforms	less than 30 CFU/g of preparation
Escherichia coli	absence checked on a 25 g sample

St. aureus[1]	absence checked on a 1 g sample
Salmonella	absence checked on a 25 g sample
Sulfitoreducing anaerobia	less than 30 CFU/g of preparation
Yeasts	maximum content 10^2 CFU/g of preparation
Total lactic bacteria	absence checked on a 10 g sample
Acetic bacteria	maximum content 102 CFU/ g of preparation
Moulds	maximum content 102 CFU/g of preparation
Antibiotic activity[2]	not detectable
Mycotoxins[3]	not detectable

9. Storage

In a solid form, the preparation can be stored for several years and in a liquid form for several months at a low temperature (+5°C).

[1] Method to be defined by the Sub-Commission of Methods of Analysis

[2] Method to be defined by the Sub-Commission of Methods of Analysis

[3] Method to be defined by the Sub-Commission of Methods of Analysis