



## **RESOLUTION OENO 14/2003**

### **ENZYMATIC PREPARATIONS**

THE GENERAL ASSEMBLY,

CONSIDERING Article 5 of the International Convention on the Unification of the Methods of Analysis and Appraisal of Wines of 13 October 1954,

UPON THE PROPOSAL of the Sub-Commission on Methods of Analysis and Appraisal of Wines,

DECIDES to replace the existing monograph by the following monograph in the International Oenology Codex:

### **ENZYMATIC PREPARATIONS**

The prescriptions described below concern all enzymatic preparations susceptible of being used during various operations that can be applied to grapes and their derivatives.

The prescriptions are based on the recommendations from the “Joint FAO/WHO Expert Committee on Food Additives (JECFA), 35th Session, Rome 29 May – 7 June 1989” and published in 1990 in the FAO Food and Nutrition Paper n° 49 “Specifications for identity and purity of certain food additives. General specifications for enzyme preparations used in Food Processing”.

#### **1. GENERAL CONSIDERATIONS**

Enzymatic preparations can be made from micro-organisms or animal tissue or vegetable tissue.

When looking for synergies between various enzymatic activities including pectinase, cellulase and hemicellulase, mixtures of preparations made from different strains can be carried out. These preparations can contain one or more active compounds, in addition to supports, diluents, preservatives, antioxidants and other substances compatible with correct manufacturing rules and in accordance with the regulation. In certain cases, the preparations can contain cells or cell fragments. Furthermore they can be in either liquid or solid form. The active substances can also be immobilised on a support admitted for oenological products. The use of glycerol is not admitted in certain countries.

## 2. LABELLING

The labelling of admitted enzymatic preparations must specify the storage conditions, additives, the nature of the enzymatic activities, batch number and the expiration date. Also the indication that the enzymatic preparations were obtained by genetic modification where relevant.

## 3. ADMITTED ENZYMATIC PREPARATIONS

All enzymatic preparations presenting a technological interest duly proven in practice and meeting the conditions and criteria mentioned above, are accepted for the treatment of grapes and their by-products.

Enzymatic preparations used must not contain any substance, microorganism, nor enzymatic activity that:

- is harmful to health,
- is harmful to the quality of the products manufactured,
- can lead to the formation of undesirable products,
- or that will give rise or facilitate fraud.

## 4. ENZYMATIC ACTIVITIES

### 4.1. General considerations

Enzymatic preparations contain many enzymatic activities. Other than the main enzymatic activities, whose technological interest has been duly proven, secondary enzymatic activities are only tolerated if they are set within the technological constraint limits for manufacturing of enzymatic preparations. They must be as limited as possible. Generally speaking, the sum of all secondary activities must not be superior to 50% of the sum of the activities necessary for the desired function. The activities are expressed in nkat. (nKat= 1 nmol of transformed substrate or product formed per second per gram of the preparation).

The secondary activities more than 10% of the main activity must be declared within the technical characteristics of the commercial product.

Enzymatic activity in a preparation and corresponding to the expressed technological

need is indicated in units of activities by preparation mass units. These units represent enzymatic activity for which the preparation is standardised.

## 4.2. Activity measurement

The enzymatic activities presented are measured in wine conditions. The incubations are carried out at 25°C for 20 minutes.

Activity measurements are carried out by measuring the initial speed of the reaction.

For each measurement, the values obtained with each preparation inactivated by boiling, (the value of white enzymatic preparations) are to be deducted from the measurements made with active enzymes.

Perform the measurements in duplicate.

The results are expressed in nanokatals.

When the sought out technological transformation results from the action of different enzymes within the same preparation, it is important to specifically measure each enzymatic activity. These activities will require special sheets, where the details of the type of measurement will be specified.

The partial undesirable activities must be sought out and identified.

## 5. SOURCES OF ENZYMES AND PRODUCTION ENVIRONMENT

The microbial sources of enzymes must be non-pathogenic, non-toxic and genetically stable, and the fermentation environments should not leave harmful residues in enzymatic preparations. In the case of microorganisms, a safety study must be conducted in order to ensure that enzymatic preparation produced by a microorganism species (e.g. *Aspergillus niger*) does not pose a health risk. This study can be based on principles brought forth on food enzyme guidelines published by the Scientific Committee for Food (SCF), or other equivalent organisations.

The techniques implemented must be compatible with the good practices of manufacturing and the prescriptions of the International Oenological Codex if yeast and/or lactic bacteria are used.

Animal tissues used in enzymatic preparations must be compatible with demands set by the official monitoring authorities. These tissues must be treated in compliance with good hygiene and manufacturing standards.

## **6. SUPPORTS, DILUENTS, PRESERVATIVES AND OTHER ADDITIVES**

The enzymatic preparations can only be diluted in substances which comply with the regulations in force in different countries for the treatment of grapes and by-products.

In the case of immobilised enzymes, the supports used must comply to standards on material in contact with foodstuffs. For this type of preparation, the content of compounds of the supports used, susceptible to spread out in musts and wine, should be determined and indicated on the label of the enzymatic preparation.

The presence of preservatives will only be tolerated for commercialised preparations in liquid form. Only preservatives authorised in wines are accepted and their contents must be clearly indicated on the label of the enzymatic preparation.

## **7. HYGIENE**

Enzymatic preparations must be produced in accordance with good practices of manufacturing and must not provoke a significant increase in germs in the treated products.

## **8. LIMITS AND TEST TRIAL METHODS**

### **8.1. Heavy metals**

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 30 mg/kg.

### **8.2. Lead**

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 5 mg/kg.

### **8.3. Mercury**

Proceed with the determination according to the method in chapter II of the International Oenological Codex.



Content less than 0.5 mg/kg.

#### **8.4. Arsenic**

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 3 mg/kg.

#### **8.5. Cadmium**

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 0.5 mg/kg.

#### **8.6. Salmonella**

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

Absence checked on a 25 g sample.

#### **8.7. Coliforms**

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

Content less than 30 CFU/g of preparation.

#### **8.8. Escherichia coli**

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

Absence checked on a 1 g sample.

#### **8.9. Total germs**

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

Content less than  $10^4$  CFU/g of preparation.

#### **8.10. Yeasts**

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

Content less than  $10^3$  CFU/g of preparation.

### **8.11. Lactic bacteria**

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

Content less than  $10^3$  CFU/g of preparation.

### **8.12. Acetic bacteria**

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

Content less than  $10^2$  CFU/g of commercial product.

The enzymatic preparations should not present antibiotic activity, nor detectable levels of aflatoxins\* (4 µg/kg), ochratoxin A\* (3 µg/kg), sterigmacycline\*, T-2 toxins\* (to be set) or zearalenones\* (10 µg/kg).

## **9. OBLIGATORY TECHNICAL SHEET TO BE SUPPLIED BY MANUFACTURER**

Each type of enzymatic preparation must be defined using a technical sheet.

It must contain at least the following information:

- Nature of the preparation (e.g. pectolytic enzymes),
- Origin (e.g. *Aspergillus niger*)
- Fields and the application procedure,
- Activity and stability of the preparation with the expiration date guaranteeing the activity and storage conditions (temperature),
- Types of reactions catalysed by the main enzymatic activities,
- Main enzymatic activities with n° IUB (for example Tannase 3.1.1.20),
- Secondary enzymatic activities with, if possible, IUB number, and their activity as a percentage of the main activity,
- Types of supports, diluents, preservatives and additives used and their respective contents,
- Whether the enzymatic preparation is genetically modified or not
- A statement identifying the batch.



FURTHER INFORMATION FOR EACH ENZYME WILL BE MADE AVAILABLE.

\* According to methods to be defined at a later date.