

ENZYMATIC PREPARATIONS
(OIV-Oeno 365-2009; OIV-Oeno 485-2012)

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 "General Specifications and Considerations for Enzymes used in Food Processing" drafted by the "Joint FAO/WHO Expert Committee on Food Additives (JECFA), 67th Session, Rome 20 -29 June 2006" published in 2006 in the FAO JECFA monographs.

1. GENERAL CONSIDERATIONS

Enzymatic preparations can be made from any safe biological sources. 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 the good manufacturing practices and in accordance with local regulations. In certain cases, 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 food use.

2. LABELLING

The labelling of enzymatic preparations must at least specify the enzyme name according to IUBMB rules (ex. polygalacturonase), the activity (in units by g or mL), the batch number storage condition for maintaining stability and the expiry date. Enzymatic preparations with multiple technological activities (cf. 4.1) should bear the name of each enzyme on which the preparation is standardized.

If there is available space, it is desirable that the label has the

additional information: recommended dose and implementation conditions, the nature of additives and carriers used, the nature of enzymatic activities. If there is not enough space, this information shall be indicated on the technical data sheet of the preparation.

The indication that enzymatic preparations were obtained by genetically modified organisms must be mentioned. If it is not mentioned in the labelling, the fact that genetic engineering was used to improve the microorganism that produces the enzyme has to be mentioned in related documentation.

3. ADMITTED ENZYMATIC PREPARATIONS

All enzymatic preparations with activities 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, particularly concerning the colour, the aroma and the taste of the wines,
- 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, (activities for which, respectively, the enzymatic preparation has been standardised) 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.]

Generally speaking, the secondary activities present in a given preparation must not become the main reason to use the said preparation unless this preparation is declared as multiple technological effects. Referring to the International Code of Oenological

Practices, Oeno 11/04 – 18/04 and 3/85, on a technological level, a distinction is made between the following types of preparations

- Maceration preparations: facilitate extraction of compounds such as colour, tannins,...
- Clarification / filtration preparations: facilitate clarification and filtration of musts and wine
- Aroma enhancers: reinforces and/or modifies aromatic profile of musts and wine
- Stabilisation preparations: facilitates extraction of macromolecules or other substances with a stabilising effect on wine (yeast mannans).

When an enzymatic preparation generates multiple technological effects, duly noted in a practice, (ex. Clarification and aroma enhancer enzymes), whether they are the result of a main and/or secondary activity, they must be declared as such on the label. Different enzymatic activities responsible for these effects must be measured and indicated in the technical preparation data sheet.

4.2 Activity measurement

The enzymatic activities presented are measured under the conditions corresponding to their biochemical characteristics. (pH, temperature) and if possible, the closest to activities encountered in the practice (grape juice, must or wine). The methods implemented must correspond to state of the art in analytical terms and, if possible, be validated in accordance with appropriate international standards (for example: ISO 78-2; ISO 5725).

Results are expressed in nanokatal/g or nanokatal/mL or in viscosity units in the case of enzymes with endo-type of activities. (nkat = 1 nmole of transformed substrate or product formed per second by g or mL of the preparation). Results should be given with reference to the method used.

When the sought out technological effect results from the action of different enzymes within the same preparation, it is necessary to measure each enzymatic activity. Each of these activities will require special Codex monograph, with the details of the analytical method.

5. SOURCES OF ENZYMES AND FERMENTATION ENVIRONMENT

The sources of enzymes must be non-pathogenic, non-toxic and genetically stable, and the fermentation broth 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 present any health risk. This study can be based on principles brought forth on food enzyme guidelines published by the European Food Safety Authority (EFSA), or other equivalent organisations.

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

6. CARRIERS, DILUENTS, PRESERVATIVES AND OTHER ADDITIVES

Substances used as carriers, diluents, preservatives or other additives must not, with a "carry over" effect, disseminate compounds in the grapes and derivative products, which are not compatible with regulations in force in different countries. Moreover, these compounds must not have a negative effect on the organoleptic properties of wine. In the case of immobilised enzymes, the carriers used must comply to standards on material in contact with foodstuffs. For this type of preparation, the content of compounds of the carriers used, susceptible to enter the musts and wine, should be determined and indicated on the label of the enzymatic preparation.

Preservatives such as KCl are added in the liquid enzyme concentrate during manufacturing. These substances prevent the development of micro-organisms during the different formulation operations of products. These substances can be found not only in liquid preparations but also in solid preparations. Given the inevitable "carry over" effect, only preservatives which are compatible to regulations in force in the different countries are authorised.

These substances must be clearly identified and indicated on the label or on the technical data sheet of the commercial product.

7. HYGIENE AND MAXIMAL LEVEL OF CONTAMINANTS

Enzymatic preparations must be produced in accordance with good manufacturing practices:

7.1 Lead

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

Content less than 5 mg/kg.

7.2 Mercury

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

Content less than 0.5 mg/kg.

7.3 Arsenic

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

Content less than 3 mg/kg.

7.4 Cadmium

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

Content less than 0.5 mg/kg.

7.5 *Salmonella* sp

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

Absence checked on a 25 g sample.

7.6 Total coliforms

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

Content less than 30/per gram of preparation.

7.7 *Escherichia coli*

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

Absence checked on a 25 g sample.

7.8 ANTIMICROBIAL ACTIVITY

Non-detectable

7.9 SPECIFIC MYCOTOXINS OF DIFFERENT PRODUCTION STRAINS

Non-detectable

8. TECHNICAL DATA SHEET TO BE SUPPLIED BY MANUFACTURER

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

It must contain at least the following information:

- Name of enzyme and biological origin (e.g. pectolytic enzymes of *Aspergillus niger* or pectolytic enzyme of *A. oryzae* expressed as *A. niger*),

- Declared activity (in nKat/g or nKat/ml of preparation)
- Fields and application mode (technological effects and useful details for the implementation of the preparation) ,
- Stability of the preparation and expiration date period based on production date guaranteeing the maintaining of activity, under the given storage conditions (temperature),
- Types of reactions catalysed by the main enzymatic activities,
- Main enzymatic activities with IUB number (for example Tannase 3.1.1.20),
- Secondary enzymatic activities with, if possible, the IUB number
- Types of carriers, diluents, preservatives and additives used and their respective contents,
- If deemed useful, further information can be added to this technical data sheet.