

ENZYMES



ENZYMES ITALY

Oenological Enzymes

Enzymes are proteins that catalyze chemical reactions, mainly the braking and formation of chemical bounds.

Oenological enzymes are classified as:

- Pectolitic enzymes
- ß-glucanase
- Lysozyme
- Glycosidase

Pectolytic Enzymes

Grapes have very low pectin levels, which are mainly localized in the skins. Must normally contains 0.5 – 1.0 g/L of pectin (as galacturonic acid). Depending on the vinification procedures the concentration can increase up to 2.5 g/L .Pectin in grape musts act as a colloidal agent that interferes with must clarification. It also reduces the filterability of the must, clogging filtration surfaces.

There are essentially two types of enzymes that possess activity towards pectins:

<u>Exolytic Enzymes</u> acting on bonds located on the external portion of pectin which require a large number of scissions in order to degrade pectin.

<u>Endolytic Enzymes</u> which act on the internal portion of the molecule resulting in a much faster affect relative to exolytic enzymes.

Pectolytic enzymes can also be divided according to their substrate:

- Intact pectin
- Demethoxylated pectin (due to the action of pectin methyl transferase PME) or pectic acid.

The first group includes the exo- and endo-pectin lyases (PL), while the second group is comprised of the exo- and endo-polygalacturonases (PG).



Factors Affecting Must Clarification

Enodogenous enzymes:

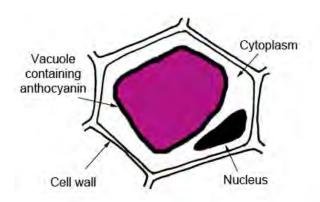
- Temperature: low temperature inhibits endogenous enzymes
- The concentration is not high enough to have a fast clarification

Concentration of pectins

• Higher in the aromatic varieties (Muscat, Traminer, Sauvignon..)

The use of PECTINASE is required for a FAST and EFFICIENT clarification

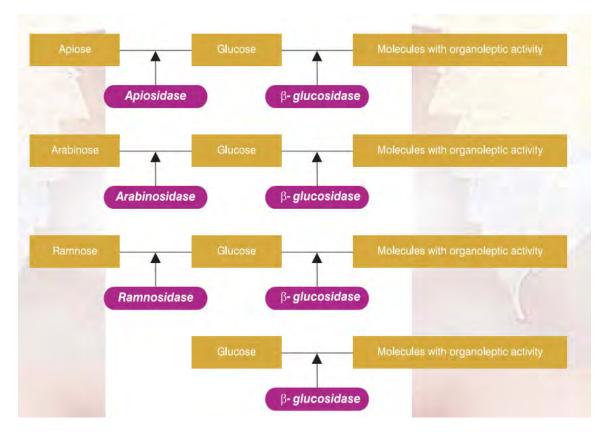
Side Activities

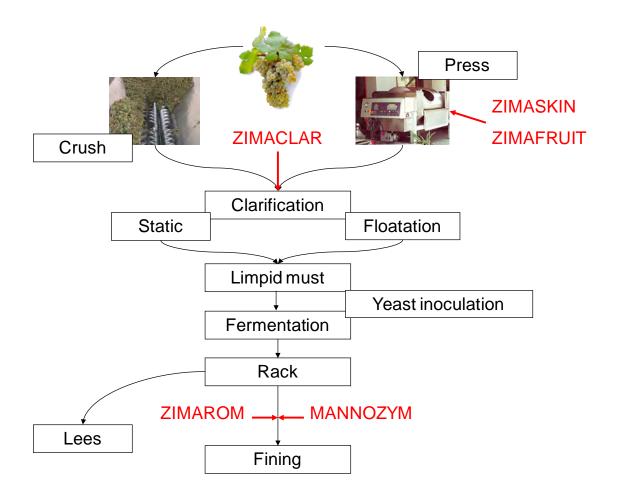


Enologica Vason enzymes have important side activities:

Cellulase Hemicellulase Protease Glycosidase

Terpens are normally bounded to 4 different sugars. To free terpens we need the four glycosidasic activities





White Vinification Summary

ZIMACLAR®

Enzymatic preparation with high pectinolytic activity for the clarification of white musts and young wines

ZIMASKIN®

Liquid enzymatic preparation for vinification of white grapes by maceration

ZIMAROM®

Enzymatic preparation for the clarification of aromatic musts

MANNOZYM®

Enzymatic preparation of beta-glucanase with lysogenic activity for clarification and filtration of wine



White Vinification Detail

ZIMACLAR®

Enzymatic preparation with high pectinolytic activity for the clarification of white musts and young wines

ZIMACLAR is an enzymatic preparation with elevated pectinolytic activity and contains polygalacturonase (PG) and pectin lyase (PL). It has been specifically formulated for the clarification of white musts and successive filtration.

Attributes:

- reduction in must viscosity
- rapid separation of solid fraction
- optimal compaction of lees
- increase in wine yield
- increase in filterability of the must

Recommended dose and uses:

From 1 to 3 g/hL for 2 hours at 20°C.

In order to avoid loss of product on stems and whole grapes, it is recommended that it be dosed on must after destemming. The use of bentonite, by adsorption, reduces the enzymatic activity. It is therefore recommended that treatment with bentonite be carried out when the enzymatic activity is complete (generally after 2 hours at 20°C).

ZIMASKIN®

Liquid enzymatic preparation for vinification of white grapes by maceration

ZIMASKIN is a pectinolytic enzyme preparation in a liquid form with various activities including PG, PL, cellulase, and hemicellulase. Use in the production of white wine during maceration with the solid fraction (cryo-maceration and maceration of the skins).

Attributes:

- specifically increases the extraction of aromatic precursors
- reduces the viscosity of must
- reduces the skin-must contact time
- improves filtration and clarification

Recommended dose and uses:

From 1 to 5 g/hL on crushed and destemmed grapes. The rate of reaction of the enzyme doubles with every 10° C interval increase in temperature. 2 g/hL at 10° C for 12 hours; 2 g/hL at 20° C for 5-6 hours.

ZIMAROM®

Enzymatic preparation for the clarification of aromatic musts

ZIMAROM is a powdered, pectinolytic preparation that contains PG and PL, which decreases viscosity in order to facilitate separation, compact waste, and increase the yield of must. The most interesting aspect is related to the glycosidase activity present in the product, which hydrolyzes aromatic precursors bound to the glycoside component and thereby releases monoterpenes and norisoprenoids that provide aroma.

Zimarom possesses activity of all four glycosidases that are required in order to increase the primary aromatic components of wine

(β-D-apiosidase, a-L-arabinosidase, a -L-ramnosidase, β-D-glucosidase).

Recommended dose and uses:

From 1 to 3 g/hL for fermentation as the presence of glucose partly inhibits the activity of b-glucosidase. At least 10-15 days of treatment are recommended, tasting the product twice a week. When the desired effect has been achieved, the enzymatic activity can be stopped by the addition of bentonite.

Red Vinification Detail

A completely new way of extracting color:

Fractioned enzymatic extraction, localization, differentiation.

Starting in 1999, the research and development sector of Enologica Vason has developed novel procedures for the vinification of red wine and has pioneered the concept of fractionation and differentiation during the process of enzymatic extraction. The process involves the use of ZIMARED PLUS in the initial phases of maceration followed by a second enzyme, during the final phases, providing differential extraction. Both doses must be concentrated on the solids portion of the must. The related product EXTRARED L is a combination of pectinolytic enzymes with various secondary enzymatic activities including hemicellulase, cellulase, and proteolyase, specific for extraction and stabilization of colored compounds.

Anthocyanins are mainly localized in the skin of the grapes, especially in the vacuole of the skin cells. (Amrani-Joutei e Glories, 1995)

Tannins are localized in the skin and in the seeds

Anthocyans and tannins evolution during fermentation

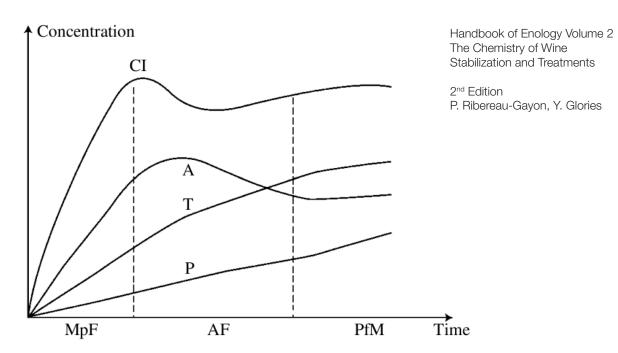


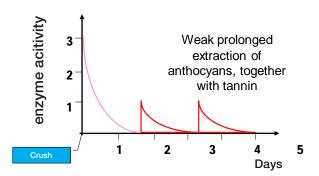
Fig. 6.41. Influence of vatting on the extraction of various compounds from grapes. A, anthocyanins: T, tannins: P, polysaccharides: CI, color intensity: MpF, maceration prior to fermentation; AF, alcoholic fermentation; PfM, post-fermentation maceration



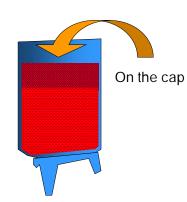
Traditional enzyme addition

Strong extraction of anthocyans, without tannin Crush Strong extraction of anthocyans, without tannin

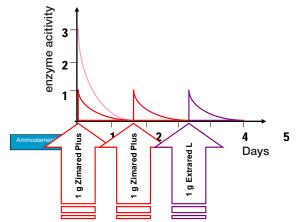
Fractioned addition

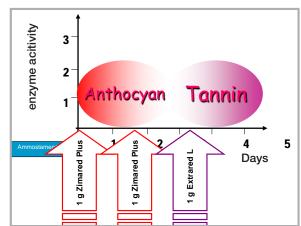












ZIMARED PLUS®

Enzymatic preparation specific for the vinification of red wine. Zimared is a powdered, pectinolytic enzyme preparation containing PG, PL, cellulase, hemicellulase, and acid protease.

Attributes:

- extraction of anthocyanins by disrupting both cells and vacuoles
- · favors the extraction of tannic polyphenols, necessary for the formation of stable complexes with anthocyanins
- reduces the skin contact time
- improves clarification and filtration
- increases the yield of wine

Recommended dose and uses:

From 1 to 4 g/hL during maceration. The preparation should be dissolved in water and added after crushing and destemming, or directly in tanks. The best results are obtained by dosing ZIMARED during pump-overs, as suggested by the protocol furnished by our technical service department.

EXTRARED L®

Liquid pectinolytic enzyme preparation with extracting and stabilizing activity for use in fractionation and differentiation during vinification of red wine

The use of EXTRARED L increases the quantity of colored substances extracted and drastically increases their stability, for fixation with tannins and polysaccharides.

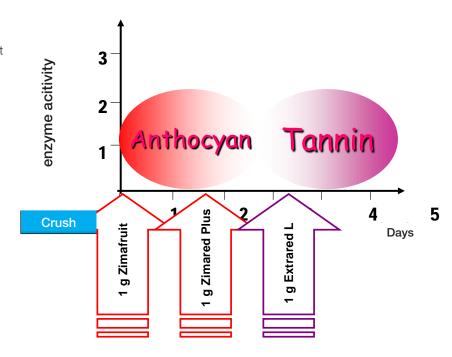
Recommended dose and uses:

During maceration whenever localized fractionation is employed with ZIMARED, 1-2 g of EXTRARED L per hL of must is added during the final phases of maceration. The maximal activity of EXTRARED occurs between 10° and 30°C. The most advantageous uses of EXTRARED L in the vinification of red wine are available from the Technical Services Department of Enologica Vason and are also outlined in the instructions provided).

Enzymes for the Release of Aromatic Compounds

ZIMAFRUIT

- Glycosidasic enzyme used on must
- Activity has been confirmed in the presence of sugars
- Can be used for red vinification (does not contain any anthocyanase activity)





BACTOZYM SG (Lysozyme)

Highly active purified lysozyme obtained from egg albumin. BACTOZYM SG is a micro granulated product that allows instant solubilization.

CHARACTERISTICS

BACTOZYM SG is a lysozyme mixture extracted from egg albumin using a chromatograph process without solvents, his makes it possible to obtain a highly active and stable mixture, and to ensure that the product is particularly pure as required for use in winemaking.

The lytic action works on Gram positive bacteria where the cell membrane is composed of glucosamine and muramic acid, the n-acetyl muramidase action intervenes breaking the glycoside bond between the acid of these two molecules, thus provoking lysis of the cell and consequent bacterial inactivation (bacteriolysis). Therefore BACTOZYM SG is used to inhibit the growth of lactic bacteria such as Oenococcus, Lactobacillus, Pediococcus; however it is ineffective against acetic bacteria (Gram negative).

BACTOZYM SG is a food additive classified as GRAS (Generally Recognized as Safe); and thus is completely harmless for humans (the lysosome is also found in tears and saliva); it also does not interfere with the multiplication and fermentation action of yeast.

DOSES 25-50 g/hL to produce lactic bacteria inhibition for around 3-6 months (500 mg/L maximum legal quantity) 10-25 g/hL to delay or control the intensity of malolactic fermentation and avoid lactic acidness.

ENYZME QUICK REFERENCE TABLE

PRODUCT	FORM	ACTIVITY	APPLICATIONS	OENOLOGICAL EFFECTS	DOSES
Zimaclar	Powder	Pectolytic	For white, red and rosé must before fermentation for clarifi- cation of must	Increases must yield Increased filtration capacity	1 - 3 g/hL
Zimaclar pH3	Powder	Pectolytic	Must with low pH (white, rosé and sparkling wines)	Improves clarification for musts from early vintage with low pH Increases yield during crush maceration	1 - 3 g/hL
Zimaskin	Powder	Pectolytic Polygalacturonase Pectin lyase	For white must, skin maceration, strong activity to breaks cell membrane	Increases aromatic precursors Reduces viscosity improves filtra- tion & clarification	1 - 5 g/hL
Zimarom	Powder	Pectolytic Polygalacturonase Pectin lyase Glycosidase	For white, red and rosé wine	Increases the terpens concentra- tion, improve the organoleptic profile	1 - 3 g/hL
Zimaclar Flot	Powder	Pectolytic	Flotation	Aids flotation process	0.5 - 5.0 g/hL
Zimared Plus	Powder	Pectolytic Cellulase Hemicellulase Protease Polygalacturonase Pectin lyase	Maceration of red must	Increases must yield Extraction of tannic polyphenols Extraction of anthocyanins Increases olfactory sensory intensity	1 - 4 g/hL
Extrared L	Liquid	Pectolytic Cellulase Hemicellulase Protease	Maceration of red must	Stable color Increases organoleptic balance	1 - 2 g/hL
Zimafruit	Powder	Pectolytic Glycosidase	Maceration of white, red and rosé must	Increases fruit aromatic precursors More stable and intense color	1 - 2 g/hL
Mannozym [®]	Powder	Betaglucanases	Treating wines from Botrytis affected grapes, on the wine to increase the rate of lees extraction	Improves filterability Increases mouthfeel Better and fast extraction of mannoproteins on sur lies	0.5 - 4 g/hL
Bactozym SG	Granulated	Lysozyme	Inhibits lactic acid bacteria		20 - 50 g/hL