USE OF ENZYMES IN WINE MAKING: A REVIEW

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Abstract:

Many years after the fruit juice industry`s recognition of the value of applying enzymes, winemakers have also come to acknowledge their usefulness. From the pre-fermentation stage, through fermentation, post-fermentation and aging, enzymes catalyzing various biotransformation reactions. These enzymes originate from the grape, yeasts and other microbes associated with vineyards and wine cellars. Today, winemakers use commercial enzyme preparations. These industrial enzymes offer quantitative benefits (increased free and press juice yields), qualitative benefits (improved color extraction in red grape varieties, improvements in the aging process of wines i.e. flavor enhancement) and processing benefits (shorter time of maceration, settling and filtration). This review article summarises the most important enzymes applied to winemaking and effects of commercial enzyme preparations on process technology and the quality of the final product. Future biotechnological advances will facilitate increased use of enzymes in these and other potential applications.

Keywords: Enzymes, Wine, Clarification, Polyphenols, Colour.

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1. Introduction

Enzymes play a pivotal role in the winemaking process. Many of these enzymes originate from the grape itself, the indigenous microflora on the grape and the microorganisms present during winemaking(Table 1). Since the endogenous enzymes of grapes, yeasts and other microorganisms present in must and wine are often neither efficient nor sufficient to effectively catalyse, commercial enzyme preparations are widely used as supplements. All these commercial enzyme preparations are obtained from microorganisms cultivated on substrates under optimum conditions and facilitate their purification. The most widely used enzymes available for commercial use in winemaking are:

- *pectinases, glucanases, xylanases* and *proteases,* to improve the clarification and processing of wine
- *glycosidase,* the release of varietal aromas from precursor compounds
- *urease,* the reduction of ethyl carbamate formation
- *glucose oxidase,* the reduction in alcohol levels

Table 1.Enzymes derived from grapes and wine associated microbes involved in winemaking [adapted from Van Rensburg & Pretorius 2000]

Enzyme	Remarks
Grapes*(Vitis vinifera)* *Glycosidases*	Hydrolyse sugar conjugates of tertiary alcohols; inhibited by glucose; optimum pH 5-6
Protopectinases	Produce water-soluble, highly polymerized pectin substances from protopectins
Pectin methylesterases	Split methyl ester groups of polygalacturonic acids, release methanol, convert pectin to pectate; thermo-stable; opt. pH 7-8
Polygalacturonases	Hydrolyse α-D-1,4-glycosidic bonds adjacent to free carboxyl groups in low methylated pectins and pectate; optimum pH 4-5
Pectin lyases	Depolymerise highly esterified pectins
Proteases	Hydrolyses peptide bonds between amino acid residues of proteins; inhibited by ethanol; thermo stable; optimum pH 2
Peroxidases	Oxidation metabolism of phenolic compounds during grape maturation; activity limited by peroxide deficiency and SO_2 in must

Fungi (*Botrytis cinerea*) *Glycosidases*	Degrades all aromatic potential of fungal infected grapes
Laccases	Broad specificity to phenolic compounds, cause oxidation and browning
Pectinases	Saponifying and depolymerising enzymes, cause degradation of plant cell walls and grape rotting
Cellulases	Multi-component complexes : endo-, exoglucanses and cellobiases; synergistic working, degrade plant cell walls
Phospholipases	Degrades phospholipids in cell membranes
Esterases	Involved in ester formation
Proteases	Aspartic proteases occur early in fungal infection, determine rate and extent of rotting caused by pectinases; soluble; thermo stable
Yeast (*Saccharomyces cerevisiae*) *β-Glucosidases*	Some yeast produce β-glucosidases which are not repressed by glucose
β-Glucanases	Extra cellular, cell wall bound and intracellular, glucanases; accelerate autolysis process and release mannoproteins
Proteases	Acidic endoprotease A accelerates autolysis process.
Pectinases	Some yeast degrade pectic substances to a limited extent; inhibited by glucose levels < 2%
Bacterial (*Lactic acid bacteria*) *Malolactic enzymes*	Convert malic acid to lactic acid
Esterases	Involved in ester formation
Lipolytic enzymes	Degrades lipids

Grape *pectinases* are inactive under the pH and SO_2 conditions associated with winemaking. Fungal *pectinases* are resistant to these winemaking conditions. The method used to produce wine enzymes for use in the European Union is regulated by the International Organisation of Vine and Wine (OIV), who have decreed that only *Aspergillus niger* and *Trichoderma species* may be used as source organisms (i.e. have GRAS, "generally regarded as safe", status) [Cannal-Llauberes 1993]. The produce the enzymes used in winemaking, selected strains are cultivated in fermentors under aerobic conditions from *Aspergillus niger*for the production of *pectinases*,

hemicellulases and *glycosidases*, while for *glucanases* from *Trichoderma species*, and *Lactobacillus fermentum* for *urease*. A well-defined composition of the growth medium induces optimal production of the enzymatic activities. After fermentation, enzymes (*pectinases*) and enzymatic side activities are isolated by centrifugation, ultra filtration and concentration. During these stages microorganisms are completely eliminated from the end product.

The main activities currently used in winemaking preparations are derived from the *pectinase* family. They include *pectin lyase* (PL), *pectin methyl-esterase* (PME) and *polygalacturonase* (PG). PL type activity, known as depolymerizing, cuts the pectin chain between two galacturonic methylated acids, while the PG prefers a non methylated substrate. PME activity does not depolymerize the pectin chain but releases the methylic group from galacturonic esterified acids.

Pectolytic enzymes have been used in the processing of fruit juices since the 1950s in order to improve juice yield and to aid clarification, but pectinase preparations have only commonly been used in the wine industry since the 1970s. Food grade industrial enzymes offer significant processing improvements. These result in overall economic benefits. Industrial enzymes offer quantitative benefits as increased free run and press juice yields.The qualitative benefits as improved color extraction in red grape varieties, color stability and phenolic extraction of red wines[Bucelli 2006; Ducasse et al. 2010; Main&Morris 2007; Parley et al. 2oo1; Romero-Cascales et al.2008; Watson et al. 1999b], and improvements in the aging process of wines, i.e. flavor enhancement.Processing benefitsresulted inshortenthe time of maceration, settling, and filtration[Canal-Llaubères 1989; Čapaunova&Drdak 2002; Plank&Zent 1993; Revila&Gonzàlez-SanJosé 2002; Rogerson et al. 2000; Villettaz&Dubourdien 1991].

The pectic enzymes play an important role in braking down grape pulp and skin cells and are able to split those chains and saccharide bonds between the chains [Whitaker 1984]. Enzymes cannot act on grapes if they are whole. Therefore, grapes should always be crushed before enzymes are added to enhance extraction.During vinification, the grape skin plays a vital role, within the skin cells are found anthocyanins, tannins and aromas or aroma precursors. The pulp represents 75 to 85% of the berry at maturity. It comprises large cells with fine pecto-cellulosidic walls offering little mechanical resistance to the grape transformation. These cells are a source of pectic polysaccharides in the must. Inside their vacuole is a concentrated solution of organic acids and fermentable sugars. The pectin is located in the primary wall and the sheath between

the skin cells and the pulp. The pecto-cellulosidic wall is a complex structure. It is comprised of cellulose microfibriles, linked together by a matrix of xyloglucan, mannan, xylan (hemi-cellulose) and pectin, all consolidated by a secondary protein network. Some neutral sugars (galactose and arabinose) make up part of the structure of the lateral pectin chains, and form macromolecules with the proteins that hinder clarification of the must. Crossed links between different pectin chains (ionic, electrostatic) determine the porosity of the cell wall. The high level of viscosity of pectin solubilized after crushing, hinder juice extraction, clarification and filtration. Soluble pectin is a major constituent of the cell walls preventing the diffusion of phenolic compounds and aromas into the must during the pre-fermentation and fermentation stages.

In red wine, tannins and anthocyanins are the most important phenolic classes. Tannins contribute to the mouthfeel of wines but they also form pigmented polymers in association with the anthocyanins to provide the stable pigments required to give red wine its longterm colour stability. Grape anthocyanins are red pigments, located in the first external layers of the hypodermal tissue and mainly in the vacuoles [Barcelo et al. 1994], as well as in special structures called anthocyanoplasts [Pecket&Small 1980]. The visual aspect of a red wine, described by its colour, brightness, turbidity or cloudiness, etc. is one of the most important attributes. This is the first characteristic seen by the consumer and it has a direct influence on the acceptance of the wine. Because are develop different enological practices which lead to attaining wines which have good visual characteristics and are stable as possible. In the last few years, some of the enological practices applied to improve the chromaticity of wines have focused on favouring the extraction of colouring materials. Among these practices has been the use of *pectolytic enzymes*. Although the former are used to reduce the turbidity and ease its clearing, it has been shown that they cause an increase in colour intensity and the extraction of phenolic compounds [Izcara et al. 2001; Sevili et al. 1992].

Commercial enzymes for wine exceptional benefits as improving profits and cutting costs, they are also eco-friendly.Enzymes enable wineries to maximize the efficiency of extraction equipment and techniques, resulting in a reduction in energy consuption. Commercial enzymes demonstrate unique benefits when used at the extraction phase during the production of red,

white, and rose short-maturation wines. Through combining increased yield and quality with cost savings, enzymes bring innovative improvements to the winery.

2. The uses and advantages of enzymes in winemaking

The most widely used enzymes available for commercial use, enologically, are *pectinases, hemicellulases, glycosidases* and *glucanases,* the singularly most important being *pectinases*, which occur naturally in grapes, and are partly responsible for the ripening process. In modern wineries is sometimes necessary to help nature by calling for biochemical assistance and adding wine enzymes in order to expedite juice extraction and to make it a much more thorough process, ensure higher quality wine, ease of processing, lower manufacturing costs and finally increased profit.

The first commercial enzyme preparations used in wine industry consisted of *pectinase*[Rombouts&Pilnik 1980]. Today, *pectic enzymes* alone account for about one-quarter of the world`s food enzyme production.

Most commercial preparations of *pectic enzymes* are obtained from fungal sources [Alkorta et al. 1994]. Over the years these became available in a variety of names, effectiveness and purity (Table 2).

Table 2. Commercial pectinases preparations used in winemaking and their effects

Enzyme preparation	Company	Applications	Advantages
Uvazym 1000S	Enartis, Italy	-Clarification of white juices-extremely effective for clarification by cold settling. -Facilitation of fining and filtration operations in pectin-rich wine.	-Reduction in vacuum filter operating time due to good lees compaction. -Increased juice yield (both free run and pressings). -Reduced need for fining agents

Progress quick	Enartis, Italy	-Must flotation.	- Rapid reduction in must viscosity, even at low temperatures. -Formation of a compact layer of floated lees that can be easily separated from the clear juice.
Uvazym couleur	Enartis, Italy	-Enhanced extraction during short macerations. -Use in all situations where there is a risk of low color and extract.	-Higher content of phenolic substances, more intense aromas -Increased color stability in red wines.
Lafazym press	Laffort, France	-Improve the yield extraction of free run juice -Facilitate settling, decrease turbidity	-Increase yield, clarification, color and tannin extraction -Improve filterability, complexity, mouthfeel and stability
Trenolin bukett DF	Erbsloh, Germany	-Aroma releasing effect -Clarification of the must after pressing	- More intense aromas -Improve filterability, complexity, mouthfeel and stability
Crystalzyme	Valley Research, USA	-Rapid clarification -Color improvement -Increased complexity -Process efficiency	-Wine is well clarified early in the winemaking process -More brilliant color -Increased color stability in red wines

2.1 Activities of commercial pectinase preparations

The activity of commercial pectinase preparations are usually reported in one of the following activity units:

- as apple juice depectinising activity (AJDU), based on the reciprocal time required to clarify apple juice at pH 3.5 and 45 ^{o}C [Brown&Ough 1981].
- as polygalacturonase activity (PGU), based on the reduction in viscosity of polygalacturonate substrate at pH 4.2 and 30 ^{o}C

- as pectin methylesterase activity (PMEU), based on the amount of enzyme required to liberate a micromole of titrable carboxyl groups per minute at pH 3.5 and 37 ^{o}C.

2.2 Factors influencing the activity of pectinase preparation

The pH of must and wine do not inhibit the activity of most commercial pectinase preparations, other temperature can reduce the efficiency of the pectolytic activity significantly. At temperature below 10 ^{o}C pectolytic activity are drasticallyreduces, and at temperatures above 50-55 ^{o}C*pectinases* are rapidly inactivated. The temperature stability of commercial pectinase preparations is another factor influencing their activity. Commercial pectinase preparations contain the active proteins (enzymes) (2-5%), sugars, inorganic salts and preservatives to stabilise and standardise the specified activity of the final products [Hagan 1996]. These compounds are important in protecting the protein during sub-optimal storage conditions and exposure to light, which decreases activity [Hagan 1996]. Because these enzymes are essentially proteins, factors inhibiting proteins in general will decrease their effectiveness. This includes juice clarification using bentonite, which adsorbs the proteins and settles them out. Alcohol levels above 17% v/v and SO_2 concentrations over 500 mg/L also inhibit pectinases [Van Rensburg&Pretorius 2000]. Wines which are high in tannins will show reduced enzyme activity as tannins react with the proteins and render them useless. Because very tanninc wines should first be treated with suitable doses of gelatin to remove the tannins that would react with the proteins.

2.3 Effects of *pectinase* additions on wine processing and quality

The *pectolytic enzymes* were the first commercial enzyme preparation used in the wine industry [Rombouts&Pilnik 1980]. Commercial *pectinases* are used to improve juice yields, release of colour and flavour compounds from grape skins and to improve clarification and filterability. See Table 3and 4 for examples of various commercially available preparations.The preparation of deliberately mixed enzymes is very useful as it performs multiple functions. The liquefaction enzymes are an example, containing *cellulases* and *hemicellulases* in addition to pectinases.

Table3. Commercial pectinase preparations to improve clarification, filtration and yield of juice and wine

Enzyme	Company	Activities	Time of addition
Rapidase Filtration	DSM	Pectolytic + β-glucanase	Add at end of fermentation
RapidaseVino Super	DSM	Pectolytic	To juice before settling
Endozyme Active	AEB Africa	Pectolytic	To juice before settling
Pectocel L	AEB Africa	Pectolytic	To grapes or juice
Endozym Pectoflot	AEB Africa	Pectolytic	To must, 4h before flotation initiation
Ultrazym	Novo Nordisk	Pectolytic	To white and red mash
Novoclair FCE	Novo Nordisk	Pectinases	To grape must
Lafazym CL	Laffort	Pectolytic	Prior to fermentation
Lafase 60	Laffort	Pectolytic	In barrel for thermo-vinification musts

Table4. Commercial pectinase preparations to improve extraction and stabilization of colour during winemaking

Enzyme	Company	Activities	Time of addition
Endozym Contact Pelliculaire	AEB Africa	Pectolytic	To juice or must
Lafase HE	Laffort	Pectolytic	During pre-fermentation maceration
Lallemand EX	Lallemand	Pectinase + hemicellulase + cellulase	To grapes before pressing

Vinozym EC	Novo Nordisk	Pectolytic, arabinase + cellulase	Into crusher or mash tank for colour and aroma extraction
Enzym'Col our Plus	Darleon	Pectolytic + Proteolytic	To juice or must
Endozyme Rouge	AEB Africa	Pectolytic + side activities	During maceration (Before SO_2)
Lallemand OE	Lallemand	Pectinase + hemicellulase + cellulase	To grapes before pressing

2.4 Effect on juice extraction, clarification and filtration

The pulp of grape varieties is rich in pectic compounds. The incomplete hydrolysis of these molecules by the endogenous enzymes may therefore cause problems during processing. If pectinases are applied to the pulp before pressing, they can improve juice and colour yield. Pectolytic enzyme preparations based on pectinase activity are recommended for clarification of musts after pressing. It's pectinmethyl-esterasic and endogalacturonic activity causes hydrolysis of pectic chains and facilitates the draining of juice from the pomace [Brown&Ough 1981; Mojsov et al. 2011 a] with an increase yield of a free-run juice with a lower viscosity. The addition of this enzyme lowers viscosity and causes cloud particles to aggregate into larger units, which settles as sediment [Chesson 1980; Mojsov et al. 2011b].The speeding up of the clarification process also produces more compact lees [Mojsov et al. 2011b]. When it is applied to pulp before pressing, it increases juice yield and colour extraction [Mojsov et al. 2011a; Mojsov et al. 2011b; Ough et al. 2010]. At the concentration of 2-4 g/hL, 15% increase in juice has been recorded over a time period of 4-10 hours [Ribereau-Gayon et al. 2000]. The enzyme treatments on red grapes mash of Vranec gives increase in free run juice yields by 4,85-6,35% compared with non-treated mash of control trials [Mojsov et al. 2011 a]. During the production of white wine the viscosity of the must is quickly reduced by the hydrolysis of pectins with *pectinases*. The enzyme treatments on white grapes mash of Smederevka gives increase in free run juice yields by 7,12% and drastic reduction in the volume of gross lees compared with non-treated mash of control trials [Mojsov et al.2011b].The time of filtration was three times shorter,

by using the enzyme preparations, and the speed ofdesliming was twofold faster, compared to the control sample [Mojsov et al. 2010].All this leading to a higher volume of clear must, and this in the more produced wine and on end the more profit.

2.5 Effect on the extraction of pigments and phenols

The pectolytic enzyme treatments of red grape musts could accelerate the extraction of pigments and phenols. Enzyme treatment produces a brighter, more brilliant colour, and the colour stability is greatly increased. The extraction of phenolic compounds usually occurs during the pulping of the mixture in the course of alcoholic fermentation. The grape skin forms a physical barrier to diffusion of anthocyanins, tannins and aromas contained in the skin cells. Thus, to release the cell content, the polysaccharides found in the pecto-cellulosic cell wall and the middle sheath of the berry must be hydrolyzed. In order to weaken the cell walls and facilitate the diffusion of the vacuole content, secondary hemi-cellulosic activities are needed in addition to the pectolytic activities required. Two enzyme preparations (Scottzyme Color Pro and Color X) produced wines with higher concentrations of anthocyanins and total phenols, and greater colour intensity and visual clarity compared with untreated control wines [Watson et al. 1999a]. Five enzyme commercial preparations (Lallzyme EX-Lallemand, Rapidase EX-Gist-brocades, Vinozyme G-Cellulo, included Scottzyme Color Pro and Color X) using both a low and a high level of addition as recommended by the suppliers [Watson et al. 1999a]. All five of the commercial enzyme preparations produced wines with greater total phenolic content than untreated controls. Wines produced by enzyme treatment were higher in polymeric anthocyanins, polymeric phenols and catechin than control wines, but not in monomeric anthocyanin content.The enzyme treated wines also had increased aroma and flavor intensity, and enhanced bitterness and astringency characteristics. Red grape mashes of Vranec were treated with different pectolytic enzymepreparations. These treatments resulted in increases on the organoleptic (colour) characteristics. Preparations Vinozym Vintage FCE and Trenolin Rot DF showed a moreintensive extraction of red grape pigments (anthocyanins) and increased colour intensity [Mojsov et al. 2010]. In 1994 the Australian Wine Research Institute conducted a review into the performance of a range of commercial available pectic enzyme preparations with

respect to effect on red must and wine colour [Leske 1996]. This investigation sought to assess the validity of the hypotheses that the use of pectic enzymes results in:

- greater colour extraction during red wine fermentation
- faster colour extraction during maceration and fermentation of red grapes
- greater colour extraction from red wines at pressing and
- improved wine clarification.

The results of the enzyme-treated musts showed no significant increase in any of the measured parameters at any stage of processing when compared to that of the control samples. In stark contrast, totally different results were obtained in a study on the effect of enzymes (Vinozym G and Lafase H.E.) during vinification on colour and sensory properties of port wines [Bakker et al. 1999]. Results showed that both enzyme preparations enhanced colour extraction during vinification, although Vinozym G was more effective than Lafase H.E. Sensory analysis after nine months maturation showed that Vinozym G treatment produced wines with significantly higher colour, aroma and flavor intensity scores than the control.

3. Conclusions:

The uses of enzymes in winemaking have been proven to be highly beneficial in various aspects, and it has caused great advances in the quality of wine. However, the application of enzymes is still in its infancy. An understanding of the interactions between enzymes is needed to in order to explore the diverse advantages this technology holds. Commercial enzyme preparations are frequently used to supplement the endogenous enzyme activity. The production process of these types of preparations makes it impossible to obtain a pure enzyme product. The result is a mixture or cocktail of enzymes, which include a variety of different activities, such as glucosidases, glucanases, pectinases and proteases. Over the last two decades commercial enzyme preparations have gained enormous popularity in the wine industry. They used in winemaking give the winemaker many advantages such as:

- speeding up settling and clarification processes,
- increased juice yield,
- improved diffusion of phenolic compounds and aroma precursors,

- improved colour stability,
- softening of the wines structure,
- increased aromatic component content
- improved wine filterability

They are effective, specific and convenient to use, and it can be expected that the search for enzymes with improved characteristics will continue.

The exploration of enzyme potential will undoubtedly help the wine industry meet the technical and consumer challenges of the 21st century.

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