ENZYMES AND WINE – THE ENHANCED QUALITY AND YIELD

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(REVIEW PAPER)
UDC 621.565

Enzymes are a natural and fundamental element of the winemaking process. These enzymes originate from the grape, yeasts and other microbes associated with vineyards and wine cellars. Grape enzymes are however 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 EU is regulated by the Office International de la Vigne et du Vin (OIV). Nowadays, they are also a commercial product found in many wineries. The most widely used enzymes available for commercial use are: pectinases, hemicellulases, glycosidases and glucanases. From the pre-fermentation stage, through fermentation, post-fermentation and aging, enzymes catalyze various biotransformation reactions. In the past years, enzymes have been increasingly used for enhancing the quality of wines. They have the potential to make more extracted and more aromatic wines and to accelerate the winemaking process. This review summarizes the most important types of commercial enzymes applied to winemaking and their effects on the process technology and the quality of the final product.

Keywords: enzymes, wine, application, processing benefits, enhanced quality.

Introduction

Enzymes were discovered in the second half of the nineteenth century, and since then they have been extensively used in several industrial processes. Enzymes are generally globular proteins and like other proteins consist of long linear chains of amino acids that fold to produce a three-dimensional product. Each unique amino acid sequence produces a specific structure, which has unique properties. Enzymes are extremely efficient and highly specific biocatalysts.

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 and will naturally influence the winemaking process (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 catalyze, 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 at a competitive cost.

The most widely used enzymes available for commercial use, oenologically, are pectinases, hemicellulases, glycosidases and glucanases, the singularly most important being pectinases. Out of the four enzyme groups mentioned above, the first three are produced from Aspergillus niger, a fungus species with GRAS (Generally Regarded As Safe) status and accepted by the Office International de la Vigne et du Vin (OIV), while glucanases emanate from Trichoderma harzianium [1]. However, by isolating microbial strains that produce the desired enzyme and optimizing the conditions for growth, commercial quantities can be obtained. This technique, well known for more than 3,000 years, is called fermentation. Yeasts are not good producers of extracellular enzymes and are rarely used for this purpose. To produce the enzymes used in winemaking, selected strains are cultivated in fermentors under aerobic conditions. A well-defined composition of the growth medium induces the 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 majority of commercial enzymes fall into two categories: those that aid in extraction and those that increase volatile aromatics. The first are enzymes known as pectinases. Used predominantly on red varieties, pectinase functions by breaking down the cell walls of red grape skins, thereby extracting anthocyanins (the color components in red grapes) and tannin [2].

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The manuscript received: February, 18, 2015.
Paper accepted: March, 30, 2015.

Table 1. Enzymes derived from grapes and wine associated microbes involved in winemaking

Enzyme	Function
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	Degrade 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	Degrade phospholipids in cell membranes.
Esterases	Involved in ester formation.
Proteases	Aspartic proteases occur early in fungal infection, determine the rate and extent of rotting caused by pectinases; soluble; thermo stable.
Yeast (*Saccharomyces cerevisiae*)	Some yeast produce *β*-glucosidases which are not repressed by glucose.
β-Glucosidases	
β-Glucanases	Extra cellular, cell wall bound and intracellular, glucanases; accelerate the autolysis process and release mannoproteins.
Proteases	Acidic endoprotease A accelerates the 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	Degrade lipids.

This then helps to improve the overall color intensity, as well as the color stability of a wine by allowing the anthocyanins to bind with the tannin, as well as its structure and improved phenolic extraction of red wines [3-5]. An additional benefit of pectinase treatment is that particles settle more quickly. The action of pectinase on negatively charged pectin molecules exposes positively charged grape solids, leading to attraction and increased flocculation. In the second category of enzymes are those that enhance or release aromas, which generally include glycosidases. Glycosidases work by releasing aromas that have bound to sugars to form odorless glycosides. For example, β-glucosidase can convert odorless bound terpenes already present in a wine into aromatic free terpenes

by cleaving bound glucose. In other words, these enzymes work to maximize the aromatic potential of a wine.

Processing benefits result in shorter time of maceration, settling, and filtration [6,7]. Commercial enzymes for wine, except for bringing benefits of improving profits and cutting costs, are also eco-friendly. Enzymes enable wineries to maximize the efficiency of the extraction equipment and techniques resulting in a reduction in energy consumption. Commercial enzymes demonstrate unique benefits when used at the extraction phase during the production of red, white, and rose short-maturation wines. Through combining the increased yield and quality with cost savings, enzymes bring innovative improvements to wineries.

Enzymes

Enzymes are high molecular weight proteins produced by living organisms to catalyze the biochemical reactions that constitute metabolisms in living systems. They accelerate the rate of the chemical reaction. The reaction happens with lower activation energy which is reached by forming an intermediate enzyme- substrate complex. The enzyme can break down particular compounds. The molecule that the enzyme acts on is known as its substrate, which is converted into a product or products. In the reaction itself the enzymes are not used up, they do not become a part of the final product of the reaction, but only change the chemical bonds of other compounds. After the reaction is complete, the enzyme is released again, ready to start another reaction.

They therefore consist of one or more polypeptide chains and display properties that are typical of proteins. Some enzymes require small non-protein molecules, known as cofactors, in order to function as catalysts. The essential characteristic of enzymes is a catalytic function. Consequently, the original attempt to classify enzymes was done according to the function. The International Commission on Enzymes (EC) was established in 1956 by the International Union of Biochemistry (IUB), in consultation with the International Union of Pure and Applied Chemistry (IUPAC), to put some order to the hundreds of enzymes that had been discovered by that point, and establish a standardized terminology that could be used to systematically name newly discovered enzymes. The EC classification system is divided into six categories of basic function:

- EC1 Oxidoreductases: catalyze oxidation/reduction reactions.
- EC2 Transferases: transfer a functional group.
- EC3 Hydrolases: catalyze the hydrolysis of various bonds.
- EC4 Lyases: cleave various bonds by means other than hydrolysis and oxidation.
- EC5 Isomerases: catalyze isomerization changes within a single molecule.
- EC6 Ligases: join two molecules with covalent bonds.

Each enzyme is described by a sequence of four numbers preceded by “EC”. The first number broadly classifies the enzyme based on its mechanism.

Generally they are active at mild temperatures. Above certain temperature the enzyme is denatured. Enzymes have a characteristic pH at which their activity is maximal. The extreme pH values influence electrostatic interactions within the enzyme leading to the inactivation of the enzyme. Other important factors that influence the effect of enzymatic processes are the concentration of the enzyme, time of treatment, additives like surfactants and chelators and a mechanical stress. Due to their efficiency, a specific action, mild conditions under which they work and their high biodegradability, enzymes are very well suited for a wide range of industrial applications.

Commercial sources of enzymes are obtained from three primary sources, i.e., animal tissue, plants and microbes. These naturally occurring enzymes are quite often not readily available in sufficient quantities for food applications or industrial use. However, by isolating microbial strains that produce the desired enzyme and optimizing the conditions for growth, commercial quantities can be obtained. This technique, well known for more than 3,000 years, is called fermentation. Several methods, such as submerged fermentation (SmF), solid-state fermentation (SSF) and whole cell immobilization have been successfully used for the enzyme production from various microorganisms [8,9].

Agro-industrial residues such as wheat bran, rice bran, sugarcane bagasse, corncobs, and apple pomace are generally considered to be the best substrates for processes [10-12]. For practical applications, immobilization of microorganisms on solid materials offers several advantages including a repeated use of the enzyme, the ease of the product separation and the improvement of the enzyme stability [9].

Specific enzymes

Today, the enzymes are commonly used in many industrial applications and the demand for more stable, highly active and specific enzymes is growing rapidly. Enzymes are a natural and fundamental element of the winemaking process. Nowadays, they are also a commercial product found in many wineries, another utility in a winemaker`s toolkit. They have the potential to make more extracted and more aromatic wines and to accelerate the winemaking process. Today, these enzymes account for approximately 20% of the world enzyme market [13].

Pectinase (polygalacturonase) breaks down pectin by the random hydrolysis of (1-4)-α-D-galactosiduronic linkages in pectate and other galacturonans. Of most polysaccharides, pectin is not a single chemical. The term pectins describes a broad family of soluble heterogenous polysaccharide. They form part of the structure of primary cell walls. Pectins are also found between the cells, in the middle lamella where they help to bind the cells together. Used predominantly on red varieties, pec-

tinase functions by breaking down the cell walls of red grape skins, thereby extracting anthocyanins (red color in red grapes) and tannin. This then helps to improve the overall color intensity, as well as the color stability of a wine, by allowing the anthocyanins to bind with tannin, as well as its structure. An additional benefit of pectinase treatment is that particles settle more quickly.

Cellulase breaks down cellulose to either simple glucose or into glucose-disaccharide. Cellulose is a polysaccharide of glucose. Hemicellulase breaks down hemicellulose. Hemicelluloses are polysaccharides composed of a broad range of simple sugar monomers including glucose, xylose, arabinose, galactose, mannose and rhamnose. Hemicellulose chains (500 to 3,000 sugar units) are much shorter than cellulose chains (7,000 to 15,000 sugar units).

Glucanase breaks down glucans. Glucans are polysaccharides of glucose. Dextran, glycogen and starch are all examples of α-glucans while cellulose is an example of a β-glucan.

Glycosidases are hydrolases which attack glycosidic bonds in carbohydrates, glycoproteins and glycolipids. The glycosidases are not highly specific. Usually they distinguish only the type of bond. Glycosidases allow a winemaker to produce the wine with more intense aromatics in a shorter amount of time. The release of bound aromatics otherwise occurs naturally in the wine by acid hydrolysis but at a much slower rate. It takes more time to release aromatics without adding enzymes. β-Glucosidase cleaves the β-D-glucosides.

Ramnosidase, apiosidase and arabinofuranosidase are used to release terpenes.

Wine production

Wine is a fermented grape juice. Wine can be made from grapes, fruits, berries etc. Most wines are made from grapes. And no matter what the wine is made from, there must be fermentation i.e. sugar transforming into alcohol. Winemaking is an old technology and has an ancient history. It can be divided into four basic phases. The first phase consists of finding and harvesting high quality grapes in an optimum condition. The second phase consists of the extraction of juice from grapes and subsequent fermentation of the juice into wine by yeast. During the third phase, the new wine is clarified and stabilized. New wine starts to clarify towards the end of the fermentation period. Some tartrates precipitate out during primary fermentation, and the wine becomes more stable. Winemakers clarify wine by fining, racking and filtration. In the fourth phase of winemaking, the winemaker ages the wine. Most high quality wines are aged in bulk and then for an additional time in the bottle.

The enzyme technology plays a central role in these processes. Many of the biochemical reactions involved in the wine production are enzyme-catalyzed. They begin during the ripening and harvesting of grapes, and continue through alcoholic and malolactic fermentation, clarification, and ageing. The addition of exogenous glucanases and related polysaccharidases are known to improve wine qualities, but also their overall production efficiency [14]. These grape and microbial pectinases are classified according to their mode of attack on the pectin molecule. These enzymes de-esterify (pectinesterases) or depolymerize (polygalacturonases, polymethylgalacturonases, pectin and pectate lyases) specific pectic substrates. The enzymes used in wine biotechnology and their function are shown in Table 2.

High quality red wine grapes have colorless juice. All red color is in the grape skins, and winemakers must leave the juice in contact with the skins for a considerable time to extract the color. Red wine is made by crushing the grapes and then fermenting the juice, the pulp, the skins and the seeds together for several days. Near the end of sugar fermentation, a wine press is used to separate the liquid from the solid materials. Pink or rose wine can be produced by removing the non-juice pumice from the must during fermentation. White wines can be made from white grapes or from pigmented grapes by removing skins, pulp and seeds before juice fermentation. First, the grapes are crushed and pressed immediately to separate the juice from the solids. After pressing, the skins, stems and seeds are discarded, and the juice is cooled to a low temperature. Then the cold juice is allowed to settle for several hours, and the clear juice is decanted off the residue before it is fermented. White wine is made by fermenting clarified juice. These are the fundamental differences between making quality red wine and white wine. The best wine grape is the European Vitis vinifera. It is considered to be optimal because it has the right balance of sugar and acid to create a good fermented wine without the addition of sugar or water.

The wine production is a biotechnological process in which both yeast cells and enzymes play a key role. In the last four decades, attempts have been made to improve the yeast strains used for fermentation of grape juice, as well as to use exogenous microbial enzymes during wine making [14]. The main benefits of using these three enzymes during wine making include:

- better skin maceration and improved color extraction;
- easy must clarification and filtration;
- improved wine quality and stability.

In the early 1980s, it was suggested that Trichoderma β-glucanase could be successfully used for wine making from grapes infected with Botrytis cinerea [15,16]. This fungus generally attacks nearly ripe grapes under conditions of certain temperatures and humidity, and produces a high molecular mass soluble β-(1,3) glucan with short side chains linked through β-(1,6) glycosidic bonds, which causes severe problems during wine filtration. A β-glucanase from Trichoderma harizanum, which specifically hydrolyses this β-glucan was identified and patented [14].

Table 2. Enzymes used in wine biotechnology and their function

Enzyme	Function	Application	Reference
Pectin metylesterase	De-esterification and gelling of pectins.	Split the methyl ester groups of polygalacturonic acids proceeding in a linear fashion along the chain, and thereby freeing methanol and converting pectin into pectate.	[14,18]
Polygalacturonases	Therefore polygalacturonases function synergistically with pectin methylesterases.	Break down the glucosidic links that connect the molecules of galacturonic acid one to another, with the absorption of one molecule of water.	[19-21]
Pectin lyases	Depolymerise highly esterified pectins.	Pectin lyase is specific for highly esterified pectin, whereas pectates and low-methoxyl pectins are the best substrates for endopectate lyase. Minimize the methanol released from methylated polygalacturonic acid during the wine production.	
Macerating enzymes (cellulases, hemicellulases and pectinases)	Hydrolysis of plant cell wall polysaccharides	Improve skin maceration and color extraction of grapes; quality, stability, filtration and clarification of wines	[14,22]
***β*-Glucosidase**	Modification of aromatic residues	Improve the aroma of wines	[23]

Significant and reproducible improvements in grape pressability, settling rate and total juice yield were achieved by using the combination of macerating enzymes compared to that using pectinase alone [17]. Galante et al. assessed the performance of Cytolase 219 (commercial enzyme preparation) in wine making. They reported the 10-35% increase in the extraction of the first wine must, the 70-180% increase in the must filtration rate, a significant improvement in the wine stability, the 50-120 min decrease in pressing time, the 30-70% decrease in must viscosity and 20-40% energy saving during cooling of fermenters. Currently, a number of commercial enzyme preparations are available specifically for improving the maceration of grapes, the color extraction, wine filtration and wine quality.

Conclusion

The use of enzymes in winemaking has been proven to be highly beneficial in various aspects, and it has made great advances in the quality of wine. The best wines are produced when the desired enzymatic activities are optimally reinforced and the negative effects restricted to the minimal level. However, the application of enzymes is still in its infancy. An understanding of the interactions between enzymes is needed in order to explore the diverse advantages that this technology holds. Over the last two decades, commercial enzyme preparations have gained the enormous popularity in the wine industry to supplement the endogenous enzyme derived from grapes and microbes present in the must and wine. 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. When used in winemaking they give the winemaker many advantages such as:

- speeding up the settling and clarification processes,
- the increased juice yield,
- the improved diffusion of phenolic compounds and aroma precursors,
- the improved color stability,
- softening of the wines structure,

- the increased aromatic component content
- the 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 the enzyme potential will undoubtedly help the wine industry meet technical and consumer challenges of the 21st century.

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Izvod

ENZIMI I VINO – BOLJI KVALITET I PRINOS

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Enzimi su prirodan i važan element procesa proizvodnje vina. Enzimi potiču iz grožđa, kvasca i drugih mikroba sa vinograda i iz vinskih podruma. Enzimi grožđa medutim nisu aktivni u prisustvu SO_2 i pH uslovima proizvodnje vina. Gljivične pektinaze su otporne na ove uslove. Metod za dobijanje enzima koji se koriste u proizvodnji vina u EU regulisan je od strane Međunarodne organizacije za vinovu lozu i vino (OIV). Danas su oni i komercijalni proizvod koji se može naći u mnogim vinarijama. Najčešće korišćeni enzimi dostupni za komercijalnu upotrebu su: pektinaze, hemicelulaze, glikozidaze i glukanaze. Od same faze pre fermentacije, preko fermentacije, do faze posle fermentacije i starenja, enzimi katalizuju različite biotransformacione reakcije.Enzimi se poslednjih godina sve više koriste za poboljšanje kvaliteta vina. Pomoću njih dobijamo aromatičnija vina a takođe ubrzavaju i proces proizvodnje vina. U ovom radu predstavljene su najvažnije vrste komercijalnih enzima koji se primenjuju u proizvodnji vina, kao i njihovi efekti na tehnologiju procesa i kvalitet finalnog proizvoda.

Ključne reči: enzimi, vino, primena, prednosti obrade, bolji kvalitet.