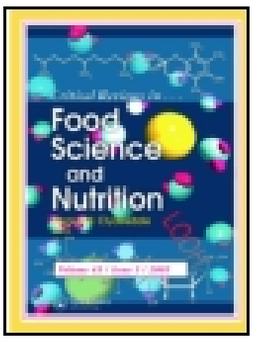

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REVIEW

The production and application of enzymes related to the quality of fruit wine

Hua Yang[a,b,c,d,e], Guolin Cai[a,b,c,d], Jian Lu[a,b,c,d], and Encarna Gómez Plaza[e]

[a]The Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, China; [b]National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, Wuxi, P. R. China; [c]Jiangsu Provincial Research Center for Bioactive Product Processing Technology, Wuxi, P. R. China; [d]School of Biotechnology, Jiangnan University, Wuxi, P. R. China; [e]School of Food Science and Technology, University of Murcia, Murcia, Spain

ABSTRACT

Grape wine is the most widely consumed fruit wine in the world. With the increasing diversification of consumers' needs, the variety of fruit wines in the market is becoming more and more abundant. Whether it is the production of grape wine or other fruit wines these processes are inseparable from the participation of enzymes. The quality of these wines is closely related to the application of enzymes in the winemaking process. Enzymes are involved in pretreatment, fermentation, filtration, flavoring, aging and storage of fruit wines. This review systematically illustrated the role of pectinase, β-glucanase, β-glucosidase, glucose oxidase, lysozyme, protease, tannase and urease in the production of wines and their current production status and also provided a theoretical basis for better application of various enzymes in the production of various fruit wines. This knowledge could be great significance to improve the quality of fruit wines and reduce the production costs in the fruit wine industry.

KEYWORDS

Application; enzymes; quality; fruit wine; production status

Introduction

Fermentation technology is widely used in the food industry. Its advantage is that it improves the physical and chemical properties, sensory quality, nutrition and flavor of foods and create new products. Fermentation technology has been mastered by humans and used in the production of alcoholic beverages for a long time. Fermented fruit wines are popular throughout the world, the most striking of which is grape wine. Although the global consumption of alcoholic beverages was declining in recent years, wine still had a relatively stable production and consumption, and the grape wine production of the world was around 260 million hectoliters (Kbilashvili 2018). In order to meet the diversified needs of consumers, other types of fruit wines with different flavors and styles have been developed. A large variety of fruits with sufficient sugar content can be used to produce fruit wine. The fruit wines that have been reported and successfully produced in the literature include litchi wine, cider, plum wine, pineapple wine, banana wine, blueberry wine, etc (Kbilashvili 2018; Chen and Liu 2016; Vedantam, Zitnick, and Parikh 2015; Ljekočević et al. 2019; Roda et al. 2017; Lyumugabe et al. 2018; Mendes-Ferreira et al. 2019). Whether it was grape wine or other types of fruit wine, enzymes play an important role in their production. The enzyme is not only derived from the fruit used for winemaking itself, but also from yeast and other microorganisms in the fermentation process. Some of these enzymes had a beneficial effect and some had an adverse effect on the quality of the wine. Naturally occurring enzymes tended to lose activity under the strict fermentation conditions of the wine, so it was necessary to artificially add enzymes during the fermentation of the wine. In addition to the general catalytic activity, the added enzymes need at least the following properties, including (a) acid resistance, most fruits contain a certain amount of acid and promote a low pH medium, so the fermentation process of fruit wine is often in the acidic environment. The added enzyme must be catalytically active under acidic conditions to meet the needs of fruit wine production. (b) SO_2 resistance, SO_2 is the most common antiseptic to be added during the fermentation of fruit wine to avoid contamination of other microorganisms except yeast. The added enzyme needs to be resistant to a certain concentration of SO_2. (c) active at low temperatures, generally, the lower the temperature of the environment during the production of fruit wine, the better the quality of the wine. Therefore, producers are more inclined to apply cold active enzymes to product wines (Sahay 2019).

Nowadays, pectinase, β-glucanase, β-glucosidase, glucose oxidase, lysozyme, protease, tannase and urease (etc.) were widely added to the production process of wine. Pectinase, β-glucanase, β-glucosidase, glucose oxidase, lysozyme, protease, tannase and urease (etc.) have great application potential in the production process of fruit wine. The enzymes

CONTACT Jian Lu jlu@jiangnan.edu.cn National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122, Jiangsu, P. R. China; Encarna Gómez Plaza encarnag@um.es. School of Food Science and Technology, University of Murcia, Campus de Espinardo, Murcia, 30100, Spain.

catalyze various biotransformation reactions during pretreatment of fruit, pre-fermentation, fermentation, post-fermentation, aging and storage of fruit wine. They increased the juice yield of the fruit, improved the wine color, promoted the release and formation of the aroma, ensured the safe drinking, accelerated the filtration and clarification speed of the wine, and shortened the fermentation process. All these positive contributions will improve the quality of fruit wine and may reduce production costs.

Commercial enzymes added during the fermentation of fruit wine are derived from animal tissues, plants and microorganisms. The extraction of enzymes from animal tissues and plants had high production costs and low yields. Therefore, researchers turned their eyes to microorganisms, isolated the enzyme-producing strains from nature and optimized the culture conditions to obtain a considerable amount of enzyme (Mojsov et al. 2015). Commercial enzymes obtained from of *Aspergillus niger* and *Trichoderma harzianum* have been widely used in the elaboration process of fruit wines (Fia, Canuti, and Rosi 2014). At present, the most advanced means of commercial enzyme production is the use of genetic engineering techniques to genetically modify enzyme-producing strains, which not only increased enzyme production but also reduced undesirable side activities (Gonzalez et al. 2016). However, the legality and security of GMO products is still a controversial issue, the law of European Union is in an uncertain state in dealing with genetically modified commodities, whereas the United States of America did not develop specific GMO legislation (Anyshchenko 2019; Custers 2017; Twardowski and Małyska 2012). This article systematically summarized the effects of enzymes from different sources on the quality of fruit wine, the new technology of enzyme production, and the new trends in the application of enzymes in fruit wine production, provided ideas for better application of enzymes in fruit wine production. It was of great significance to reduce the production cost of fruit wine and improve the quality of fruit wine.

Pectinase

Pectin is a generic term for a class of soluble polysaccharides that are part of the plant cell wall and are also present in the intermediate layer between cells. Homogalacturonan is the main structure of pectin, accounting for about 80% of the polymer. It is a long-chain of galacturonic acid residues. The galacturonic acid residue may be acetylated on the hydroxyl group or methyl esterified on the carboxyl group (Rollero et al. 2018). The high-viscosity pectin limits the fruit juice yield and when extracted (after the fruit is crushed), hinders the clarification and filtration of the wine. Pectinase is a general term for enzymes that hydrolyze pectin, including protopectinases, which dissolves protopectin and forms soluble pectin; pectin methyl esterases and pectin acetyl esterases, which eliminate the methoxy and acetyl residues of pectin to produce polygalacturonic acid; and the polygalacturonases, which hydrolyze the α-(1-4) glycosidic bond between the galacturonic acid residues. Also, pectin lyases and pectate lyases transfer α-(1-4) glycosidic bonds between galacturonic acid residues. The use of pectinase in the red grape winemaking process destroy the cell wall of the grape skin and release tannins and anthocyanins (Del Rocío Castro-López et al. 2016). This process improves wine color and the combination of tannins and anthocyanins, that at the same time improves the color stability of red wines. Also, the use of pectinases promotes the release of flavor substances (Mojsov et al. 2015).

The application of pectinase

Traditional fruit wine processing methods generally start by processing fruit into juice, crushing the fruit, pressing out the turbid raw juice, and filtering out the residue. This process is usually achieved by using mechanical presses. However, due to the presence of pectin, a high rate of juice yield is not always achieved. The use of enzymes maximizes extraction efficiency and reduces energy consumption in terms of equipment and technology. Egwim, Ogudoro, and Folashade (2013) examined the effects of pectinase addition on the juice yield and sensory evaluation of banana and paw-paw juices and wines. They found that when pectinase was added to bananas and paw-paw, respectively, the juice yields were 63.4% and 78.7%, while bananas and paw-paw without enzyme added, the juice yielded were 38% and 43%, respectively. The concentration at which the enzyme exerts maximum performance was 6 mg/mL, which resulted in a paw-paw juice density of 0.940, and resulted in a juice density of 1.003 for banana juice. The reducing sugars of banana and paw-paw juice after pectinase treatment were 1098.2 mg/100 g and 968.8 mg/100 g, the titratable acidity of banana and paw-paw juice were 2.0% and 0.8%, respectively. The pectinase-treated banana and paw-paw juice were used to ferment the wine, the pH of the wines was not significantly affected by the concentration of the enzyme used in the juice extraction ($p < 0.05$). And after two weeks of aging, the sensory analysis of banana wine was improved (Egwim, Ogudoro, and Folashade 2013). The addition of pectinase also reduces the viscosity of the juice, which is beneficial to the filtration of the juice and increases the clarity of the juice. Byarugaba-Bazirake, van Rensburg, and Kyamuhangire (2013) applied four commercial enzymes with pectinase activity, OE-Lallzyme, Rapidase TF, Rapidase CB and Rapidase X-press to the clarification of three types of banana wines. The results showed that the clarity of the banana wine treated with the three enzymes was improved. The OE-Lallzyme had the best clarification effect on all banana wines (Byarugaba-Bazirake, van Rensburg, and Kyamuhangire 2013). Borazan et al. studied the effect of pectinase addition on the phenolic components of Okuzgozu red wine during the winemaking process. The results showed that the wine treated by adding pectinase had lower monomeric flavan-3-ol content than other wines. The content of anthocyanins does not increase with the addition of the enzyme (Borazan and Bozan 2013).

Researchers used different pectinase preparations to produce white wine. When the enzyme preparations Trenolin Mash DF (Geisenheim, Germany) and Rohavin LX (Darmstadt,

Germany) were used, the filtration time was reduced by a factor of two. By using the enzyme preparation Vinozym Process (Bagsvaerd, Denmark), the desliming speed was three times faster, and Trenolin Mash and Rohavin LX were used, the desliming speed was twice as fast as the control sample. These pectinase treatments increased the clarity, filterability, and settling solids content of the white wine. The visual and overall quality of the wine were improved (Mojsov et al. 2011).

Production practice requires that the pectinase applied to the fermentation of fruit wine has activity at low temperatures. In the fermentation process of most fruit wines, the lowest the fermentation temperature is, the best for volatile aroma components are retained, thus improving the flavor characteristics of the wine (Rollero et al. 2015; Martín et al. 2019; María and Morata De Ambrosini 2014). However, if the temperature is low during the production of red grape wine, it is not conducive to the release of pigments in the grape skin, resulting in insufficient color of the wine. The application of cold active pectinases can just solve this contradiction (Merín et al. 2011). It is worth noting that when using bentonite to clarify fruit wine, it will adsorb and eliminate the proteins. Pectinase is also a protein, so in the case of using both bentonite and pectinase, it is necessary to use pectinase first, in order to prevent it from being adsorbed by bentonite and losing its efficacy. Tannins in fruit wine also react with proteins. Therefore, in the production of fruit wine with higher tannin content, sometimes it is necessary to treat the wine with gelatin to remove excess tannin, and then add pectinase and other kinds of enzymes (Sahay 2019).

In recent years, research on the application of non-*Saccharomyces cerevisiae* in the production of wine and fruit wine has gradually emerged. Non-*Saccharomyces cerevisiae* often had fermentation characteristics not found in *Saccharomyces cerevisiae*, such as the production of certain enzymes. Studies have found that a small number of non-*Saccharomyces cerevisiae* have strong pectinase activity (Merín et al. 2011; Belda et al. 2016).

Rollero et al. (2018) added the strain *K. marxianus,* which possesses pectinase activity, during fermentation. Since pectinase also helped to release the aroma and its precursor component of the fruit, thereby increased the aroma of the wine, the results showed that the content of some volatile aroma components such as phenylethanol and phenylethyl acetate in the wine of added strain *K. marxianus* was increased compared with control (Rollero et al. 2018). However, it is risky to directly add non-*Saccharomyces cerevisiae* as an enzyme source during the production of fruit wine since this process may introduce yeast metabolites other than the expected enzyme. Therefore, it is not the best choice to add a non-*Saccharomyces cerevisiae* as an enzyme source directly in the fermentation process of fruit wine.

The production of pectinase

Microbially produced pectinase has been largely used in industrial production, and microorganisms has used to produce many industrially interesting enzymes in relatively inexpensive and environmentally friendly processes. Advances in biotechnology such as molecular biology and microbial genetics have led to significant improvements in enzyme production technology, and the development of new strains and microbial enzymes has also benefited from the development of these technologies. Discovery of gene sequences that coded the enzymes, protein engineering, and molecular biology tools resulted in defined microbial strains that over-produced the enzymes for cost-effective technologies. Conditions such as alcohol levels above 17% (v/v) and SO_2 concentrations over 500 mg/L will inhibit pectinases (Van Rensburg and Pretorius 2000), however, the microbial pectinases, especially the pectinase derived from fungi are resistant to the fermentation conditions of the wine, and could be used in the fermentation process of the wine to improve the product quality (Mojsov et al. 2015). So far, commercial enzymes all produced by fungi, mainly *Aspergillus*.

The expression of pectinase gene in yeast, especially in *Pichia pastoris* has attracted the attention of researchers (Sieiro et al. 2012). Byaruagaba-Bazirake, Rensburg, and Kyamuhangire (2013) research found that the main problematic polysaccharide in kayinja banana fruit was pectin, so, they treated the pulp with pectinase secreted by a genetically modified yeast. The yield of banana wine was 60.3 ± 0.28% (v/w) with 35% increase compared to the wine obtained with the control yeast and the transgenic yeast enzyme-treated banana wine also had better clarity (Byaruagaba-Bazirake, Rensburg, and Kyamuhangire 2013). In recent years, some researchers have found that a few non-*Saccharomyces cerevisiae* have strong pectinase activity (Merín et al. 2011; Belda et al. 2016). These non-*Saccharomyces cerevisiae* are also potential sources of pectinase production.

Singh et al. (2012) isolated six strains of fungi with cold pectinolytic activity in the Jammu and Kashmir areas, three enzymes with pectinolytic activity were isolated from six cold-active fungi, namely pectin esterase, exo-galacturanase and endo-galacaturanase. The highest specific activities of the three enzymes were 47.2, 31.4 and 64.8 U/mg, respectively. At 12 °C and 28 °C, pH 3.5 (closed to the winemaking environment), the percentage activities of the three enzymes were 40%, 60% and 39% (12 °C); 45%, 60% and 42% (28 °C), respectively. The pectinolytic enzymes produced by these cold active fungi had great commercial application prospects, and could be tried to apply them to the processing of fruit wine (Singh et al. 2012). Pectinase produced by *Aspergillus niger* has been widely used in industrial production, based on the enzyme activity pattern required for pectin in industrial applications, the research goal was to overproduce enzymes based on specific microbial hosts. According to the 97 pectinase genes that have been annotated and the 60 new genes identified from the complete genome of *Aspergillus niger* and the biological techniques such as homologous recombination, pure pectinase without side activities has been produced (Khan, Nakkeeran, and Umesh-Kumar 2013; Pel et al. 2007). Although the enzyme production efficiency of microorganisms has been greatly improved by genetic engineering techniques, current reports indicate that only two types of genetically modified microorganisms were allowed to be used

for winemaking in a few countries and they are not pectinases. One strain approved by the appropriate food safety agency (US and Canada) is malolactic wine yeast ML01. The GMO carried the *Schizosaccharomyces* pombe malate permease gene (mae1) and the Oenococcus oeni malolactic gene (mleA). The other one strain ECMo01 constitutively expresses the urease gene to prevent the formation of urethane (Gonzalez et al. 2016). However, although the enzyme produced by genetically modified micro-organisms is absolutely safe under the legal framework, it cannot be used in the food industry (Pariza and Johnson 2001). Some studies have publicly provided very effective methods for assessing the safety of food ingredients from genetically modified organisms, which provide rigorous guidelines and frameworks for the safety assessment of microbial enzymes in foods (E. EFSA Panel on Food Contact Materials, Aids, Silano et al. 2018; E. EFSA Panel on Food Contact Materials, Processing Aids, Silano et al. 2019; Sewalt et al. 2016).

β-glucanase

Glucans are polysaccharides derived from glucose. Among them, β-glucan is an important polysaccharide in the production process of fruit wine. It comes from the fruits and from the cell wall of yeast. The main component of yeasts cell wall is β-glucan, which accounts for 60% of the cell wall composition. Secondly, in the process of fruit wine production, it is inevitable that some lactic acid bacteria will be involved, and some of them, especially *Pediococcus spp*., produce viscous capsular or extracellular polysaccharides. These polysaccharides prevent the filtration of the wine and cannot be removed from the wine by the flocculant (Dimopoulou, Lonvaud-Funel, and Dols-Lafargue 2017). Glucanase breaks down glucans, the exo-β-1,3 glucanase cleaves the β-glucan chain by sequentially cleavage of the glucose residue from the non-reducing end and releasing glucose as the sole hydrolysate. Endo-β-1,3-glucanase catalyzes the intramolecular hydrolysis of β-glucan while releasing oligosaccharides (Claus and Mojsov 2018). The application of a commercial product having β-glucanase activity to the fermentation of fruit wine has a positive effect on reducing the viscosity of must and wine and increasing the filtration efficiency of the wine. In addition, glucose, oligosaccharides and mannoproteins from the yeast cell wall are released into the wine to improve the flavor of the wine.

The application of β-glucanase

Blättel et al. (2011) studied the ability of extracellular β-1,3-glucanase from *Delftia tsuruhatensis* strain MV01 to hydrolyze β-1,3-glucan from pediococcus and yeast cell walls, found that the β-1,3-glucanase showed the best activity at 50 °C and pH 4.0. At 50 °C, there was about 50% viscosity reduction effect, while at 20 °C, the enzyme reduced the initial viscosity by 35%. It also had high enzyme activity under high ethanol, sulfite and phenol concentrations and low pH. The enzyme was expected to be applied to wine production to prevent the formation of mucus and the growth of harmful yeast (Blättel et al. 2011). For wine fermentation, the most important glucan was β-1,3-1,6 glucan (Sahay 2019). The review of Mojsov et al. (2015) mentioned that *Botrytis cinerea* was a fungus that infected almost all mature grapes under specific temperature and humidity conditions and produced high molecular soluble β-(1,3) glucan linked by β-(1,6) glycosidic bonds, which caused serious filtration problems during wine production. The β-glucanase from *Trichoderma harizanum* specifically hydrolyzed the β-glucan produced by grape infection with *Botrytis cinereal* (Mojsov et al. 2015). Schwentke et al. (2014) purified the multifunctional exo-β-1,3-glucanase (WaExg2) from the culture supernatant of yeast *Wickerhamomyces anomalus* AS1. WaExg2 effectively decomposed various glycosylated polyphenols and laminarin, had the ability to increase the bioactive polyphenol content and hydrolyze glucan. It was active in the condition of pH was 3.5-4.0, the sugar concentration was as high as 20% (w/v), the ethanol concentration was 10-15% (v/v), the sulfite concentration was 2 mM and various cations (K, Ca, Mg, Mn, Cu, Zn) were present. Therefore, the enzyme had the potential to be applied to the wine fermentation process (Schwentke et al. 2014).

In the production of sparkling wine, the aging process was crucial, the yeast death and autolysis occurred at this stage, which imparted sparkling's roundness and characteristic aroma and flavor, together with their foaming properties. Accelerating the aging process and keeping the quality of the wine unchanged has always been the pursuit of researchers (Rodriguez-Nogales, Fernández-Fernández, et al. 2012). The autolysis ability of the yeast used in the secondary fermentation had an important influence on the quality of sparkling wine. During the cleavage of yeast after death, endo- and exoglucanases hydrolyze the β-O-glycosidic linkage of the β-glucan chain, thereby releasing glucose, oligosaccharides and other cytoplasmic and wall compounds, improving the sensory sense and foaming properties of sparkling. Added β-glucanase to sparkling wine not only increased the aging characteristics of traditional sparkling wine, but also improved the antioxidant properties of sparkling wine (Rodriguez-Nogales, Fernández-Fernández, et al. 2012). Rodriguez-Nogales, Fernández-Fernández, et al. (2012) studied effects of a β-glucanase enzymatic preparation on yeast lysis during aging of traditional sparkling wines and found that β-glucanase was slightly inhibited by ethanol, the addition of enzymes has not a substantial effect on the total protein content or foam characteristics. Added β-glucanase catalyzed cell disintegration and promoted the release of yeast components into sparkling wine, effectively accelerated the aging characteristics of traditional sparkling wines (Rodriguez-Nogales, Fernández-Fernández, et al. 2012). Recently, researchers have also tried to explore the inhibitory effect of β-glucanase on wine spoilage bacteria. Enrique et al. (2010) applied a commercial β-glucanase to the anti-spoilage yeast experiment for wine, and the spoilage yeast involved in the experiment included *Cryptococcus albidus, Dekkera bruxellensis, Pichia membranifaciens, Saccharomyces cerevisiae, Zygosaccharomyces bailii,* and *Zygosaccharomyces bisporus.* These yeasts exhibited different sensitivities to β-glucanase, β-glucanase had an antibacterial effect on *D. bruxellensis* and

Z. bailii. The addition of β-glucanase did not affect the winemaking parameters of the wine. This study showed that β-glucanase had potential applications as an antibacterial agent in wine (Enrique et al. 2010).

The production of β-glucanase

β-glucanase is usually synthesized by species of *Trichoderma*. Song et al. (2017) isolated the gene CaCel of endo-β-1,4-glucanase that provided high temperature, cold and acid resistance. The CaCel gene product exhibited the best activity at pH 3.5, maintained 80% activity at 0–10 °C, and maintained a half-life of 4 hours at 70 °C. CaCel gene product efficiently digested green algae (Ulva pertusa) under acidic conditions at 50 °C. The production of wine often required acid-resistance and cold-active β-glucanase. The CaCel gene screened in this study was transferred to mold or yeast to produce a cold-active and acid-resistant β-glucanase that may have potential for fruit wine production. However, it is still controversial whether the enzymes produced by genetically modified microorganisms can be used in the food industry because of safety and legal issues (Song et al. 2017; Sewalt et al. 2016).

β-glucosidase

Although the aroma and flavor components of most wines are produced during the alcoholic fermentation process, the aroma of the wine is also determined by the volatile components of the grapes. In addition to the free volatile compounds found in berries, there are a large number of aroma and flavor precursors in the form of odorless nonvolatile glycosides, which are mostly combined with terpenic, norisoprenoids, alcohols, or phenolic compounds. Terpenes such as linalool, geraniol, nerol, citronellol, α-terpineol and linalool oxide are components that favor the aroma diversity of wine (Palmeri and Spagna 2007). β-glucosidases (1,4-β-D-glucoside glucohydrolases, EC 3.2.1.21) are mostly extracellular hydrolases, that break glycosidic bonds to release nonreducing terminal glucosyl residues from glycosides and oligosaccharides (Ketudat Cairns and Esen 2010; Baffi et al. 2012). During the fermentation process, β-glucosidase is used for the hydrolysis of glycoside precursors, especially the hydrolysis of monoterpenyl β-D-glucosides bounds with sugar molecules, to enhance the aroma characteristics and aroma diversity of the wine. However, the use of β-glucosidases may also have side effects on the quality of the wine, such as the hydrolysis of anthocyanins. Anthocyanins are the stable glycosidic form of the anthocyanidins. The use of β-glucosidases leads to the cleavage of glucosidic bonds, and the free anthocyanidins are quickly degraded into colorless compounds, resulting in poor color or even loss of color of red wine. Therefore, glycosidases for the liberation of bound aroma compounds are mainly used in white wine technology.

The application of β-glucosidase

Winemaking processes occur in the usual pH ranges of around 3.0–3.8 and in temperatures of 18–25 °C. A purified extract of extracellular β-glucosidase from *Issatchenkia terricola* was highly active in the presence of glucose (100 g/L), ethanol (18%) and metabisulfite (60 mg/L). It was active and relatively stable at pH 3.0. Immobilization of this β-glucosidase onto Eupergit C greatly improved its stability, allowed an increase of the aroma in a white Muscat wine in a 16-day experiment. The composition of the volatile aroma compounds of the control and enzyme treated wines was analyzed by GC-MS. The results showed that after enzyme treatment, the effective release of geraniol was promoted, and the content of monoterpenes and isoprenoids in wine increased significantly. The content of monoterpenes increased from 1420 to 1914 ug/L ($p < 0.05$), and the content of norisoprenoids increased from 27 to 99 ug/L ($p < 0.01$) (González-Pombo et al. 2011). Wang et al. (2013) studied the effects of three different sources of glycosidase on the aroma of Cabernet Gernischt, BG1, BG2 from *Trichosporon Asahii*, AS from *Aspergillus Niger*. The wine treated with β-glucosidase BG1 had the highest content of volatile aroma components in 19 of the totals of 23 volatile aroma components, and 8 anthocyanins were isolated from the wine, of which BG1 had a minimum effect. β-glucosidase BG1 showed the higher hydrolysis ability for delphinidin 3-glucoside. Besides delphinidin 3-glucoside, β-glucosidase BG2 also had a high hydrolysis ability for peonidin 3-glucoside and petunidin 3-glucoside, higher than other enzymes. The results showed that β-glucosidase BG1 was considered to have the highest application potential for fermentation of Cabernet Gernischt wine (Wang et al. 2013). Gaensly et al. (2015) has found that β-glucosidase from yeast increased the concentration of free resveratrol in wine without changing its composition or sensory properties (Gaensly et al. 2015).

In recent years, some researchers have used yeast with β-glucosidase activity directly into the fermentation process of wine. Vernocchi et al. (2011) used four yeast strains AS11, AS15, BV12 and BV14 with β-glucosidase activity in experiments to enhance the aroma characteristics of Sangiovese wine. The results showed that wines fermented with As 11 and As 15 had low levels of volatile acidity, accompanied by high levels of linalool and nerol, however, As 11 and As 15 caused changes in the content of certain anthocyanins. The addition of BV12 and BV14 resulted in a decrease in the residual sugar content and volatile acidity of the wine, an increase in the content of nerolidol and citronellol, and no adverse effect on the color of the wine (Vernocchi et al. 2011). Baffi et al. (2013) isolated a strain produced β-glucosidase from the grape ecosystem. The optimum pH of the crude enzyme AP-β-GL was 5.5, and the optimum temperature was 45 °C and 70 °C. AP-β-GL had a wide range of pH stability, low temperature stability and resistance to ethanol. At 10% (W/V) ethanol concentration and 15% (W/V) ethanol concentration, the enzyme activity was maintained at 94% and 71%, respectively. Glucose inhibited AP-β-gl, at sugar concentrations of 100 and 400 mM, AP-β-gl had 48% and 12% residual enzyme activity,

respectively. In this study, the terpienol evidenced an increasing of around 27 times with respect to the untreated control. Terpene alcohol was one of the main monoterpenoids, gave wine a fresh and fruity aroma (Baffi et al. 2013). In order to maintain the stability, the β-glucosidase immobilization technology can accurately manage the conversion rate, achieve rapid and controlled release of terpenoids, and retain a small amount of bound aroma as a reserve, which is released over time. Romo-Sánchez et al. (2014). immobilized β-glucosidase in the diverse supports of alginate-chitin and chitosan-chitin. The most appropriate matrix was chitosan, the immobilized β-glucosidase had the advantages of low dosage, high stability, reusability, and the good performance in wine aroma release (Romo-Sánchez et al. 2014). The β-glucosidase immobilized on silica gel retained 80% relative activity after repeated use for 20 times (Jung et al. 2012). Su et al. (2010) immobilized β-glucosidase on sodium alginate, after repeated use for 50 times, the residual activity was about 93.6% of the initial activity (Su et al. 2010).

The production of β-glucosidase

Cloning of the gene of β-glucosidase and expression in a heterologous strain was beneficial because of β-glucosidase had a low level of expression in its parental strain. Genetically engineered strains produced β-glucosidase in an optimal manner. The β-glucosidase gene directly cloned into the other expression system, and then overexpressed to obtain a large amount of β-glucosidase. Yang et al. (2015) cloned and overexpressed the thermotolerant β-glucosidase gene from bacteria, the β-glucosidase activity was enhanced and its enzymatic properties were improved (Yang et al. 2015). *Pichia pastoris* was an ideal gene expression system for the production of industrial enzymes due to its particularly high extracellular protein production capacity and was used for heterologous protein expression. *Pichia pastoris* had the ability to produce β-glucosidase and has been successfully used as a host for cloning and expressing recombinant proteins from fungi (Ramani et al. 2015; Chen et al. 2012). In the future, it is promising to overexpress the β-glucosidase gene in *Pichia pastoris* and to improve the enzymatic properties of β-glucosidase by biotechnology to produce β-glucosidase suitable for fruit wine and wine fermentation.

Glucose oxidase

The rising of temperatures around the world has led the grapes to have a higher sugar content, which in turn has led to an increase in the alcohol content of the wine. High-alcoholic wines have an impact on the health of drinkers, decreasing the quality of wines and increasing the cost of consumers in certain countries (in some countries, the tax on alcoholic beverages is related to the alcohol content) (Ozturk and Anli 2014). So, research on low alcohol wines is emerging in recent years. The addition of glucose oxidase (Gox, β-D-glucose: oxygen 1-oxidoreductase; EC 1.1.2.3.4) could be used to reduce the alcohol content of the wine by reducing the glucose content in the musts. First, Gox catalyzes β-D-glucose into D-glucono-δ-lactone and produces H_2O_2, then D-glucono-δ-lactone is converted into gluconic acid. The glucose in the must is converted to gluconic acid, and the decrease in the glucose content leads to a decrease in the alcohol content of the wine.

The application of glucose oxidase

Röcker et al. (2016) applied both Gox and catalase to the fermentation of wine to examine their ability to convert glucose to gluconic acid. They performed a Gox repeat addition experiment on a 220 L scale in must. The results showed that Gox oxidized glucose of 30.1 g/L and 32.5 g/L to gluconic acid of 29.1 g/L and 30.6 g/L in 43 hours, the alcohol content was reduced by 1.72% (vol) and 1.52% (vol), respectively. In this study, although the enzyme treatment achieved the purpose of reducing the alcohol content of the wine, the acidity and fermentation cycle of the wine were significantly affected, and the flavor and typicity of the wine were also affected (Röcker et al. 2016). Vivas et al. (2015) pointed out that Gox remained active in wine and promoted the formation of color and aroma characteristics. Gox oxidized tartaric acid, ethanol and glycerin to glyoxylic acid, acetaldehyde and glyceraldehyde in must and wine, respectively, and then reacted with catechins and proanthocyanidins to form new compounds by nucleophilic addition under acidic conditions, some of the new compounds were colored and had an impact on the visual quality of the wine, this effect is harmful in white wines (Vivas et al. 2015). In addition, Gox also has a strong inhibitory effect on wine browning. Gómez, Martínez, and Laencina (1995) added four different products to rose wine: ascorbic acid, citric acid, glucose oxidase and Riduxhigh (a commodity containing ascorbic acid, citric acid, metatartaric acid and potassium metabisulfite). After storage for 9 months at 30 °C, the browning and color characteristics of the different treated wine samples were examined. The results showed that the wine treated with Gox had the lowest degree of browning and maintained the best color quality (Gómez, Martínez, and Laencina 1995).

The production of glucose oxidase

Gox was produced in a variety of fungi, mainly from *Aspergillus* and *Penicillium*. At present, low production efficiency, large amount of by-products, and high sensitivity to metal ions in raw materials were major problems encountered in industrial production of fungal enzymes. To overcome these obstacles, Gox could be produced in a heterologous expression of gene (Derakshan et al. 2017). Gong et al. (2015) cloned a variant containing the two amino acid substituted Gox genes from *Aspergillus niger* CICC40179. The Gox gene was expressed in *Pichia pastoris* and obtained approximately 16.31 U/mL of Gox after 3 days of fermentation in an optimized medium (Gong et al. 2015). Derakshan et al. (2017) successfully expressed the GOX gene of *Aspergillus niger* in *Yarrowia lipolytica* for the first time

using a single integration vector containing a strong hybrid promoter and secretion signal, after 7 days of culture, the highest Gox activity was 370 U/L, the optimal pH and temperature of recombinant Gox were 5.5 and 37 °C, respectively. *Yarrowia lipolytica* was a suitable and highly efficient eukaryotic expression system in conjunction with other yeast expression systems for the production of recombinant Gox (Derakshan et al. 2017).

Lysozyme

Lysozyme (EC 3.2.1.17) is officially described as N-acetylhexosaminodase was classified as a muramidase, and widely used in soluble form to control lactic acid bacteria in different foods. Lysozyme hydrolyzes the cell wall of Gram-positive bacteria, increasing its permeability and rupturing cells. Lysozyme has little activity against Gram-negative bacteria and no activity on eukaryotic cell walls. Lysozyme has selective antibacterial activity against the hydrolysis of peptidoglycan of lactic acid bacteria (LAB) cell wall, so lysozyme is used to control spontaneous LAB, many of which causes wine spoilage. The major LAB species consist of *Pediococcus spp.*, heterofermentative *Lactobacilluss spp.*, *Oenococcus oeni.*, and *Leuconostoc mesenteroides spp.* (Liburdi, Benucci, and Esti 2014). Adding lysozyme to must and wine may help to control malolactic fermentation ad reduces the risk of increased volatile acidity and biogenic amines caused by these bacteria.

The application of lysozyme

Guzzo et al. (2011) used lysozyme for the control of LAB in the process of wine biological aging, mitigating or preventing heterolactic fermentation. They found that an amount of lysozyme of 6.25 g/hl could successfully control LAB populations in wines with high gluconic acid content. In addition, the combination uses of lysozyme and flor velum yeast reduced the volatile acidity of the wine. Therefore, the addition of lysozyme was an effective method to correct and prevent the fermentation of malic acid in biological aging of wine (Guzzo et al. 2011). The study of Guzzo found that phenolic compounds in grape pomace reduced the lethal rate of lysozyme to LAB, and proanthocyanidins extracted from grape seeds strongly inhibited lysozyme activity. This study showed that the antibacterial activity of lysozyme to LAB was closely related the content of proanthocyanidin in red wine (Guzzo et al. 2011). Benucci et al. (2016) studied the inhibitory effect of ethanol, sulfur dioxide and proanthocyanidin tannin on the antibacterial activity of lysozyme in model wine and obtained similar results, proanthocyanidin tannin had the strongest inhibitory effect on lysozyme (Benucci et al. 2016). Because tannins react with proteins, wines containing higher amounts of tannins must be treated with gelatin to remove excess tannins prior to adding some of these enzymes.

The production of lysozyme

At present, egg white is still the main source of commercial lysozyme. Leśnierowski and Cegielska-Radziejewska (2012) separated the lysozyme by direct crystallization, ion exchange chromatography and ultrafiltration, modified the lysozyme by ion exchange, chemical, chemical-thermal, thermal and membrane. In this study, the recovery of lysozyme was 20–85%. After modification, the mass fraction of the polymerase was 50–70% and the dimer content was 30–40%. The method of isolating and modifying in this study could be successfully used to produce highly active lysozyme. Lysozyme monomer, especially its modified form showed the possibility of wider use (Leśnierowski and Cegielska-Radziejewska 2012). Cappannella et al. (2016) covalently immobilized egg white lysozyme on a microbial chitosan spherical carrier, and established an enzymatic hydrolysis system of continuous, high-efficiency and food-grade of lysozyme in white and red wine, and successfully reduced the amount of SO_2 used to control LAB during fermentation. Lysozyme from hen egg white was more effective in the immobilized form than in the free form, the reason was that covalent immobilization made the lysozyme less sensitive to the inhibition of wine flavonoids (Cappannella et al. 2016).

Application of other enzymes related to the quality of fruit wine

The application of proteases

Proteases are commonly used in the fermentation of fruit wines. Byarugaba-Bazirake, van Rensburg, and Kyamuhangire (2013) treated a banana wine with the commercial proteases Zumizyme (Biobright Japan) and papaine (Novozymes SA), the turbidity of the banana wine was 27.5 and 18 NTUc after one week, and the turbidity of the control sample was 47 NTUc; the turbidity after treatment for four weeks were 18 and 10 NTUc, and the turbidity of the control sample was 29 NTUc. The original turbidity of banana wine before enzyme treatment was 58.3 NTUc. Both enzymes had obvious effects on the clarification of banana wine (Byarugaba-Bazirake, van Rensburg, and Kyamuhangire 2013). Zhang, Zhang, and Xu (2016) treated the jujube wines with protease, and the results showed that protease could improve the intensity and complexity of wine aroma due to the increase of the assimilable nitrogen (Zhang, Zhang, and Xu 2016). If white wine suffers of high temperatures during transport and storage, some proteins are prone to turbidity due to denaturation and affect the quality of the wine. These proteins should be removed prior to bottling. Chitinase is the main protein that forms turbidity in wine and has no heat denaturation. Van Sluyter et al. (2013) used the protease BcAP8 from the grape fungal pathogen *Botrytis cinerea* to treat chitinase in white wine, which effectively removed the protein under normal brewing conditions (Van Sluyter et al. 2013).

The application of tannase

Tannin acyl hydrolase (tannase, EC 3.1.1.20) catalyzes the hydrolysis of ester bonds in hydrolyzable tannins such as tannic acid, thereby releasing glucose and gallic acid, is a clarifying agent used to reduce the haze of wine (Byarugaba-Bazirake,

van Rensburg, and Kyamuhangire 2013). In addition, in the process of wine aging, hydrolyzable tannins are released from oak barrels, which a major source of wine astringency. The tannase hydrolyzes the ester bonds in these polyphenolic compounds to avoid their polymerization, and therefore, a high-quality wine with a high aromatic content and suitable color may be obtained (Yao et al. 2014). Selwal et al. (2011) used the newly isolated tannase-producing fungal strain *Penicillium atramentosum* KM for tannase production under solid-state fermentation (SSF), and obtained the largest extracellular tannase production with Jamoa and Keekar leaves as substrates were 170.75 U/gds and 165.56 U/gds. Applied the obtained tannase to wine clarification, it resulted in 38.05% reduction of tannic acid content in case of jamun wine, 43.59% reduction in case of grape wine (Selwal et al. 2011).Tannins (found in many fruits) could combine with the proteins by hydrogen bonds and hydrophobic interaction to induce haze in wines, the application of tannase in the production of banana wine was very beneficial to improve the clarity of banana wine (Byarugaba-Bazirake, van Rensburg, and Kyamuhangire 2013).

The application of urease

Ethyl carbamate (urethane, EC) is naturally formed during the fermentation or storage of fruit wine, EC in foods are related to carcinogenic potential. Urea is considered to be an important precursor to EC in wine. Therefore, researchers often use urease to reduce the urea content and thus reduce the EC content in wine. Cerreti et al. (2016) treated 6 kinds of white wine, red wine and rose wine rich in urea with purified acid urease prepared by fermenting lactic acid bacteria. The results showed that the hydrolysis of urea successfully reduced the EC concentration in wine (Cerreti et al. 2016).

The potential application of glutathione reductase

Glutathione reductase (GR, EC 1.6.4.2) is an important enzyme that can reduce glutathione disulfide bonds to thiol forms in the presence of a NADPH-dependent system. The oxidized glutathione (GSSG) is reduced to reduced glutathione (GSH) through the pentose phosphate pathway, which is a key antioxidant mechanism (Tandoğan and Ulusu 2007). Recently, more and more researchers used GSH for fruit wine production or storage. GSH played an important role in preventing browning and flavor loss caused by oxidation of white wine (Coetzee and Toit 2012; Kritzinger, Bauer, and Du Toit 2013; Rodríguez-Bencomo et al. 2014). But only reduced GSH has the function, and GSH is easily oxidized to GSSG. GSSG can also be reduced back to GSH by the enzymatic action of GR (Carmel-Harel and Storz 2000). With the application of GSH in fruit wine, people pay more attention to it, and GR can effectively guarantee the reducibility of GSH. In the future, GR is likely to be combined with GSH in fruit wine production.

Conclusions

Enzymes were widely used in the production of fruit wine. The addition of enzymes was of great significance for improving the quality of fruit wine and reducing the production cost of fruit wine. Pectinase reduced the viscosity of must and wine, which was conducive to the clarification of wine, improved the color strength and stability of red wine, promoted the release of phenolic substances and flavor substances. β-glucanase reduced the viscosity of must and improved the filtration efficiency of wine, accelerated the aging characteristics of sparkling wine. β-glucosidase enhanced the aroma characteristics and aroma diversity of the fruit wine, however, the use of β-glucosidase might also have side effects on the quality of the fruit wine, such as the hydrolysis of anthocyanins. Glucose oxidase was mainly used to reduce the alcohol content of fruit wine. Lysozyme was used to control lactic acid bacteria that cause deterioration of wine. Proteases and tannase were commonly used to increase the clarity of fruit wines. Urease could reduce the content of urethane in wine. Glutathione reductase has potential applications in stabilizing the sensory properties of fruit wine because it can maintain the reducing properties of glutathione. The enzyme added during the brewing process needed to have high catalytic activity in the presence of ethanol, SO_2 and relatively low temperature conditions. Finding suitable enzyme-producing strains from environmental biodiversity and using microbiological or metabonomic approaches was an important and effective way to address these constraints. These enzymes for winemaking were environmentally friendly and could be mass produced by biotechnological means such as genetic engineering and metabolic processes. The enzymes produced by genetically modified micro-organisms may have the great potential to be applied to fruit wine production within the framework of ensuring absolute safety and legal approval in the future. However, the application of enzymes in the production process of fruit wine is still in its infancy. Researchers need to understand the interactions between different enzymes, the advantages of various enzymes and the characteristics of different fruit wines. Combining the use of multiple enzymes to reduce production costs and improve quality of fruit wine is the development trend in the future.

Disclosure statement

No potential conflict of interest was reported by the authors.

Compliance with ethical standards

The authors declare that they have no conflict of interest to this work. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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