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APPLICATION OF ENZYME PREPARATION IN ALCOHOLIC INDUSTRY

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Abstract

The use of enzymes in industrial production has increased greatly, especially in the alcoholic industry, namely beer, grape wine, Huang jiu, and liquors. Diacetyl produced in the fermentation process of beer affect the essence of beer in which α-acetyllactate decarboxylase plays a good inhibitory role. Glucosidase, pectinase, and lysozyme play their respective roles in the process of grape wine fermentation. Huang jiu is produced through traditional technology by taking glutinous rice and japonica rice as the main raw materials and employing wheat koji or a variety of microorganisms contained in rice koji. Wheat koji provides rich enzyme systems such as amylase and protease for the brewing process of rice wine. Enzymes are widely used in various stages of liquor production, such as making koji, fermentation of grains, distillation blending, and waste utilization of distillers' grains. This paper mainly describes the application of enzymes in the production of various wine industries, the improvement of enzyme stability through enzyme immobilization, the treatment of waste enzymes in the wine making process and the future challenges and development.

Keywords: Enzyme, Alcoholic drinks, Production, Immobilization

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Introduction

With the improvement of people's living standards and the rapid development of science, people are not only satisfied with the most basic sense, but also begin to have a higher level of demand for flavor and quality. Enzyme preparations, such as α-acetyllactate decarboxylase, glucose oxidase, beta-glucanase and so on which possessed characteristics of high efficiency, specificity and high safety, effectively improve the quality, taste and shelf life of food, thus it was widely used in food, especially in the alcoholic industry at different industrial processes. Besides, it played a crucial role, such as the saccharification in the early stage of wine making, the regulation of the wine body composition and the alcoholic fermentation in the later period. Gradually, wine was not just a drink on the table, it was also a cultural symbol. Countries have developed distinctive cultures by brewing their unique alcoholic drinks, such as Japanese sake, Mexican tequila, Chinese rice wine, etc. Among them, enzyme preparations play a significant role. This review focuses on the application of enzyme preparations in alcoholic industry.

APPLICATION OF ENZYMES IN BEER INDUSTRIAL PRODUCTION

α-acetyllactate decarboxylase

α-acetyllactate decarboxylase, the main application in beer is to reduce the content of diacetyl produced during beer fermentation. That is because diacetyl, one of the important by-products produced by yeast metabolism, can make beer produce bad flavor during the process of beer fermentation. Diacetyl is considered as an odor compound in beer because of its buttery taste (Choi et al. 2015). It was usually necessary to store the beer for a period of time to reduce the content of diacetyl, but this slow process led to a prolonged production cycle, and α-acetyllactate decarboxylase converts the precursor of diacetyl

- acetolactic acid into acetoin, thereby reducing the production of diacetyl (Cejnar et al. 2016). Costa G. P., Spolidoro L. S. and their teammates found that the stability coefficient of α-acetyllactate decarboxylase covalentiously fixed by chitosan spheres and activated by glutaraldehyde at 60°C was almost 7 times higher than traditional free enzymes. Moreover, the immobilized enzyme-made catalyst was repeatedly tested for the conversion of α-acetyllactic acid to acetylacetone in beer. Moreover the activity of the enzyme recovered to 80% of the initial activity, which made the enzyme widely used in beer industrial production (Costa et al. 2022).

Glucose Oxidase

Glucose oxidase is a kind of aerobic dehydrogenase. According to the study on beer flavor test, another important factor affecting the flavor of beer was the content of oxygen in beer. The premature oxidation of brewed wines phenomenon is usually occur and not drinkable at this time (Romanini et al. 2019). It leads to oxidative aroma degradation, including premature loss of variety aroma (Silva Ferreira et al. 2003). Also, a further investigation had been carried out in terms of the mechanisms involved in oxidation over at least three decades, using a simple model system showing that the main affected substances focus on phenolic compounds, especially flavonoids and their subsequent polymers, as well as flavonoid conversion products turned pink (Andrea-Silva et al. 2014). Therefore, reducing the oxygen content in beer products and removing some oxygen floating around the bottleneck were effective measures to ensure the stability of beer taste. Glucose oxidase effectively slow down the oxidation process of beer, reduce the turbidity of beer significantly, and extend the shelf life of beer by at least three years. Compared with other chemical deaerators such as hydrogen peroxide, glucose oxidase was used as a natural food additive, which had higher safety and more than ten times greater stability. Furthermore, glucose oxidase is a mild enzyme that does not cause changes in environmental acidity and basicity. Hence, it basically could not affect other substances in beer, which contributed to the preservation of beer (Baek et al. 2020).

beta-glucanase

β-glucanase is an enzyme that can specifically decompose various barley β-glucans with high viscosity. The β-glucan content has a negative effect on the quality of malt and beer, mainly in the wine filtration stage. Barley is one of the main crops in China and is also used as the main raw material in brewing beer. However, the barley cultivated in our country for brewing beer generally had the disadvantage of high β-glucan content, which had brought great influence on beer industry production. Based on studies of barley and β-glucanase, Baek I, Choi H, it had been found that microbial β-glucanase enzyme preparations were expected to reduce the content of high molecular weight β-glucan in barley (Goode et al. 2005) (Hattingh et al. 2013). In addition, β-glucanase also decomposed glucan in barley, loosened the endosperm cell wall of barley, and increased the outflow of some cell contents. In a research related application of β-glucanase, 50 mg/kg β-glucanase was added to malt beer after germination for 64 h, it was found that it was similar to malt beer fermented for 96 h by traditional process, which greatly improved the industrial production efficiency and increased the output of beer (Brazil et al. 2019).

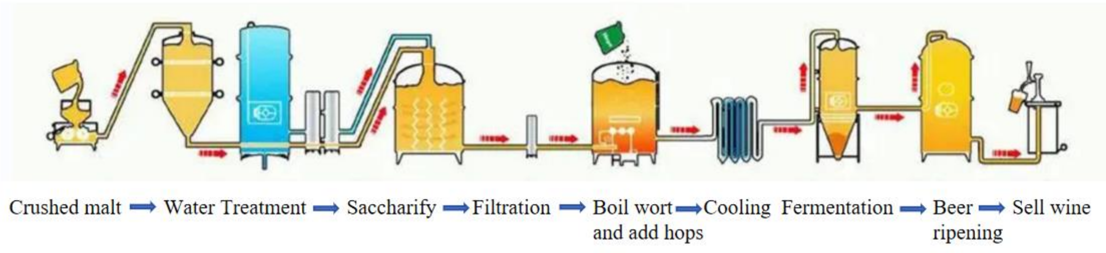

Figure.1. Beer equipment brewing process flow chart

APPLICATION OF DIFFERENT ENZYMES IN WINE INDUSTRY PRODUCTION

Glycosidase

Glycosidase is an important enzyme in wine- manufacturing, which could hydrolyze the glycoside bond and release fructose and glucose to improve the quality of wine (Schmidt et al. 2011). The fragrance of wine is one of the important manifestations of its quality and characteristics. The fragment substance was derived from the grape fruit, the fermentation process and the aging process. The fragrance of wine would change gradually over time, it is not only an important factor in the quality of the reaction but also a very important part of the tasting process. Through the research about the perfumed substances in wine, some scientists found that the main perfumed glycosides in grapes were terpenes, mainly including monoterpenes and isoallenes. While, many phenolic compounds such as anthocyanins usually existed in the form of glycosides (Parker et al. 2018) (Garrido et al. 2013). In addition, some phenolic glycosides in smoked grapes are also be hydrolyzed by beta-glucosidase, releasing volatile phenols that are detrimental to most wines, such as cresol, guaiacol and eugenol (Hayasaka et al. 2010) (Zhang et al. 2021). By mixing glucosidase with wine, the fragment substance changed from the bound state to the free state by hydrolyzing the glucoside bond. The contents of monoterpene substances in wine were twice as high as the original content namely α-terpinol, citronellol, geraniol, linalol and nerolol. Among them, Nerolol made the wine with rose and neroli aromas, was relatively peaceful, with slight lemon-like fruit notes; Linalol with a fragrant and sweet smell, like lily of the valley, gave the wine a more fruity flavor (Jiang et al. 2021).

Pectinase

Pectinase is one of the important enzymes in the wine-manufacturing industry, which can break down pectin into smaller molecules such as galacturonic acid, methanol and pectinic acid. A large amount of pectin contained in the raw grapes of wine would increase the viscosity of the fruit wine, which was not conducive to filtration and consequently leading to low juice yield. When pectinase was added in the impregnation stage of wine making, not only the total fruit juice production would be greatly improved, but also the contents of benzene derivatives, ethyl acetate and phenylethyl acetate in wine would be significantly increased, thus well improve the quality of wine (Espejo & Armada, 2021). Additionally, the effect of pectinase pretreatment on the fermentation of red dragon fruit wine was studied. In the study, pectinase (0.1% v/v) was used after pasteurization, and then fermented with T. delbrueckii at 20°C for 14 days. Jiang X, Lu Y, Liu S Q found that pectinase pretreatment did not affect yeast growth, nor the yield of ethanol (8% v/v) and glycerol (6 g/L) (Armada et al. 2010). However, pectinase significantly increased juice or wine production to 16% v/v (Jiang et al. 2020). Tannins and pigments are mainly distributed in grape skins and seeds. Compared with the movement speed of the two under the same conditions, it was found that pectinase was more conducive in the diffusion of tannins and pigments. It was found that different pectinases had different effects on the extraction and color change of raw grape pigments. Lafase HE pectinase was more beneficial to the extraction of tannins and pigments. Optizyme pectinase was beneficial to the clarification of wine (Osete-Alcaraz et al. 2021). Adding an appropriate amount of pectinase in the processing process can completely decompose pectin into small molecules, improve the juice yield of wine, enhance the filtration ability, deepen and stabilize the color of red wine, and prevent the occurrence of wine turbidity.

Lysozyme

Lysozyme is a natural antibacterial substance that can attack bacteria by hydrolyzing mucopolysaccharides from bacterial cell walls, thus making an impact on Gram-positive bacteria. Nawaz N, Wen S, Wang F found that lysozyme played an important role in wine manufacture (Nawaz et al. 2022). In the wine-manufacturing industry, a variety of enzyme preparations were used, whose components were mainly proteins. some microorganisms present in wine would change the pH in wine, even cause some enzymes to denaturate and deactivate. Adding from100 mg/L to 150 mg/L lysozyme to grape juice could inhibit the growth of lactic acid bacteria. When alcoholic fermentation was completed, malic-lactic acid fermentation could be started by normal inoculation, and the dosage of lysozyme in white wine increased to 500 mg/L, and malic-lactic acid fermentation were completely inhibited. If alcohol fermentation was stopped, adding 250 mg/L to 350 mg/L lysozyme inhibit the growth of lactic acid bacteria during the restart alcohol fermentation stage, thus simplifying the process of removing excess volatile acids from the wine. When lactic acid bacteria exceed 160 CFU/mL, the amount of lysozyme should be increased to 500 mg/L. As long as malic-lactic acid fermentation was completed, if the concentration of lysozyme is added between 250 mg/L and 300 mg/L , the SO_2 required to stabilize the wine could be greatly reduced. Also the environmental pH of the enzyme preparation was suitable for the production of wine.

Urease

The application of urase in wine production is to reduce the content of nitrite in wine and degrade urea in wine. In the process of wine fermentation, nitrite is produced in the process of wine fermentation and yeast would produce urea in the process of arginine metabolism. When urea content in wine is too high, alcohol mixed with urea forms carbamate, which has a strong carcinogenicity. Urease breaks down nitrite and converts urea into ammonia ions and carbon dioxide, in order to degrade urea in wine and inhibit the production of carbamate. The optimal pH for most ureases is from 6 to 7, and generally do not show activity in wine. Suzuki and his teammates first found a urease in the Lactobacillus genus with an optimal pH of 2.4 in wine fermentation (Suzuki et al. 1979). When the urea content of wine exceeds 3 mg/L, it was necessary to use urease to treat with rewrite using scientific writing style. Later, study showed that Andrich and other people covalently bound acid urease to chitosan particles of different sizes, and when the conjugate was placed at 4℃ for 150-170 days, its activity decreased by about 5% (Andrich et al. 2010). Therefore, the use of this enzyme would greatly improve the efficiency of enzyme use and save more costs. Urease plays an important role in wine production, not only improving the quality of wine, but also making an indispensable contribution to its compliance with national food safety.

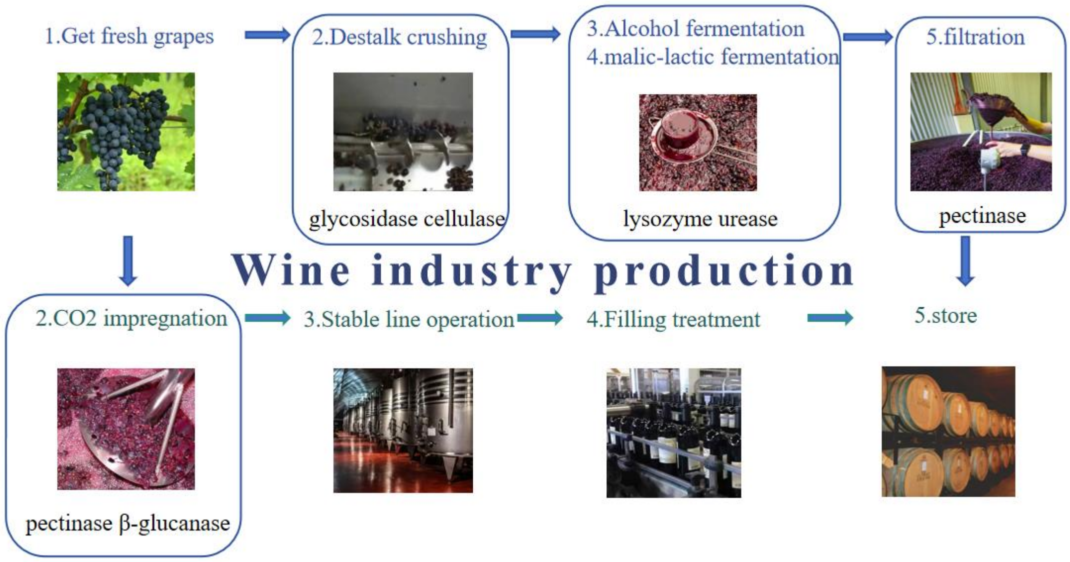

Figure.2.Wine industry production

THE APPLICATION OF VARIOUS ENZYMES IN HUANG JIU

Cellulase

The cellulase complex, consisting of exoglucanase, endoglucanase, and β-glucosidase, is naturally produced by various microorganisms and plays a crucial role in the recycling of cellulose in the biosphere (Ejaz et al. 2021). Black rice is rich in bioactive compounds that contribute to its high nutritional value when used to make black rice wine. However, the dense cortex of black rice hinders fermentation rate and reduces the dissolution of active ingredients. In an experiment conducted by Yang Liu, the effects of pregelatinization (PG) before cooking and the addition of cellulase (CE) during fermentation increased the alcohol content, free amino acid content, volatile flavor substance content, and total antioxidant activity of black rice wine by 90.81%. These values were 38.05% higher than those observed in the control group (Yang et al. 2022). Scanning electron microscopy, low field nuclear magnetic resonance analysis, and texture analysis revealed that pregelatinization treatment improved starch gelatinization during cooking and reduced bound water content in black rice grains. It also promoted the release of unbound water. Cellulase disrupted the structure of the aleurone layer, leading to enhanced free water release and exposure of rice starch particles, significantly increasing the reaction area and exudate content, thus facilitated microbial growth and fermentation process. Simultaneously, the incomplete aleurone layer promoted dissolution of anthocyanins, polyphenols, and other active substances, thus elevating antioxidant activity levels within black rice wine. Fermentation time was reduced and quality characteristics were improved (Yang et al. 2022).

Liquefaction Enzyme

The role of liquefaction enzymes is to hydrolyze α-1,4 glucoside bonds in long amylopectin molecules, primarily yielding small dextrins and a minor amount of oligosaccharides, maltose, and glucose. For example, Shichao Bian incorporated heat-resistant A-amylase into Huang jiu's fermentation process (Bian et al. 2021). Experimental results showed that extrusion pretreatment methods enhanced Huang jiu fermentation. This resulted in increased gelatinization degree (84.23-99.99%), water solubility index (28.09-62.75%), and reducing sugar content (74.82-263.39 mg/g) of horsetail while decreasing its water absorption index (3.25-209 g/g) and viscosity (<20 cP), thereby facilitating fermentation.

X-ray diffraction and Fourier transform infrared spectroscopy revealed a decrease in starch structure order with increasing enzyme concentration. Scanning electron microscopy observations indicated that enzyme extrusion led to an increase in phenol content and antioxidant properties of the samples. Using 5% enzyme as a fermentation material yielded a fermentation efficiency of 74.23%, along with antioxidant properties measuring 75.87, 76.21, and 203.32 mg Trolox equivalent/L for DPPH, ABTS, and FRAP values respectively. These results demonstrate that enzymatic extrusion method can be effectively applied to enhance the fermentation process of Huang jiu by promoting starch degradation and improving its antioxidant performance (Bian et al. 2021).

A significant amount of bioamines is produced during the rice soaking process, resulting in an excessively high concentration of bioamines in Huang jiu. In Wang Lan's experiment, raw materials were directly crushed and liquefied before saccharification and fermentation to produce Huang jiu, as shown in Figure 1. The alcohol content of Huang jiu produced through this procedure was 14.0%vol, with a bioamine content at 71.59% lower than the control (Bian et al. 2021).

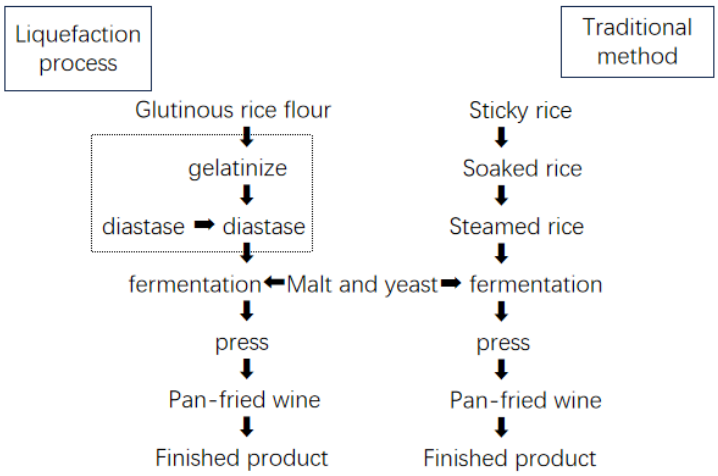

Figure.3.Comparison between liquefaction method and traditional method of Huang jiu brewing technology

Acid protease

The acid protease is a type of aspartic acid protease that possesses remarkable chemical and physical properties, exhibiting exceptional resistance to acidic conditions without succumbing to microbial contamination during hydrolysis. It is widely used in proteolytic processes in the food and brewing industries (Tang et al. 2002) (Yuang et al. 2003) (Theron & Divol, 2014). The instability of Huang jiu primarily arises from protein-polyphenol interactions, leading to oxidation or heavy metal-induced effects (Ferreira et al. 2001) (Siebert, 1999).

Amylase (AM), glucose amylase (GAM) and acid protease (AP) were added to japonica rice starter culture, and mixed fungi, such as Rhizopus chinensis R01, Aspergillus niger A20, and Saccharomyces cerevisiae S10 to produce high the efficiency of fermentation, were used to fermentate japonica rice starter culture resulted in the fermentation ability and quality of japonica rice were higher than control group. The ethanol content, free amino acid (FAA) and volatile flavor substance concentration were

increased by 49.22%, 85.54% and 25.71%, respectively. In order to understand the role of enzymes in Huang jiu brewing, the effects of hydrolase on rice physicochemical properties, yeast growth and mold enzyme production were measured. Both AM and GAM could effectively hydrolyze rice starch, the viscosity of starch was significantly reduced, the contact area of enzyme was expanded, and the japonica rice was further hydrolyzed. The increase of hydrolysate promoted the growth of fungi and increased the ability of mold to produce enzyme. Among them, AP improves the protease activity of pusillus M05 and A. niger A20, improve the protein utilization, increase the FAA content, and form A. niger A20 higher level of flavor substances in Huang jiu.
The bitter polypeptide (PGP) is a crucial component of Huang jiu and responsibles for its bitterness. Xie Guangfa conducted an experiment using six proteases to degrade PGP, evaluating the degradation rate and its impact on the quality of yellow Huang jiu. The result showed that flavor protease, xyloglutinase, and acid protease exhibited superior degradation effects, with acid protease achieving a degradation rate of 24.7% (Ferreira et al. 2001).
To enhance the stability of Huang jiu, Wei Taoying incorporated acid protease at various stages (Siebert, 1999). The content of sugar, composition, acidity, amino acid nitrogen and other parameters of rice wine treated with different amounts of protease were determined and analyzed. Findings revealed a proportional relationship between the amount of produced compounds and the quantity of added protease; notably affecting amino acid nitrogen levels. Cold turbidization demonstrated that when adding 10-20 u/g of protease, it resulted in approximately 0.3 units lower than blank raw wine; with an optimal amount observed at 15u/g.

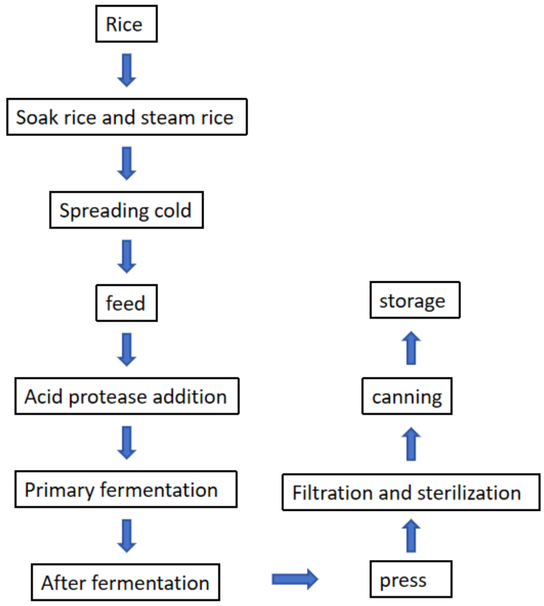

Figure.4.Huangjiu brewing process

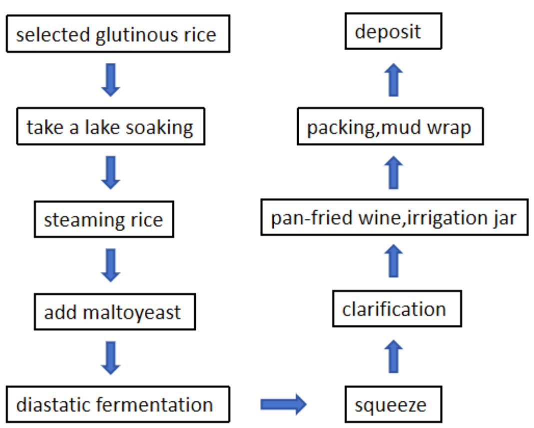

Figure.5.Huangjiu making process

APPLICATION OF DIFFERENT ENZYMES IN LIQUOR

xylanase

Xylanase is a complex enzyme system that could degrade xylan hemicellulose.And xylan hemicellulose exists in large quantities in nature (Chaudhary et al. 2023). Research and development on the structure and function of xylanase from other sources, such as plants, promote the industrial application of xylanase in the field of brewing (Wang et al. 2020). Xylanase can break down hemicellulose outside the starch layer of cereals, and adding xylanase at the early fermentation stage can increase alcohol yield. Ogasawara H, a Japanese scholar, found that xynC, a xylanase used in the production of barley shochu in Japan, it was isolated and purified from Aspergillus albicans, improved fermentation efficiency and alcohol yield at optimum condition such as pH2.0 (Beg et al. 2001). Zheng Bin's study revealed that starch utilization during ethanol fermentation of maize was significantly increased when compound enzyme preparation was used, which included acid protease, xylanase, and pullulanase as raw materials, compared to the control group (Bin et al. 2019). This finding suggests that the application of such a compound enzyme preparation can enhance starch utilization efficiency and shorten the fermentation period. Xu Man's research, Based on specific fermentation time, demonstrated through an alcohol fermentation verification experiment that the alcohol content and residual sugar showed significant fluctuations. His results showed that the alcohol content was 130.47% of the control group and the residual sugar content was 7.4% of the control group after adding xylanase, highlighting the substantial advantages of using xylanase in corn alcohol fermentationv (Xu et al. 2019). Therefore, it can be inferred that incorporating xylanase into the alcohol fermentation process can enhance juice yield and optimize fermentation efficiency.

esterifying enzymes

The esters present in alcoholic beverages not only confer a unique aroma to the beverage but also regulate its sensory properties and quality. These esters, aside from the raw materials, are produced during the brewing process. It is widely accepted that esterification catalyzed by enzymes is the primary mechanism for ester formation in alcoholic beverages. Among various esterification enzymes, lipase exhibits superior esterification capabilities and is one of the key enzymes involved in this catalytic process (Fan et al. 2021). Lipase can regulate the content and proportion of various flavor compounds in the beverage, such as ethyl acetate, which has a solvent-like aroma, ethyl caproate, which has an apple-like fragrance, and ethyl caprate, which has a floral scent, thereby enhancing the quality of the beverage (You et al. 2015). During the production and fermentation of koji, organic acids such as acetic acid and propionic acid, which are beneficial to the taste of the beverage, is produced, thus enriching its flavor. The glycerin improves the flavor quality of the beverage, alleviates the harshness and spiciness, and renders the flavor of the beverage. During the fermentation process, various acids are produced and esterified with the alcohol produced in the fermentation stage to create the compound "ester", which has the greatest impact on the flavor of the beverage. Therefore, esterifying enzymes play a crucial role in enhancing liquor flavor.

acid protease

The application of acid proteases is ideal for the fermentation of jiupei, as they can provide amino acid substrates for Maillard. By-products of protease degradation also contribute significantly to the production of soy sauce. In the acidic environment of liquor brewing, acid protease efficiently hydrolyzes proteins into amino acids, thereby enhancing the total acid and ester content of the liquor, ultimately improving its flavor. Hu Xuezhi's research showed that adding complex acidic protease to bran promoted the production of alcohol, and adding enzymes improved the production of liquor. (Zhi et al. 2005) Acid protease promotes grain metabolism, increases wine yield, increases the amino acid content in fermented grains, enhances esterification, and produces a unique flavor. Liu Xin's study showed that, compared to the control group, the metabolic rate of reducing sugar was significantly accelerated, and the alcohol content was also significantly increased (Xin et al. 2017). In conclusion, the integration of acid protease in the liquor-making and brewing process is importance for enhancing the aroma and stability of the final product. Specific applications of enzymes can be seen in Table 1 below.

Table.1.Function of enzymes in different stages of wine production

Type of enzyme	Function	Action flow	References
Beer			
α-acetolactate decarboxylase	Reduce the diacetyl content	Alcoholic fermentation	(Choi et al. 2015)
Glucose Oxidase	Reduce the oxidation of other substances	stockpile	(Romanini et al. 2019)
β-glucanase	Reduce the amount of glucan	Wine filtration	(Goode et al. 2005) (Hattingh et al. 2013)
Red Wine			
glycosidase	Hydrolyze glycoside bonds and release fructose and glucose	Wine fermentation	(Schmidt et al. 2011)
pectinase	Breaks down fructose into small molecules	Grapes stem and break	(Espejo & Armada, 2021) (Armada et al. 2010)
lysozyme	An antimicrobial substance that hydrolyzes bacterial cell walls	Early stage of wine fermentation	(Nawaz et al. 2022)
urase	Reduce nitrite and urea content	wine fining agent	(Suzuki et al. 1979)
Huang jiu			
cellulase	Degradation to monosaccharides or small oligosaccharides	Rice wine fermentation	(Yang et al. 2022)
liquefaction enzyme	Hydrolysis of starch into dextrin and sugar	Liquefaction of rice wine	(Yang et al. 2022)
Acid protease	Improve the ability of saccharification and fermentation	Early or late stage of rice wine fermentation	(Ferreira et al. 2001) (Siebert, 1999)
Liquor			
xylanase	Improve yield and fermentation efficiency	Initial fermentation	(Beg et al. 2001)
esterifying enzymes	Adjust various flavor substances to improve the flavor	Liquor brewing and fermentation period	(You et al. 2015)
acid protease	Enhances aroma and stability	Fermented grains in fermentation	(Zhi et al. 2005)

THE APPLICATION OF ENZYMES IN OTHER WINE INDUSTRY PRODUCTION

Different enzymes play different roles in the wine-manufacturing process, which results in the unique taste and fragrant substance of various wines. Sake was known as the "national wine" of Japan. According to the experiment of adding cellulase reagent in sake mash, the activity of amylase and protease in cellulase reagent was very low, and the main components of each brewing sake sample were similar. The concentration of ethanol was from 18.40% to 18.95%, and the content of extract was from 6.5% to 7.8%. Titrable acidity and amino acid acidity were both from 1.55 to 1.60. Even under low temperature and high ethanol conditions, adding cellulase reagents also increase ferulic acid and ethyl ferulate. The ratio of ethyl ferulic acid to ferulic acid was an important index to measure the quality of sake. In 40% Akizumachi koji mash, the ratio of ethyl ferulic acid to ferulic acid was significantly increased, while in 70% Yinshan koji mash, the ratio of ethyl ferulic acid to ferulic acid did not change (Hashizume et al. 2013) (Ito et al. 2019).

Tequila was a distilled spirit made from the tequila plant and produced native to the Mexico. In the production of tequila, the application of enzymes was mainly reflected in the decomposition and filtration of sugars. The sugars in the agave plant was mainly stored in the bulbs of its stem. In order to finish this step, the bulbs were first removed and ground to release the sugars inside. Amylase, which broke down starch into sugar, and glycosylase, which released sugar from the agave fiber, were added to the ground agave. In this way, the brewer could obtain more fermented sugars, thus increasing the alcohol content (Singh et al. 2021).

In the production of whisky, for example, a multi-enzyme complex called Diastaticus played a key role. This complex could convert the starch and protein in the malt into sugars and amino acids, which in turn produced a rich aroma and tasted during fermentation. In the test about the influence of pectinase treatment on the quality of cider wine, Cheng Zhenggen and his team found that the clarification degree of the wine samples treated with pectinase was about 95%, while the clarification degree of the wine samples without pectinase was lower, and the content of fusel oil in cider after pectinase treatment was reduced. So it was concluded that the addition of pectinase in cider brewing had a great effect on improving the quality of cider (Zheng et al. 2017).

APPLICATION OF IMMOBILIZED ENZYMES

Enzyme biocatalysis plays a crucial role in the advancement of chemical industries, including energy, food, and fine chemistry. In order to achieve this, enzyme immobilization is often a prerequisite for obtaining reusable biocatalysts, thereby reducing the cost of this relatively expensive compound (Garcia ‑ Galan et al. 2011). Enzyme immobilization refers to the method and technology that utilizes organic or inorganic solid materials as carriers to encase or bind enzymes onto their surfaces and micropores (Homaei et al. 2013), enabling them to retain catalytic activity while being recoverable and reusable. There are various methods available for enzyme immobilization; traditional techniques include adsorption, covalent binding, embedding, and cross-linking methods. Compared to free enzymes in solution, immobilized enzymes exhibit enhanced vitality and greater resistance against environmental changes. Moreover, the heterogeneity of immobilized enzyme systems allows for easy recovery of enzymes and products, multiple reuse of enzymes, continuous operation of enzymatic processes, rapid termination of reactions, and a wider range of bioreactor designs. Despite these advantages, the limitations associated with immobilized enzymes should also be considered， as shown in Table 2. Due to diffusion constraints when immobilized, natural enzymes such as amylase or protease experience significantly reduced kinetics, resulting in less economical for utilization.

Table.2.Advantages and disadvantages of immobilized enzymes

Advantages	Disadvantages
Easy separation of biocatalysts	Lower activity than natural enzymes
Better stability, especially at higher temperatures	Reaction rate is lower than natural enzymes
The downstream processing cost is reduced	Pollution of the environment by discarding immobilized enzymes
Can be fixed with other enzymes	Vulnerable to pollution

In the field of winemaking, the use of immobilized enzymes is prevalent. In a scholarly investigation by Xin Xing, the actual utility of GAPDH@Fe3O4 was evaluated in the maturation phase of Kirsch wine. The influence of this material on key physico-chemical indices, volatiles, and non-volatile phenolics in the body of wine was examined after repeated use. A spectrum of eight biogenic amines, including histamine, tyramine, and putrescine, were identified in the wine matrix. Upon initial exposure to the immobilized enzyme, the degradation rate of each biogenic amine fluctuated between 18.6% and 55.2%; in particular, after ten reuses, the degradation rate maintained values ranging from 6.4% to 17.1%

(Xin et al. 2023) (Tong et al. 2021). Thus, through enhancing batch repetitions and cost reduction, GAPDH@Fe3O4 has shown potent efficacy in mitigating biogenic amines but not compromising wine integrity.

Subsequently, in a separate study led by Li Tong, chitosan served as a scaffold for immobilizing neutral urease (Basso et al. 2019). This encapsulated urease subsequently catalyzed the degradation of urea present in rice wine. In contrast, when treated with free enzymes at 30 ºC for 24 hours at an enzyme dose of 1000 units/L, urea levels in wine dropped significantly by 95% or more. The integration of enzyme selectivity, stability, and kinetics with the physico-chemical characteristics of the supports ensures optimal physical and enzymatic stability of the bio catalyst (Basso et al. 2019). The creation and application of novel immobilization technology provides unexplored pathways for targeted enzyme immobilization, opening avenues for broad industrial implementations.

WASTE ENZYMES PRODUCED IN THE PROCESS OF WINE MANUFACTURING

Disposal of waste enzymes produced during the wine manufacturing process usually requires compliance with environmental regulations and sustainability principles. Here are some solutions:

Recycling of waste enzymes

Optimization of processes and equipment, as well as recycling, were employed to minimize waste enzymes during the wine manufacturing process. For instance, improvements in filtration and centrifugation technology have been made, leading to an improved utilization rate of raw materials. Additionally, the utilization of waste enzymes with reusable value has been integrated into the production of feed, fertilizer, or other industrial raw materials.

Biological and chemical treatment

The application of biotechnology, such as microbial degradation, is suitable for treating waste enzymes in wastewater by converting organic matter into harmless substances. For waste enzymes that cannot be recycled or are unsuitable for biological treatment, chemical methods may be considered, including chemical precipitation and flocculation.

Incineration and landfill treatment

Incineration and safe landfill methods can be employed for waste enzymes that are unmanageable by the aforementioned techniques. Incineration reduces the volume of waste enzymes and generates heat energy for power generation or heating, while landfills must adhere to environmental standards to prevent pollution.

In the treatment of waste enzymes, factors such as technical feasibility, economy, and environmental impact should be considered to select the most appropriate method. Furthermore, technological innovation and process improvement can minimize the generation of waste enzymes and promote sustainable development.

CHALLENGES AND FUTURE DEVELOPMENT OF ENZYMES IN WINE MANUFACTURING ENGINEERING

Development and application of new enzymes

Advances in fermentation technology and strain breeding have deepened our understanding of enzymes and led to the discovery and modification of new enzymes. Each enzyme technology upgrade and new enzyme application makes a significant contribution to the alcohol industry. Duan Gang's research on rice alcohol production using a novel granular starch hydrolase found that Genenke's STARGENTM grain starch hydrolase, when used with rice as raw material, significantly enhances fermentation efficiency. Additionally, GC-MS analysis of the fermentation mash reveals a significant reduction in impurities in the fermentation products compared to the traditional process (Gang & Sophia, 2008).

The influence of intelligence and bioengineering technology on wine manufacturing engineering

With the rapid development of economy and science, intelligent technology has penetrated into every field of human life. Intelligent wine manufacturing has gradually become a new direction for the development of the wine manufacturing industry, the use of intelligent control systems can achieve precise control in the production process, thereby reducing the loss of raw materials. After using the intelligent system, the output and quality of the workshop increased by 4.89 percentage points and 2.32 percentage points, steadily improving the production quality of the brewing workshop and reaching the expected goal. At the same time, the optimal temperature stabilized each discharge, thus contributed to efficient brewing production（Yu et al. 2017).

Future development of environmentally friendly and sustainable wine manufacturing

Currently, there is an array of more than twenty distinct enzymes that are integral to the wine industry, primarily including amylases, esterification enzymes, proteases, and xylanases. Despite their prevalence, these enzymes possess substantial instability, rapidly diminishing functionality in extreme heat and pressure environments and exposure to strong acids or bases. Therefore, there is a need to study enzymes that can maintain high activity under extreme conditions such as extreme acids and alkalis, thereby contributing to the sustainability of the agrochemical industry. Enzyme formulations have been shown effectively reduce greenhouse gas emissions within the wine sector, contribute positively to environmental conservation, and amplify productivity levels while simultaneously curbing waste production. Recently, with the increasing prevalence of China's global standing, enzymes are expected to play a crucial role in the progress of environmental conservation and sustainable practices within the wine industry. Below are some of the potential improvement areas for advancement:

Improve the utilization rate of raw materials

Enzymes can break down complex carbohydrates during the wine manufacturing process, increasing the conversion of raw materials, thereby reducing waste and reducing costs. The yield of raw materials with low yield can be improved by adding additional enzymes such as amylase, protease, and complex enzymes. Yang Yunshang discussed the influence of different conditions on wine yield. The results showed that under the condition of pH 5, the reaction temperature was maintained at 55℃, and the mixture of cellulase and pectinase at a concentration of 4.5mg/g, the ratio of which was 1:2, the maximum yield achieved after 120 minutes of reaction. The yield was increased by 25.56% compared to without the enzyme reaction under the similar conditions（Yun et al. 2013).

Waste disposal

Enzymatic agents can aid in treating byproducts from the vinification procedure. For instance, lees derived from white wines are abundant in organic constituents and represent an exceptional biomass resource of high quality, which holds significant importance for achieving sustainable and reduced carbon emission cycles in the wine manufacturing trade. Furthermore, these enzymes facilitate the decomposition of organic compounds found in wastewater generated throughout the vinification operation, thereby reducing effluent outflow and reducing environmental pollution.

Reduce energy consumption

Under standard conditions of room temperature and pressure, this remarkable enzyme enables reaction, thus minimizing energy expenditure throughout the wine manufacturing process. Typically, processes such as raw material cooking, alcoholic fermentation, alcohol distillation, concentration and drying. High energy usage is needed during ethanol manufacturing. The use of this thick mash fermentation method greatly reduces the use of steam. When the potency of ethanol in the fermented solution increased 1%, the distillation phase generated a large number of steam, conserving about 150 kg of carbon steam, thereby reducing carbon emissions and advancing sustainable progression（Qiang et al. 2014).

Development of new brewing technology

Enzyme technology can provide new possibilities for the wine manufacturing process, for example by catalyzing the fermentation of non-traditional raw materials, developing new wine manufacturing processes, and broadening the sources of winemaking raw materials.

Conclusion

Enzyme preparation has been widely used in food for hundreds of years because of its advantages of strong enzyme activity, small dosage and convenient use. In the process of saccharification, esterification, fermentation and curing, the application of enzymes plays an important role in improving the quality, taste and aroma of wine. At the same time, with the development of science and technology, new enzyme varieties and enzyme application technology continue to develop, providing more possibilities and innovation space for wine manufacturing process. Adopting energy-efficient technologies, reducing water waste and using renewable energy will help reduce the brewing industry's environmental impact during production. Liquor-making industry is an industry full of opportunities and challenges, need constant innovation, advance with The Times, to adapt to the change of market and consumer demand. Relevant production enterprises need to pay attention to the development trend of the industry, and actively adjust their strategies to achieve sustainable development.

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Author Contribution

Wang Sihan-Article writing, revision and editing;Li Jing-Literature research, experimental design;Song Leyi-Experiment, data analysis, chart making.

Conflict of Interest

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

Declaration on the Use of Generative AI

Authors declare no generative AI used in the manuscript

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