Research Progress on the Mechanism of Action and Screening of Acid-lowering Yeast in Wine

Yunhan Zhou*

School of Food Quality and Safety, Huazhong Agricultural University, Hubei, 430070 China

*Corresponding author: yunhanzhou1226@webmail.hzau.edu.cn

Abstract. The acidity of wine is a crucial factor affecting its quality and stability, serving as an important index for wine evaluation. The paper reviewed the application of acid-lowering yeast pathways and screening methods in wine, kiwi wine, and milone. As a biological acid-lowering method, acid-lowering yeast primarily relies on the lactose-lactate fermentation pathway and the malate-lactic acid fermentation route to reduce acidity. The method of acid reduction varies among the three types of beverages. Additionally, the paper explored the screening pathways for acid-lowering yeast. Beyond traditional screening methods, molecular biology techniques, gene editing techniques and adaptive evolution have further improved the screening efficiency and accuracy. By analyzing the fermentation conditions of different alcoholic beverages, different screening methods can be employed to improve the fermentation effect of acid reduction. Acid-lowering yeast has broad application prospects in the alcohol industry. Combined with gene technology to further study and innovate the yeast acid-lowering way, apply it to more alcohol, and promote the development and progress of the industry.

Keywords: Biological acid reduction; acid-lowering yeast; metabolic pathways; screening method; gene editing technology.

1. Introduction

In the brewing industry, the acidity of wine significantly influences the quality of the beverage. Organic acids not only reduce sweetness and enhance flavor but also extend the shelf life of the wine. Adequate acidity contributes to heightened thickness of body flavor of wine and enhances the tasting experience for consumers [1]. However, excessive acidity often leads to unfavorable taste characteristics. Thus, effectively controlling wine acidity poses a significant challenge in the brewing industry.

During alcohol fermentation, wine acidity is influenced by various factors. Organic acids primarily originate from fruit materials and dissolve into the wine during pressing and maceration processes, thereby increasing the total acid of the wine [1]. Additionally, acid substances may arise from yeast metabolic activity during fermentation, impacting the sensory characteristics of the wine. While conventional methods for acid reduction encompass physical, chemical, and biological approaches. Although these methods can reduce the acidity to a certain extent, they are low in efficiency, difficult to control and easy to be disturbed by the external environment [2].

The emergence of modern biotechnology has paved the way for innovative approaches to acid reduction. It is a new research direction to reduce the acidity of wine by using acid-reducing saccharomyces. Utilizing molecular biology and genetic engineering techniques, these yeast strains can be modified to mitigate acid production during fermentation, consequently reducing overall acidity [3]. Furthermore, with the development of gene editing technology, it has become possible to achieve the targeted reduction of acidity of acid-lowering saccharomyce. The advent of gene editing technologies has further empowered researchers to achieve targeted reductions in acidity levels, expanding the potential applications of acid-lowering saccharomyces in the brewing industry.

The review aims to discuss the mechanism of action and the research progress in screening of acid-lowering saccharomyce. It entails a comprehensive analysis of yeast metabolic pathways. It includes an overview of the metabolic pathways of yeast, the action mechanism of key metabolic enzymes, and the difference in acid reduction pathways in different wines. By comparing beverages with

varying acidity requirements, the review further explores optimization strategies for acid-lowering saccharomyce and assesses their applications and prospects within brewing production [4]. Through these endeavors, the review endeavors to furnish novel technical insights into the brewing industry, fostering the widespread adoption of acid-lowering saccharomyce to enhance the quality and competitiveness of alcoholic products.

2. Mechanism of Action of Acid-lowering Saccharomyces

The common biological method for reducing acidity in fermented foods involves leveraging the metabolic activities of microorganisms such as molds, lactic acid bacteria, or yeasts. The study found that mold can have a strong acid-lowering effect, but because most mold will produce toxins, such as ochratoxin A (OTA), the feasibility is low [5]. Lactic acid bacteria can reduce acid through malate-lactic acid fermentation route (MLF), but it is not applicable due to the high citric acid content in some fruit wine. Yeasts, particularly Saccharomyces cerevisiae, are the preferred organisms for biological acid reduction in alcoholic fermentation. A thorough understanding of the mechanisms by which these yeasts reduce acidity is essential for the development of enhanced yeast strains [5].

2.1. Introduction to Yeast Acid Reduction Pathways

2.1.1 Overview of Metabolic Pathways

In alcoholic fermentation, yeast strains capable of lowering acidity utilize biochemical pathways such as decarboxylation, aminolation, or assimilation to reduce the concentration of organic acids.

The decarboxylation pathway is the most common method in acid-lowering yeast. Yeast can convert organic acids (such as malic acid) into ethanol and carbon dioxide through decarboxylation reactions, reducing overall acidity. Additionally, yeasts can lower acid levels through aminidation reactions. Yeast uses amino compounds to react with organic acids to generate the corresponding amino acids, which reduce the acidity. This transformation typically occurs in nitrogen-rich environments, enabling yeast not only to alleviate acidity but also to facilitate nutrient conversion.

It has been documented that some yeast strains have been genetically engineered to utilize certain organic acids as carbon or energy sources, supporting their growth and reproduction. This method of assimilation directly consumes acids, effectively reducing the acidity of the medium. For instance, Liu et al. genetically modified Oriental yeast (Issatchenkia terricola) WJL-G4, which greatly reduced the content of citric acid in alcoholic drinks through the assimilation of yeast, while improving phenolic compounds and antioxidant activity [6].

Acid-lowering yeasts exhibit varied biochemical reactions under different fermentation conditions, often acting in concert rather than in isolation. By understanding these biochemical pathways, acid-lowering yeasts can be better selected and applied to meet specific industrial needs.

2.1.2 Mechanisms of Key Metabolic Enzymes

Lactate dehydrogenase (LDH) is the most important enzyme in the acid-reduction process, catalyzing the conversion of lactate to pyruvate. During this reaction, LDH oxidizes lactate while concurrently reducing NAD+ to NADH, a critical step in cellular respiration and energy production [7].

The reaction mechanism is as follows:

$$\text{Lactate} + \text{NAD}^+ \rightarrow \text{Pyruvate} + \text{NADH} \quad (1)$$

Furthermore, the function of LDH in acid reduction is supported by various auxiliary enzymes, including transporter proteins and other enzymes integral to cellular energy metabolism. These components collectively enhance the efficiency and effectiveness of the acid-lowering reaction, playing vital roles in maintaining cellular homeostasis.

2.1.3 Analysis of Differences in Yeast Acid Reduction Pathways Across Different Types of Alcohol

This paper explores the similarities and differences among acid-lowering yeasts utilized in the fermentation of wine, kiwi wine, and milone (Table 1).

Wine comes from grapes, with high sugar and acidity that often require acid-lowering yeast to reduce body acidity. Saccharomyces cerevisiae is employed for this purpose due to its capacity to ferment under acidic conditions and reduce acidity through MLF. The strains with strong acid reduction ability can be obtained through isolation and screening or genetic engineering technology. For instance, Cao Ying and colleagues isolated 63 microorganisms from commonly cultivated wines in Shandong, ultimately identifying five strains that demonstrated resistance to acid and ethanol while effectively reducing acidity [8].

Kiwi fruit wine, produced from the juice of orange kiwi fruits, is characterized by its low alcohol content and high native acidity, which can lead to bad flavor and a bitter aftertaste. In response to this problem, Zhong et al. selected a fermented Pichia JT 1-3 that can metabolize citric acid, and the acid reduction rate was 10.57% [9]. The use of mixed microbial fermentations has been shown to enhance the flavor profile of the wine.

Peosake, derived from whey fermentation, presents unique challenges due to the high nutrient and lactose content of whey. Most yeasts struggle to ferment lactose into alcohol, often resulting in low alcohol yields and poor alcohol tolerance, complicating the production of higher-alcohol serosake [10]. Ji et al. employed ultraviolet mutagenesis breeding technology to develop a yeast strain, QY-6, which can decompose lactose and reduce acidity, thereby decreasing the acidity of serosake by 10.7% and increasing the alcohol content by 41.8% compared to the original strain [11].

Table 1. Comparison of the three acid-lowering routes of alcoholic yeast.

The type of Wine	Raw Material	Yeast Type	Acid Lowering Pathway
Wine	Grape	Saccharomyces Cerevisiae	Malic Acid-lactic Acid Fermentation
Kiwi Wine	Yangtao	Pichia Yeast JT 1-3	Malic Acid-lactic Acid Fermentation Mixed Fermentation
Milone	Whey	Kluvis QY-6	Lactose-lactate Fermentation

2.2. The Impact of Yeast Metabolic Products on the Acidity of Alcohol

Yeast metabolites, including carbon dioxide, acetic acid, and other compounds, significantly influence the flavor and acidity of wine.

During alcoholic fermentation, yeast continuously produces carbon dioxide. However, due to the limited solubility of carbon dioxide, the formation of carbonated acid has little impact on the acidity of alcohol, and the appropriate carbon dioxide content can add a refreshing sour taste for some special alcohol such as sparkling wine and beer [12]. The solubility of carbon dioxide is influenced by temperature and pressure. In addition, some studies have shown that higher fermentation temperatures can lead to increased ester and higher alcohol content in beer, altering its taste. Conversely, low fermentation temperatures may hinder yeast activity and extend fermentation duration while potentially increasing internal pressure, which can adversely affect yeast vitality and the resulting flavor profile [13].

In conditions where there is anaerobic stress or contamination by non-target bacteria, the ethanol produced by yeast can be further metabolized into acetic acid. Excessive acetic acid not only

heightens the acidity of the wine but also causes sedimentation and produces off-flavors, such as rancidity [14]. In order to prevent the production of acetic acid, should do a good job in the environmental hygiene of the brewing distillery and personal cleaning and disinfection work, in addition to choose no mildew deterioration of starch high good raw materials, but also use good quality raw wine and fresh and normal leasse liquid. Additionally, selecting robust yeast strains that are well-suited to the specific conditions and raw materials of the fermentation can further refine the flavor and stability of the wine.

Yeast can also be other kinds of organic acids, the appropriate amount of acid can increase the wine body flavor. The effect of yeast metabolites on acidity depends on the specific type of fermentation, the yeast strains used, and the raw materials, etc. Reasonable control of these factors can optimize the taste and flavor of wine.

2.3. Overview of Other Acid Reduction Pathways

In addition to the biological method of acid reduction, the chemical and physical methods are also commonly employed, though each comes with significant drawbacks.

The chemical acid reduction method involves using chemical substances to mitigate the organic acids in alcoholic beverages. For instance, in the production of wine and fruit wines, calcium carbonate is frequently utilized to neutralize excessive organic acids. While effective, the use of strong alkaline substances in industrial settings can be hazardous. These chemical reactions can cause turbidity in the wine, altering its taste and color, and may introduce a high concentration of metal ions [15].

On the other hand, the physical acid reduction method offers alternative solutions. Ivic et al. have employed electrodialysis in fruit wine to enhance the efficiency and feasibility of acid reduction [16]. Similarly, Akyildiz et al. utilized ion exchange resins to decrease acidity in fruit wines [17]. However, this technique significantly impacts the volatile compounds in the wine.

3. Screening methods and optimization of acid-lowering yeast

3.1. Screening Method for Common Acid-Lowering Yeast

3.1.1 Traditional Screening Method for Acid-Lowering Yeast

In traditional experimental approaches, samples can be sampled by looking for sites or objects in nature that meet the growth conditions of acid-lowering yeast, and then sampled in culture. Samples are then collected and cultured. The initial screening of strains involves adjusting the pH of the culture medium to a higher level to domesticate the yeast. Upon achieving a certain level of acidity, liquor is introduced into the culture medium to select strains that thrive under high acidity conditions. For acid tolerance testing, yeast was inoculated in medium with different pH values and their outgrowth and fermentation performance were observed. The yeast strains that could remain active at lower pH values or the strains that increased the most pH, that is, good acid suppression ability, were selected for further analysis [16]. Further evaluate the growth ability and fermentation activity of yeast in a high acid environment (Figure1).

The selected strains were used for molecular identification and to complete the molecular phylogenetic information of their acid-lowering yeast. More tolerance experiments can then be further completed to determine the physiological and biochemical indicators of the colonies.

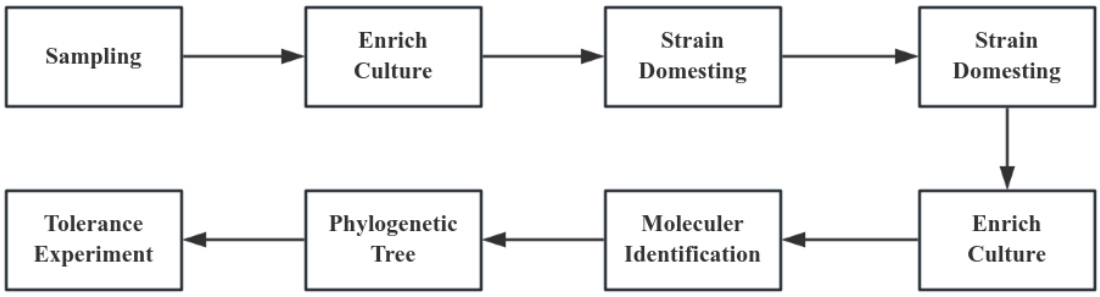

Fig.1 Traditional screening flow for acid-lowering yeast

3.1.2 Genetic Engineering and Mutagenesis Breeding Methods

With the development of genetic engineering and molecular biology, researchers have increasingly applied mutagenesis breeding technology to the screening of yeast strains. Common mutagenesis methods include physical, chemical, and compound mutagenesis [18]. For instance, using Atmospheric and Room Temperature Plasma (ARTP) mutagenesis breeding techniques, Feng et al. developed strain Ho C32-2-72, which achieved a 10.26% reduction in ethanol yield during fermentation [19].

However, most mutagenic agents, particularly chemical mutagens and radiation, are recognized as highly toxic carcinogens that may compromise product quality. These inherent defects also lead to genetic engineering mutagenesis breeding has become the most important development direction, combined with whole genomics, transcriptomics and other technologies can effectively improve the mutation frequency, providing new ideas and new methods for the development of acid-lowering yeast.

3.2. Optimization Strategies for Screening Acid-reducing Yeast

3.2.1 Application of Gene Editing Technology in Screening

Combining the screening of acid-lowering yeast with gene editing technology allows for precise knockout of genes associated with high acidity, altering specific processes in the acid metabolic pathway and further reducing the acidic products of yeast. The modified genes were introduced into the plasmid and followed the plasmid transformation into the host. These recipient cells are then cultured to select strains with positive mutations that meet the requirements for acid reduction. Alternatively, the modified genes can be subjected to agarose gel electrophoresis to select out the linearized fragments, ligated with T4 DNA ligase and form the plasmids, and then select the positive clones to further extract the plasmid DNA. Ms. Xie et al. used CRISPR-Cas9 mediated genome editing to transform Saccharomyces cerevisiae, resulting in a 10% reduction in acidity from the modified S. cerevisiae NaDUR1,2-Δ car fermentation [20]. In addition to knockdown, gene editing techniques can enhance the metabolism and decomposition of acidic products by increasing the expression of target genes through the insertion of enhancers or the use of strong promoters (figure2).

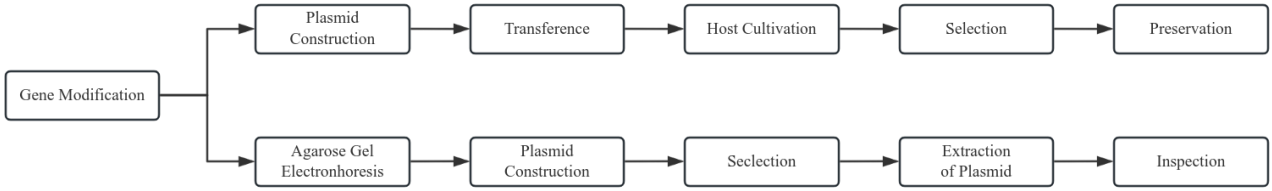

Fig. 2 Selection process for acid-lowering yeast in the application of gene editing technology.

3.2.2 Adaptive Evolution of Acid-reducing Yeast

Adaptive evolution of acid-lowering yeast refers to the method that selects engineered strains that exhibit excellent properties by giving a cell-specific pressure to evolve over a long time [21]. This

method is often utilized in industrial fermentation processes to optimize yeast performance, improve product quality, and enhance production efficiency. By applying environmental stress, adaptive evolution can screen for microorganisms with increased resistance and higher yields of the target product, thereby maximizing economic benefits [22]. Li Ziying et al. obtained the evolved strain CEN.PK 1B-7D 385 through adaptive evolution, which has demonstrated tolerance to high concentrations of furfural (4 g/L) and a reduced acidity [23].

3.3. Application of Acid-Reducing Yeastin Three Types of Alcohol

In recent years, acid-lowering yeast has been employed in the production of wine, kiwi wine, and milone. In wine, CRISPR-Cas9 technology is frequently used for gene editing of Saccharomyces cerevisiae to knock out or regulate genes associated with lactate and malate metabolism, thereby reducing the formation of these acidic substances [24]. For kiwi wine, the adaptive evolution method is commonly utilized, involving continuous subculturing in high-acidity media to select yeast strains that can adapt to the acidic environment. For milone, QY-8 and S. cerevisiae BY-6 showed strong acid reduction effect, reducing the production of acid substances.

3.4. Future Developments of Acid-reducing Yeast

According to the traditional method, the efficiency of yeast selection is low, and the selection conditions are complicated. Advances in gene editing technology have provided more efficient approaches for screening yeast. This method not only shortens the selection time but also broadens the development prospects of acid-lowering yeast. Future development should focus on integrating molecular biology, gene editing technology, and further screening of acid-lowering yeast to elucidate its metabolic mechanisms, optimize fermentation conditions, and improve acid-lowering efficiency.

Additionally, developing specific acid-lowering yeast strains tailored to different types of wine can lead to the creation of low-acidity, uniquely flavored wine products that meet consumer demands for diversified and personalized alcoholic beverages. The use of acid-lowering yeast also aligns with environmental protection requirements. Traditional acid reduction methods in alcohol production often rely on chemical additives, which can result in the overuse of chemical reagents and environmental issues. In contrast, the application of acid-lowering yeast aligns with the principles of green brewing by reducing the use of chemical agents and contributing to environmental protection. With the continuous progress of genetic technology, acid-lowering yeast is expected to become an indispensable tool in the wine brewing industry.

4. Conclusion

This paper examines the mechanism of acid-lowering yeast in wine and the screening methods for yeast, discussing the similarities and differences in the metabolic pathways of yeast across different alcoholic beverages. The acid reduction pathway is divided into physical, chemical and biological methods. As a biological way, acid-lowering yeast mainly reduces the content of organic acids by adjusting the biological metabolism of yeast, improves the flavor and taste of wine, and further improves the quality and stability of wine body. Different acid-lowering yeast have different mechanisms, and their metabolites can further affect the acidity of alcohol. Therefore, it is crucial to screen for yeast strains that are both efficient and suitable for specific alcoholic beverages.

Yeast screening methods include traditional approaches and molecular biology techniques. Studies have shown that gene editing technology and adaptive evolution can significantly enhance the efficiency and accuracy of yeast screening compared to traditional methods. With advancements in gene technology, future research should focus on elucidating the metabolic mechanisms of acid-lowering yeast and selecting more efficient strains by integrating metabolomics, molecular biology, and optimized fermentation conditions.

In conclusion, gene technology will improve the screening efficiency of acid-lowering yeast, leading to significant technological breakthroughs and development opportunities in the wine brewing industry.

References

[1] Xiaochun Yu, Jing Xu, Ming Li, et al. Progress on biological acid lowering mechanism and fermentation strategy in wine . Brewing in China, 2023, 42(08): 28-32.

[2] Bingbing Dong, Tian Fang, Jing Liu, et al. Progress on acid lowering process of fermented fruit wine and its influence on flavor . Food Industry Technology, 2023, 44(22): 368-376.

[3] Gao Y, Liu Y, Sun M, Zhang H, Mu G, Tuo Y. Physiological function analysis of Lactobacillus plantarum Y44 based on genotypic and phenotypic characteristics . Journal of Dairy Science, 2020, 103(7): 5916-30.

[4] Hirayama S, Furukawa S, Ogihara H, Morinaga Y. Yeast mannan structure necessary for co-aggregation with Lactobacillus plantarum ML11-11 . Biochemical and Biophysical Research Communications, 2012, 419(4): 652-5.

[5] Ge C, Sheng H, Chen X, Shen X, et al. Quorum sensing system used as a tool in metabolic engineering . Biotechnology Journal, 2020, 15(6): 1900360.

[6] Liu B, Yuan D, Li Q, Zhou X, Wu H, Bao Y, Lu H, Luo T, Wang J. Changes in organic acids, phenolic compounds, and antioxidant activities of lemon juice fermented by Issatchenkia terricola . Molecules, 2021, 26(21): 6712.

[7] Yingxue Zhong, Xiaofang Zhang, Ruiqun Yang, et al. Effect of acid-lowering yeast combined with lactic acid bacteria on the acidity of 'black tiger flavor' wine . Chinese and Foreign Grapes and Wine, 2019(06): 26-33.

[8] Ying Cao, Yujuan Zheng, Longxiang Liu, et al. Screening and fermentation performance analysis of excellent strains for malate-lactate fermentation in wine . Food Research and Development, 2021, 42(11): 138-144.

[9] Hong W, Chen T, Yang H, Li EH. Isolation and selection of non-Saccharomyces yeasts being capable of degrading citric acid and evaluation its effect on kiwifruit wine fermentation . Fermentation-Basel, 2020, 6: 25-25.

[10] Bajpai P. Utilization of cheese whey for production of α-amylase enzyme . Biotechnology, 1991, 18(3): 265-270.

[11] Tiepeng Ji. Breeding of cruwe yeast and process condition optimization of fermented sersake . Journal of Inner Mongolia Agricultural University (Natural Science edition), 2015, 36(03): 82-87.

[12] Choowong A. Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation . Journal of Bioscience and Bioengineering, 2017, 124(2): 133-42.

[13] Luzarowski M, Vicente R, Kiselev A, et al. Global mapping of protein–metabolite interactions in Saccharomyces cerevisiae reveals that Ser-Leu dipeptide regulates phosphoglycerate kinase activity . Communications Biology, 2021, 4(1): 1-15.

[14] Suryani AE, Anggraeni AS, Istiqomah L, Damayanti E, Karimy MF. Isolation and identification of phytate-degrading yeast from traditional fermented food . Biodiversitas Journal of Biological Diversity, 2021, 22(2).

[15] Tang BL, Yang J, Chen XL, Wang P, et al. A predator-prey interaction between a marine Pseudoalteromonas sp. and Gram-positive bacteria. Nature Communications, 2020, 11(1): 1-14.

[16] Ivić I, Kopjar M, Jukić V, Bošnjak M, et al Aroma profile and chemical composition of reverse osmosis and nanofiltration concentrates of red wine Cabernet Sauvignon . Molecules, 2021, 26: 874-874.

[17] Akyildiz A, Onur E, Agcam E, Kirit BD, Turkmen FU. Changes in quality parameters of orange juice deacidified by ion exchange resins. Food Chemistry, 2022, 375: 131837-131839.

[18] Mohammad PN, Meisam T, Mortaza A, et al. Progress toward improving ethanol production through decreased glycerol generation in Saccharomyces cerevisiae by metabolic and genetic engineering approaches . Renewable and Sustainable Energy Reviews, 2019, 115: 109353.

[19] Feng WQ. Breeding of Indigenous Non-Saccharomyces cerevisiae with Low Ethanol Yield [D]. Shanxi: Northwest AF University, 2022.

[20] Wenjuan Xie, Dianhui Wu, Xiaomin Li, et al. Construction of CRISPR/Cas9 mediated engineered bacteria producing low urea rice production . Food and Fermentation Industry, 2019, 45(13): 45-51.

[21] MIF. Study on Screening and Acidic Adaptive Evolution of ε-poly-L-lysine Producing Strains [D]. Jinan: Qilu University of Technology, 2019.

[22] Fong SS, Burgard AP, Herring CD, et al. In Silico Design and Adaptive Evolution of Escherichia coli for Production of Lactic Acid . Biotechnology & Bioengineering, 2010, 91(5): 643-648.

[23] Ziying Li, Wenshuang Du, Mengyan Li, et al. Adaptive evolution to improve furfural tolerance and its mechanism in Saccharomyces cerevisiae . Chinese Journal of Paper Making, 2023, 38(04): 24-30.

[24] Nobrega I, Pereira J, Paiva JE, et al. Ethylcarbamate in cachaça (Brazilian sugarcane spirit): Extended survey confirms simple mitigation approaches in pot still distillation . Food Chemistry, 2011, 127(3): 1243-1247.