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REVIEW ARTICLE

Comparative Study Between Mixed Culture of *Schizosaccharomyces Pombe* with *Saccharomyces* and Single Culture *Saccharomyces* on Wine End Product: A Systematic Review

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ABSTRACT

The wine industry has always been searching for a way to improve the quality of its produce. Lately, the trends of using a mixed culture of yeast to improve the wine qualities in the wine industry are increasing. The aim of this systematic review is to determine whether or not the mixed culture of S*chizosaccharomyces pombe* and *Saccharomyces cerevisiae* actually improves the quality of the wine**.** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used to conduct the systematic review. Studies performed from 2010-2020 were collected from MDPI, ScienceDirect, Semantic Scholar, and PubMed databases. A total of 54 studies are systematically reviewed in this paper. Pure Fermentation of *S. cerevisiae* produced generally acceptable wine qualities with desirable amounts of ethanol and an acceptable amount of secondary metabolites; however, in recent findings, *S. cerevisiae* cannot naturally degrade malic acid, leading to a too sour-taste wine. Meanwhile, pure Fermentation of *S. pombe* results in the high production of polysaccharide, pyranoanthocyanin, glycerol, pyruvic acid, urease, reduction of malic acid and gluconic acid, altogether considered as desirable traits in wine production. Mixed Fermentation with *S. cerevisiae* and proper strain selection of *S. pombe* are the solutions for the suppressed production of acetic acid, acetaldehyde, and acetoin, which are the undesirable compounds highly produced by *Schizosaccharomyces*. The hypothesis is proven to be true as mixed Fermentation of *S. cerevisiae* and *S. pombe* results in enhanced wine quality, especially contributed by the compounds produced from *S. pombe* fermentation.

Keywords: ***Mixed Culture Fermentation; Wine Quality; Saccharomyces cerevisiae; Schizosaccharomyces pombe***

HIGHLIGHTS

- Mixed culture fermentation of wine improves its quality compared to pure fermentation.
- Pure fermentation of *S. cerevisiae* and *S. pombe* produces acceptable amounts of ethanol and secondary metabolites (both good and bad).
- Mixed culture fermentation reduces the bad secondary metabolites production to make sweeter wines.

INTRODUCTION

Since the ancient period, wine production and consumption had taken a role, approximately between 8500 - 4000 BC (Varriano, 2011). The bioconversion in grape into wine was first studied by Louis Pasteur in 1866 and continues to be studied until now (Neil et al., 2014). Microorganisms (such as yeast, fungi, lactic acid bacteria (LAB), and acetic acid bacteria) have significant roles that contribute to wine fermentation, starting the process of harvesting, fermenting, packaging, and distributing. The exposure of grapes to the environment shows the naturally occurring microorganisms to contribute to the fermentation process (Lonvaud-Funel, 1999; Neil *et al*., 2014). Officially referred to as the wine yeast, *Saccharomyces cerevisiae,* they are utilized as the starter culture in the fermentation process. They are deeply correlated with the alcoholic fermentation process, which converts the grape's sugar into sugar ethanol and CO_2. A study also reported that *S. cerevisiae* yeasts are found to be dominant during both non-spontaneous and spontaneous Fermentation (Benito et al., 2016). At the same time, the non-*Saccharomyces* are reported as one of the contributors to wine spoilage (Padilla et al., 2016). Although, when the non-saccharomyces had been well-studied, results showed contrasting observations. For instance, *Wickerhamomyces subpelliculosus* are used for the low alcohol beer production, and S*chizosaccharomyces pombe* are found useful in the rapid malic acid deacidification, leading to flavor changes and health promotion (Basso et al., 2016). Yeast contributions are studied for an extended period to produce a flavorful and high-quality wine.

Unlike *Saccharomyces* yeast, problems arise when using non-*Saccharomyces* due to their inability to complete the fermentation process properly. *Schizosaccharomyces*, considered a promising non-*Saccharomyces* yeast, has been known to reduce the acidity in wines while transforming the L-malic acid into ethanol and carbon dioxide. This term is commonly known as deacidification. The reduction of malic acid and pH increase in the winemaking will impact the flavor. Therefore, winemaking that utilized *Schizosaccharomyces* resulted in a sweeter wine in some wine production. Researchers propose the use of the mixed culture of *S. cerevisiae* and *Schizosaccharomyces* to ensure there is no remaining residual sugar and produce a better wine quality (Benito et al., 2016). In this review, single culture fermentation is also referred to as pure fermentation.

The end-quality of wine products refers to the wine flavor. Wine flavors result from a series of complex interactions between chemical compounds inside the wines. Research has shown that about 1000 chemical compounds formulate both the taste and aroma of wine (Styger et al., 2011). Some measurable aspects of those compounds include but are not limited to acidity, sweetness, alcoholic strength, fizziness, astringency, and bitterness (Robinson, 1994). Along with the aroma produced by the wine, these aspects will be compared in this review.

Due to the uprising trend of using mixed culture fermentation in wine manufacture, this systematic review aimed to analyze the influence on performing mixed culture fermentation, specifically using the *Saccharomyces cerevisiae* and S*chizosaccharomyces pombe* yeast, towards the end-quality wine product. Alongside the analysis of the wine quality, the health aspects of using mixed culture fermentation will also be discussed. This systematic review provided a comparative analysis between the usage of *S. pombe* and *S. cerevisiae* in mixed culture fermentation and pure fermentation.

METHODS

This systematic review was conducted using the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Moher, 2009). It was hypothesized that the usage of *S. pombe* in mixed culture fermentation would enhance the quality of the wine.

Search strategy

Systematic searches were conducted using the following databases: MDPI, ScienceDirect, Semantic Scholar, and PubMed. In addition to these databases, searches were also performed through Google Scholar for the secondary references. The keywords used for the systematic searches were classified according to the subtopics discussed: (i) general information: mixed fermentation, non-Saccharomyces, *Schizosaccharomyces pombe*, and *Saccharomyces*; (ii) quality of wine: red wine, white wine, wine color, flavor and aroma, phenolic compounds, tannins, anthocyanins; and (iii) health aspects: type 2 diabetes, cardiovascular disease, biogenic amines, histamine, resveratrol. Meanwhile, the studies are screened further based on the title corresponding to one or more of the keywords searched.

Eligibility criteria

Selected studies are limited to those published in the recent 10 years, up to December 2020. The included studies are only those delivered in English and in the form of research articles, research reviews, and books. Studies were included based on the inclusion criteria that contained the relevant information stated in their abstract. The discussion of the studies, considered as the variables for the systematic review, should have either (i) the general information of yeast (*S. cerevisiae* and non-*saccharomyces*), (ii) the attributes of using mixed culture fermentation, (iii) factors affecting the quality of the wine (i.e., metabolites), (iv) the desirable wine characteristics; such as flavor and aroma, and (v) the health impact on the usage of *S. pombe* in wine fermentation.

Data extraction

The collected studies were reviewed using Mendeley and Google Sheet, similar to Excel. The Mendeley software helped to organize the collected studies from the databases and search for duplicates. Data extractions were conducted through analyzing the full-text articles with the following components: title, year of publication, types of fermentation conducted, *Saccharomyces cerevisiae* and/or *Schizosaccharomyces pombe*, red and/or white grape must as substrate, and the relevant discussions reported that corresponds to the quality of the wine in respect to the flavor, aroma, color (appearance), and acidity. The selected references are categorized as primary references, while the secondary reference and *others* are used as supporting references. In addition, the secondary reference and *others* were included for in-depth discussions (Supplementary Table 1-Reference).

RESULTS AND DISCUSSION

Analysis of the included studies

The analysis flowchart uses the PRISMA template, and a total of 53 studies published between 2010 and 2020 are systematically reviewed in this study (Fig.1). The excluded articles are due to their insufficient information regarding the *Schizosaccharomyces pombe* performance in mixed fermentation, health-related compounds produced, and its influence on the wine quality in respect to the flavor, aroma, and color. A total of 22 studies are considered as secondary references, in which they are obtained from the primary reference or searched by the individual authors for an in-depth discussion (Supplementary Table 1-Reference).

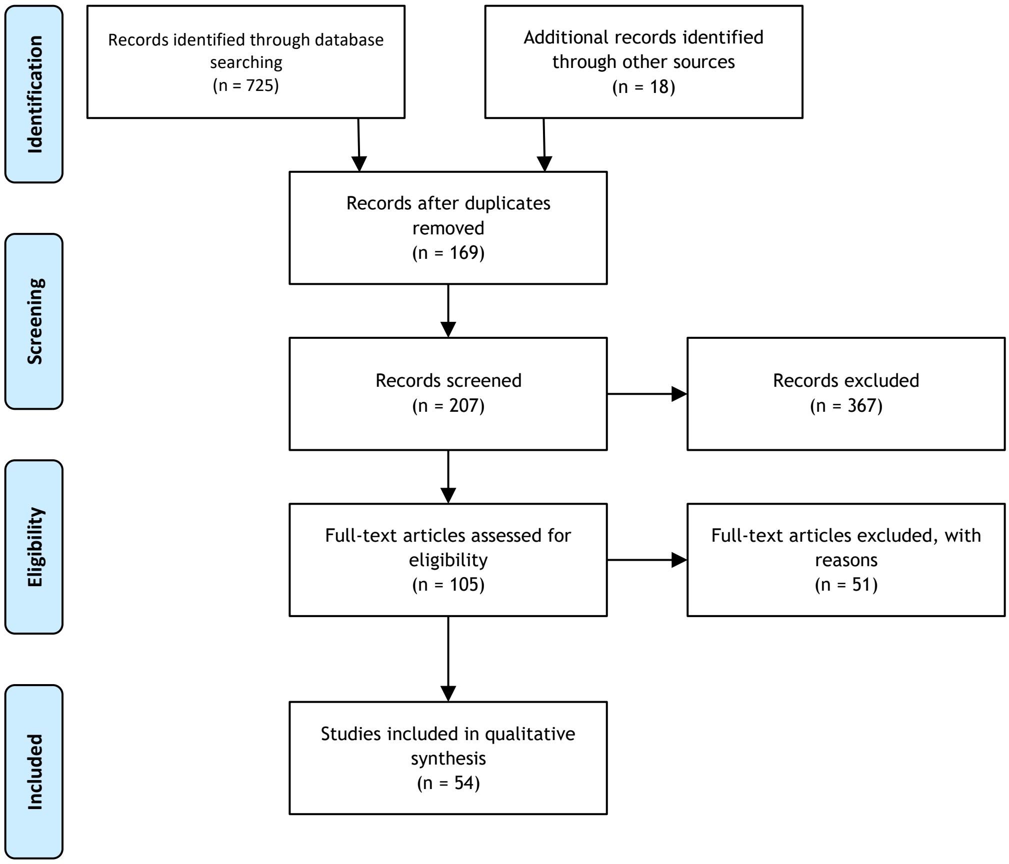

Figure 1. Flowchart of systematic review for the comparison of mixed culture fermentation *S. pombe/S. cerevisiae* and single culture fermentation *S. cerevisiae*.

Quality of wine

Wine quality is assessed by a sensory test covering flavor, aroma, texture, astringency, and taste (mouthfeel and after taste) (Grohmann et al., 2018; Heras Roger et al., 2016; Hornedo Ortega et al., 2020; Li & Sun, 2017). The most important parameter determining a good quality is by assessing wine appearance and the organoleptic properties. All of these are closely related to the phenolic compounds, including anthocyanins, flavan-3-ols, flavonols, stilbenes, and wine acids (Hornedo Ortega et al., 2020; Puértolas et al., 2010). Sensory test wine will be carried out after the downstream processing (filtration and maturation) to ensure the taste and end quality.

Flavor and aroma. The organoleptic of wine is the most important parameter for assessing wine quality. Several factors affecting the flavor and aroma of wine come from the overall processes, including vinification, maturation, aging, and the raw materials used. It is worth noting that the final various odor active components (OAC) concentration depends on the yeast fermentation (Hornedo Ortega et al., 2020; Mina & Tsaltas, 2017).

The influence of wine aroma on yeast during the fermentation starts from the biocontrol of the yeast; de novo biosynthesis of the alcoholic fermentation along with the natural grape flavor that activates the flavor and aroma. Lastly, post-fermentation conditions also influence the malolactic fermentation associated with bacteria spoilage and alteration of the wine quality. Not only limited to this, but studies by Cordente et al. (2012), McRae et al. (2019), and Mina & Tsaltas (2017) also reported yeast strain that affects the formation of various compounds contributing to wine flavors. Moreover, reported compounds, such as methoxypyrazine, ester, aldehydes, and ethanol, produce distinct aromas, namely floral, pepper, fruit, woody, etc. (Bloem et al., 2006; Styger et al., 2011; Wang et al., 2018). These compounds affect the quality and organoleptic of wine by having a balanced acidity flavor, sweetness/bitterness, and alcohol through complex interactions, hence, affecting the wine end-quality (Wang et al., 2018).

Texture and taste. The taste of the wine with high quality should be a balance that affects the mouthfeel. Tannin, one of the phenolic compounds that contribute to the taste of the wine, causes the astringent and bitter taste in the wine (Teissedre & Jourdes, 2013; Szabo, 2013). The balance of the astringent and bitter taste of red wine is found to be suitable for wine to be served with fatty or salty food (Szabo, 2013). It is worth noting that the bitterness of the wine is impacted by the ethanol content, whereas too bitter tastes are not desirable. Moreover, too high alcohol produced during the fermentation will cause gustatory disequilibrium, which causes an unbalance in the wine taste (Jordão, Vilela & Cosme, 2015). Tannins levels are suggested to decrease along with the increase of aging processes to minimize the bitterness (Ma et al., 2014; Teissedre & Jourdes, 2013).

Acidity in the final red wine products also impacts the quality, as the acceptable range of acetic acid presence should be in the range of 0.3 g/L to 0.8 g/L. Otherwise, too high an acetic acid concentration will make the wine taste like vinegar (Fernández de Ullivarri et al., 2011). Different ranges of wine texture impact the final sensory and consumer preferences. The soft wine texture is achieved due to the presence of high polysaccharides in the wine composition (Benito et al., 2018a). Therefore, a balance in tannins, acids, ethanol, and polysaccharides needs to be achieved to produce good quality wine.

It is reported that the main function of anthocyanins is tightly correlated with the wine color intensity (Azevedo et al., 2020; McRae, Teng & Bindon, 2019). Yet, the anthocyanin's properties that contribute to the taste and texture of wine are still controversial (Ferrero-del-Teso et al., 2020; Ma et al., 2014). Studies by Ferrero-del-Teso et al. (2020) and Gonzalo Diago (2014) conclude that anthocyanins may have contributed to astringency and bitterness. Thus, how anthocyanins may affect the astringency and bitter taste will depend on its derivatives on the wine, as some of the wine may have a bitterness suppressor.

Appearances. The color of wine had been known to be influenced by several factors, such as the phenolic (i.e., anthocyanins and tannin) compounds in the grapes and the viticultural factors. As the color of red wine is taken from the grape's skin, several parameters should be considered. Anthocyanin's color depends on the pH of the solution. In a pH environment of <2, it will appear red. Meanwhile, the blue quinoidal base will dominate in the higher pH (4.5-6). The decrease of anthocyanins due to the reduction process will result in the formation of stable wine pigments derived from anthocyanins, which are proanthocyanidins and pigmented polymers. These may as well be formed and affected due to the yeast metabolites and fermentation temperature, which will contribute to the overall wine color and stability (McRae et al., 2019). The interaction between anthocyanins-tannin influences the color properties by the formation of a stable color of the wine (García-Estévez et al., 2017; Nel, 2018).

The concentration of anthocyanins and tannins in grapes is also affected by viticultural factors: genetic origin, sunlight exposure, ripeness of the grape, temperature, soil type, and the plant's water source/ quality. Delayed harvest has the potential to increase both concentrations as it is more extractable, leading to the increase of wine color. Moreover, studies found the increase of UV exposure and less water given will give the same result in the increase of anthocyanins and tannins (McRae et al., 2019; Nel, 2018).
Winemaking conditions, such as oxygen exposure, maceration, additives, and aging, may contribute to the wine color, aside from the effect of raw materials. The addition of potassium or sodium metabisulfite is known to prevent wine spoilage and preserve the natural color (Day, 2015; McRae, 2019).

Single culture fermentation using *S. cerevisiae*

Saccharomyces cerevisiae is a single-cell fungus that has 16 chromosomes that organize 12,068 kilobases of nuclear genomic DNA. It has been widely used in winemaking due to its fermentative ability, mainly to ferment hexose sugars, such as glucose, fructose, and mannose, with glucose fermentation being the most favored by the yeast (Johnson & Echavarri-Erasun, 2011). In wine fermentation, *S. cerevisiae* is responsible for the determination of the chemical and sensory of the wine due to its presence as predominant yeast in the fermentation process (Camarasa et al., 2011). Genetic variability between *S. cerevisiae* will induce a flavor and aroma shift in wine end products due to a significant change in volatile compounds produced by different strains (Callejon et al., 2010). The genetic variability will also determine how each strain will have different susceptibility to different stress conditions induced in the fermentation process. *S. cerevisiae* has the ability to grow and strive between 8-12 % ethanol (v/v), while temperature shift and the presence of unsaturated fatty acid could improve its alcohol tolerance. *S. cerevisiae* in wine fermentation may produce secondary fermentation metabolites that will be separated into two distinctive groups of compounds that are categorized as general wine composition and flavor compounds.

Fermentation parameters. There are a few fermentation conditions that are favored by the *S. cerevisiae;* thus, when the conditions are fulfilled, the viability and growth rate of the yeast will be better. Growth factors are required by the *S. cerevisiae* to be taken up during biosynthesis in order for them to fully perform the fermentation rather than dividing their metabolic process to produce growth factors. Growth factors help *S. cerevisiae* to perform specific catalytic or structural processes. Biotin, pantothenate, inositol, thiamin, and pyridoxine are the growth factors that could affect the growth and cell division of *S. cerevisiae*. Minerals, such as zinc, phosphate, and magnesium, also play an important role in determining the fermentation rate. Zinc and magnesium deficiencies led to slow and ineffective fermentation, while phosphate is responsible for the production of ATP, phospholipid, and nucleic acid (Udeh et al., 2014). *S. cerevisiae* could grow in temperatures ranging from 5-40^{o}C; however, the most optimum growth rate is achieved at 25–35^{o}C (Fleet, 2011). Oxygen is another vital parameter in maintaining the viability of *S. cerevisiae,* but it could be tweaked to enhance the alcoholic fermentation rates by limiting the oxygen concentration. On the other hand, anaerobic conditions would cause the build-up of metabolites that would eventually stop cell growth. The water activity of a_w 0.89-0.92 is required for optimum *S. cerevisiae* growth as water activity lower than that will trigger glycerol synthesis, which lowers the osmotic pressure difference of the yeast plasma membrane that would lead to polyol leaks into the medium (Stewart, 2014).

Fermentative ability of *Saccharomyces cerevisiae*. In wine fermentation, *S. cerevisiae* often becomes the predominant yeast species as observed on the surface of the winery equipment (Padilla et al., 2016). Most of the glucose present in the grapes will be processed through glycolysis by *S. cerevisiae* to form pyruvate sequentially. The pyruvate will then be processed in alcohol fermentation to produce ethanol. The amount of ethanol will constitute the alcohol concentration inside the wine. Each strain of *S. cerevisiae* has different tolerance to ethanol and other compounds added during fermentation could also improve the strain tolerance to alcohol. Nearing the end of the fermentation process, *S. cerevisiae's* ability to ferment fructose will determine the ability to maintain a high rate of fermentation, as at the later stages of wine fermentation, most glucose would already be consumed, and fructose would act as the alternative sugar source (Eldarov et al., 2016). *On the other hand, S. cerevisiae's* low sugar uptake rate will result in the incomplete sugar fermentation that produces wine with low ethanol and microbiologic stability. The selection of suitable grapes is also essential as the grape natural inhibitors' content, such as lipid and vitamins, could potentially affect the viability and metabolism of *S. cerevisiae* (Mendoza et al., 2018). In the fermentation process, various stress factors are implied on the *S. cerevisiae.* The *S. cerevisiae* must endure osmotic stress, high acidity content, ethanol, sulfite content, temperature fluctuation, low oxygen content, and deficient nitrogen content. Lastly, the nitrogen content affects the rate of how the fermentation is carried out and the content of the volatile compound, which accounts for the wine flavor and aroma (Marsit and Dequin, 2015).

Compounds produced by *Saccharomyces cerevisiae*. In order to produce desirable organoleptic wine attributes, various strains of *S. cerevisiae* were utilized, as different strains will result in different types and amounts of secondary metabolites produced (Barrajón et al., 2011; Scacco et al., 2012). Overexpression of the secondary metabolites such as malic acid could produce an off-balance wine with an unpleasant sour taste. The overexpression could be avoided if there is an effective degrader of the malic acid compounds;

however, *S. cerevisiae* cannot effectively degrade malic acid. Thus, an unpleasant sour wine could be produced in a single culture fermentation. In order to avoid off-flavor wine production, the addition of biological deacidification such as *S. pombe* is recommended. Sulfite (SO2) Is often added to the fermentation as an antioxidant and antimicrobial agent; thus, the utilization of *S. cerevisiae* strains that have sulfite resistance is important to ensure a successful fermentation process (Divol et al., 2012). In nitrogen deficient media, another sulfuric compound will be produced due to the catabolization of the sulfuric amino acid of the yeast producing H_2S (hydrogen sulfide). The amount of H_2S compounds produced varies between *S. cerevisiae* strains. H_2S is an undesirable compound in wine end products as it induces a rotten egg odor into the product (González-Barreiro et al., 2014). This problem could be resolved by providing fermentation media with sufficient nitrogen sources, such as ammonium phosphate.

Fernández de Ullivarri et al. (2011) evaluated 5 different *S. cerevisiae* strains isolated from 2 wineries in Argentina's northwest region. The strains were isolated from the winery's equipment surface and analyzed for their oenological properties, such as the ability to produce glycerol, acetic acid, and tartaric acid, which are categorized as the general wine composition. The glycerol produced was about 4.4-5.1 g/L which is enough to enhance the flavor and wine taste but too low to affect the wine body. Glycerol is the third most-produced metabolite in wine fermentation after CO_2 and ethanol. Glycerol is produced through the redox-balancing solution or as a solute osmotic stress response. The acetic acid produced was about 0.38-0.68 g/L, which is below the 0.8 g/L mark where a vinegar flavor could be perceived, but enough to induce a complexity improvement of the flavor compound, such as ester. The tartaric acid produced was about 3.66-4.46 g/L which has the ability to enhance microbiological stability, wine color, and taste. The strains have also been shown to have high resistance to antimicrobial compounds and produce a negligible amount of H_2S compounds. It also produces high ethanol content and has a high yield with each fermentation exceeding (10% v/v ethanol) and 39.34-48.59 P_g/S_g yield.

Aside from the general wine composition, wine also consisted of flavor compounds that account for the odor and taste of the wine. The compounds originated from the *S. cerevisiae* metabolism during fermentation and some from the grape itself and wine maturation process. According to Walker & Stewart (2016), wine flavor compounds are differentiated into 5 general groups: higher alcohol, esters, fatty acids, phenols, and carbonyl compounds. Higher alcohol, usually known as fusel oils, is produced through the Ehrlich Pathway, a deamination and decarboxylation route of excess amino acid. The Ehrlich Pathway will turn into an anabolic system deriving higher alcohol from α-keto acid intermediates when the system is deficient in amino acid. Higher alcohol favors higher temperature; thus, fermentation with higher temperature yields more higher alcohol. Higher alcohol imparts desirable flavor and aroma to wine when present in the threshold value. The threshold value differs for different higher alcohol. Isoamyl alcohol and 2-phenyl ethanol are 2 of the most produced higher alcohol with 60-180 mg/l and 25-105 mg/l threshold, respectively. Both higher alcohols impart desirable sensory value to the wine end product. 2-phenyl ethanol has been reported to be responsible for rose flavor in the wine.

Esters also produced fruity/floral flavor and aromas in wine fermentation. It is usually produced in the fermentation process between alcohols and acyl-CoA reactions. Its amount depends on the number of alcohols and acyl-CoAs present in the fermentation process. However, some esters (i.e., ethyl lactate) are synthesized during malolactic fermentation, not depending on the reaction of alcohol and acyl-CoA. Aside from promoting ester production, acyl-CoA derivatives are hydrolyzed to form fatty acids. Fatty acids impart fatty, cheesy, rancid, and fruity notes to wine and improve its complexity when present in its threshold value (Liu et al., 2019). Phenolic compounds produced by *S. cerevisiae* are often considered POF (Phenolic Off-Flavors) that produce undesirable phenolic flavor and aromas. However, 4-vinylphenol and 4-vinylguaiacol are two phenols that are naturally present in the wine and constitute the formation of aroma characteristics in wine. Both compounds are produced by *S. cerevisiae* through the conversion of P-coumaric acid, hydroxycinnamic acid, and ferulic acid to 4-vinylphenol and 4-vinylguaiacol. Carbonyl compounds also constitute the flavor compound that makes up the wine flavor and aroma. Carbonyl compounds in wine are present in different forms; acetaldehyde, acrolein, ethyl carbamate, formaldehyde, and furfural, with acetaldehyde being the compound that is present in the largest amount. Acetaldehyde is usually present in Syrah wine from Brazil at a concentration of 213 µg/L. Acetaldehyde is metabolized in the wine fermentation process via the pyruvate decarboxylase and alcohol dehydrogenase (Jackowetz et al., 2011). Alcohol oxidation pathways of acetic acid bacteria, as well as storing and aging, could also produce acetaldehyde compounds in wine. Wine astringency, aroma, and color are modulated by acetaldehyde as long as the threshold value of (51-635 µg/l) is not surpassed. Sulfur dioxide (SO_2) could be added to regulate the amount of acetaldehyde present in the wine as SO_2 when induced in the wine with pH 3-4 would spontaneously turn into bisulfate ion (HSO_3^-), which bind with acetaldehyde and form α-hydroxyethanesulfonic acid (Lago & Welke, 2019).

Mixed culture fermentation using *S. cerevisiae* and *S. pombe*

Schizosaccharomyces pombe, which is a well-known fission yeast and spoilage microorganism, is unicellular and has a rod shape with a size of 3–5 × 5–24 µm (Benito, Calderón, & Benito, 2016). Strains of *S. pombe* are not commonly found, and ABCD is the reported strain available for winemaking. With high fermentative power, the *S. pombe* yeast is able to perform fermentation in low water activity, high level of sugar environment, a low pH range between 3.0-3.6, and a wide range of temperature; commonly at room temperature (Benito, Palomero, Gálvez, et al., 2014; Benito, Calderón, & Benito, 2016; García et al., 2017; Liu et al., 2019; Loira et al., 2018; Mylona et al., 2016). However, the less occurrence and available commercial strains in the market have become one of the limitations on the application of *S. pombe* within the wine industry (Benito et al., 2018a). Moreover, the production of high volatile acidity concentrations is considered undesirable in winemaking. Nevertheless, the problem can be overcome through proper strains selections and the mixed or sequential Fermentation with *Saccharomyces cerevisiae*. Thus, the application of *S. pombe* can be a great advantage within the wine industry in the future.

Compounds produced by *Schizosaccharomyces pombe*. *Schizosaccharomyces pombe* has several advantages in the process of winemaking, contributing to the food's safety through malic acid degradation and urease activity: maintaining and improving the wine's quality through elevated production of pyruvic acid, polysaccharide, pyranoanthocyanin, and glycerol. Amongst other studied non-saccharomyces yeast, the *S. pombe* constantly results in its strong fermentation ability and malic acid degradation/deacidification (Bañuelos et al., 2016; Benito et al., 2018a). Malic acid, one of the primary acids in wine, contributes to the sourness in the wine taste. An excessive amount of malic acid in the process of wine fermentation was studied to lead to wine spoilage, often caused by lactic acid bacteria that promote malolactic acid fermentation. Winemakers often wish to avoid malolactic fermentation caused by natural lactic acid bacteria (LAB) due to biogenic amines and histamine production (Del Fresno et al., 2017, Mylona et al., 2016). Histamine, a biogenic amine, has become one of the most considerations on wine’s food safety due to the allergic reaction it causes towards the consumer. The risk of histamine production in wine can be eliminated when fermenting using the *S. pombe* yeast, as malolactic fermentation is inhibited. The LAB uses the malic acid to perform the malolactic fermentation, in which the biogenic amines are synthesized (Benito et al., 2018a). Thus, the malic acid degradation performed by *S. pombe* is associated with increased wine quality and food safety. The urease activity performed by *S. pombe* helps to maintain wine safety by reducing the urea concentration occurring in the fermentation process. The low concentration of urea in wine can minimize the growth of LAB, which eventually leads to a lower concentration of biogenic amines. Furthermore, urea is a precursor for the formation of ethyl carbamate, a carcinogenic compound that is unwanted in wine quality. Hence, *S. pombe* is a powerful yeast in maintaining wine food’s safety due to the reduction of toxic compounds, such as ethyl carbamate and biogenic amines (Benito et al., 2012; Benito et al., 2018a; Mylona et al., 2016). In terms of wine quality, high polysaccharides release in the aging over lees, or a process of wine maturation, increases the wine mouthfeel quality (Benito et al., 2018a; Mylona et al., 2016). Benito et al. (2018a) mentioned that the high polysaccharide release causes the inhibition of "inhibit negative sensory sensations produced by astringent compounds resulting in rounded soft wines''. Good wine quality results from the quality of the good grapes. Gluconic acid is often used as an indicator of grapes quality, whereas a high concentration of gluconic acid decreases wine quality. The presence of gluconic acid higher than 1 g/L causes off-flavors and microbial de-stability. *S. pombe* is able to decrease the gluconic acid concentration and reduce the volatile spoilage compounds, resulting in higher wine quality compared to wine that has high gluconic acid (Benito et al., 2018a). The ability of *S. pombe* to produce a high concentration of pyruvic acid and pyranoanthocyanin is associated with the stabilized color of the red wine during Fermentation (Benito et al., 2012; Benito, Jeffares, et al., 2016; Benito et al., 2018a; Del Fresno et al., 2017; Kulkarni et al., 2015; Mylona et al., 2016). The production of pyruvic acid during the fermentation process promotes the formation of a compound called vitisin A that has a role in maintaining the stability of the wine's pigment (Benito et al., 2018a). This attribute is considered as an advantage in using the *S. pombe* yeast. Moreover, the study by Benito et al. linked high glycerol production through the glyceropyruvic pathway with the production of pyruvic acid. Not only that it assists the production of pyruvic acid, but the mouthfeel in sensory profiles or mouth smoothness of the wine is also developed from the glycerol compounds (2016; Benito et al., 2018a).

On the other hand, there are undesirable compounds produced by *S. pombe*, such as acetaldehyde, acetic acid, and acetoin. In this study, these compounds will be referred to as the negative oenological characteristics. Although the formation of acetaldehyde within a relatively low adequate amount is associated with buttery aroma and red wine color stability (Benito, Palomero, Gálvez, et al., 2014; Del Fresno et al., 2017), an excessive amount of acetaldehyde produced by *S. pombe* is causing undesirable off-flavor of the wine, especially white wine (Benito, Palomero, et al., 2013; Benito, Jeffares, et al., 2016; Benito, 2019; Del Fresno et al., 2017; Liu et al., 2019). In the production of red wine, acetaldehyde produced by *S. pombe* can be considered tolerable due to its ability as a precursor for vitisin B in stabilizing the wine color (Benito, Palomero, Gálvez, et al., 2014). However, the high yield of acetic acid produced by *S. pombe* in pure culture causes the off-flavor in the wine quality (Benito, Jeffares, et al., 2016). A concentration of acetic acid above 0.8–1 g/L will cause a vinegar-like taste causing the off-flavor wine tasting, as reported by (Benito et al., 2018a). Meanwhile, unselected strains of *S. pombe* are studied to produce acetic acid above 1 g/L (Benito et al., 2018a). A high concentration of acetoin is often reported as a cause of off-flavor in wine produced by *S. pombe* (Benito, Jeffares, et al., 2016; Liu et al., 2019). The oxidation of acetoin will derive the compound into diacetyl and 2,3-butanediol, as they are often associated with the off-flavor in wine (Ciani et al., 2010).

Application of mixed culture fermentation. Recently, the wine industries are studying the method of mixed and sequential fermentation of *S. pombe* and *S. cerevisiae*. A mixed fermentation method uses the same inoculation time for both strains. In contrast, the sequential fermentation method uses different inoculation times in which the *S. cerevisiae* culture will be inoculated after the inoculation of the *S. pombe* culture (García et al., 2017). Amongst other studied non-saccharomyces yeast, the *S. pombe* yeast constantly results in its strong fermentation ability. It is also believed that performing mixed and sequential fermentation will increase the wine fermentation efficiency, reducing the undesirable compounds produced by *S. pombe* yeast, and enhancing the aromatic properties of wine from the yeasts' interactions (Benito, Palomero, et al., 2013; Benito, Palomero, Gálvez, et al., 2014; Ciani et al., 2010; Liu et al., 2019; Mylona et al., 2016). Research proposing the mixed Fermentation of *S. pombe/S. cerevisiae* used immobilized *S. pombe* cells to allow the uptake of remaining malic acid, while *S. cerevisiae* performed the must fermentation with nearly all the available sugars. The ProMalic®, the immobilized *S. pombe*, is the only *S. pombe* commercialized on the market. It supports that *S. pombe* carries malic deacidification and minimizes the production of negative oenological characteristics (Ciani et al., 2010). A research study conducted by Benito, Palomero, Gálvez, et al., (2014) suggested the usage of *S. pombe* 938 strains in mixed and sequential fermentation with

S. cerevisiae to be an alternative to the pure fermentation that solely uses *S. cerevisiae* (Table 1.). The *S. pombe* in their study resulted in a major decrease of malic acid, urea concentration, and alcohol content; and high glycerol content that leads to the higher pyruvic acid concentration—specifically concerning the polysaccharide compounds, the mixed Fermentation of *S. pombe/S. cerevisiae* resulted in the high production of polysaccharides and glycerols that led to the increase of mouth-fullness, richness and aromatic persistence, and color stability of the wine, as studied by (Romani et al., 2010). The application of mixed Fermentation of *S. pombe/S. cerevisiae* can as well be applied to reduce the negative oenological characteristics of *S. pombe*, namely the reduction of acetic acid and acetoin (Benito, Palomero, Gálvez, et al., 2014; Ciani et al., 2010; Liu et al., 2019).

Table 1. Strains used in the research studies

	Research Studies	*Schizosaccharomyces pombe* Strains	Type of Fermentation	Substrate	Distribution (Location)
1	García et al., 2017	CLI 1079	Sequential	*Vitis vinifera* L. grapes	Madrid, Spain
2	Roca-Domenech et al., 2018	*S. pombe* strain CECT11197	Fed-batch	Riesling must	Tarragona, Spain
3	Benito et al., 2016	JB899, JB873, JB917	Pure	Wine, Brewer's yeast	Malta & Sweden
4	Liu et al., 2018; Liu et al., 2019	*Schizosaccharomyces pombe* 3796 (SP3796), *Schizosaccharomyces pombe* 70572 (SP70572)	Pure, Sequential, Mixed	Bilberry fruit	Braunschweig, Germany
5	García et al., 2017	*S. pombe* CLI 1085	Pure, Sequential, Mixed	*Vitis vinifera* L. grapes	Madrid, Spain
6	Ciani et al., 2010	ProMalic®	-	-	-
7	Bañuelos et al., 2016; Benito et al., 2013; Benito, Palomero, Gálvez, et al., 2014; Del Fresno et al., 2017; Kulkarni et al., 2015; Loira et al., 2018; Mylona et al., 2016	938	Pure, Sequential, Mixed	*Vitis vinifera* L. grapes	Madrid, Spain
8	Benito et al., 2012	936, 938 and 2139	Pure	*Vitis vinifera* L. grapes	Madrid, Spain
9	Mylona et al., 2016	V1 and 4.2	Pure	*Vitis vinifera* L. grapes	Madrid, Spain
10	Benito, Palomero, Calderón, et al., 2014; Scansani et al., 2020	15.4, 4.5, 31, V1 and V2	Pure	14 honeycombs, 21 unpasteurized organic honeys, 7 concentrated grape musts, white grape juice	Madrid, Spain

Additional solutions to reduce the production of the negative oenological characteristics. Aside from the application of mixed culture fermentation, the proper selection of the *S. pombe* strains and fed-batch technique will allow decreasing or eliminating the negative oenological characteristics of winemaking by *S. pombe* (Table 1.). The proper selection of the *S. pombe* strains can be performed through the isolation of the strains in a selective medium. The selective medium is formulated as actidione up to 100 mg/L as an antibiotic, benzoic acid as an inhibitory agent, malic acid for its malate reductase activity as a differential agent, and SO_2 up to 120 mg/kg at pH 3.5. The incubation should be performed at 30°C (Benito, Gálvez, et al., 2013; Benito, 2018b; Loira et al., 2018). Several selected *S. pombe* strains are studied for having the ability to produce low concentrations of acetaldehyde and acetic acid (Benito et al., 2012; Benito, Palomero, et al., 2013; Benito et al., 2018a; Benito, 2019; Mylona et al., 2016). The fed-batch technique can be applied to the fermentation of wine as it was proven to significantly decrease the acetaldehyde and acetic acid formation (Benito, 2019; Roca-Domenech et al., 2018). In the study conducted by Roca-Domenech et al. (2018), the fed-batch technique successfully eliminated acetic acid while maintaining a high level of glycerols. The study suggested that the change of *S. pombe* and *S. cerevisiae* metabolisms were induced by osmotic stress through the fed-batch fermentation, causing the significant reduction of acetic acid and acetaldehyde. Therefore, the combination of selected *S. pombe* strains and the fed-batch technique can reduce or eliminate negative oenological characteristics as the final quality of the wine.

Comparison of single culture fermentation and mixed culture fermentation

A single culture fermentation and mixed culture fermentation of wine will have a very distinctive difference in forms such as its fermentation process, alcohol content, composition, flavor, and aroma. A single culture fermentation with *S. cerevisiae* and mixed culture fermentation with *S. cerevisiae and S. pombe* will have a different metabolism, in such where *S. cerevisiae* will induce a flavor and aroma shift in wine end products due to a significant change in volatile compounds produced. A mixed culture fermentation with *S. pombe/S. cerevisiae* will result with a strong fermentation ability and malic acid degradation/deacidification, where the outcome will differ from a single culture fermentation in its content and profile. A mixed culture fermentation has an aging process of around one to two months (Benito, Palomero, Gálvez et al., 2014; Benito et al., 2019). A study by Loira et al. (2018) suggested a decrease in the aging period by *S. pombe* in comparison to *S. cerevisiae*, where its aging period can take more than six months, through the polysaccharide's concentration found at the wine end products. Another study by Tika et al. (2021) also found that the aging on the lees of *S. cerevisiae* was optimal after 4 months. Not only the concentration of polysaccharides released during the aging period indicates the end of the process, but it also contributes to the complexity of the flavor of the wine (Benito et al., 2018a; Mylona et al., 2016).

Compounds such as alcohol, esters, fatty acids, phenols, and carbonyl compounds are considered to determine the aroma and flavor profile of the wine, in which the single culture and mixed culture fermentation will have a different level of compound yield. One example of this is the malic acid yield from both cultures, where an *S. cerevisiae* pure fermentation will yield a higher malic acid than mixed culture fermentation, which is responsible for the sour flavor. This is due to the inability of *S. cerevisiae* to degrade malic acid (Vilela, 2017). Not only that it causes the acidity flavor in wine, but the malic acid content in wine can also induce the growth of lactic acid bacteria, spoiling the wine product (Benito, Jeffares, et al., 2016; Benito et al., 2018a).

Comparing *S. pombe* yeast to *S. cerevisiae, S. cerevisiae* has a stronger fermentation ability, as it is commonly used in winemaking. Therefore, performing a mixed and sequential fermentation will increase the wine fermentation efficiency, reduce the undesirable compounds produced by *S. pombe* yeast, and enhance the aromatic properties of wine from the yeast's interactions (Benito, Palomero, Gálvez, et al., 2014). A mixed fermentation of *S. pombe/S. cerevisiae* can also result in a reduction of negative oenological characteristics produced by *S. pombe*, namely the reduction of acetic acid, acetaldehyde, and acetoin, which are undesirable in wine. Although, low acetaldehyde in wine has an effect on buttery aroma and color stability in wine (Ciani et al., 2010; Liu et al., 2019; Romani et al., 2010).

The alcohol level in wine can also affect the taste and aroma profile of the wine; therefore, a single and mixed fermentation will result in a different profile as well. A smoother wine profile can also be achieved more with mixed fermentation, as a higher kind of polysaccharide is more likely to be achieved. Another aspect that can affect the wine's taste, aroma, and flavor profile is in the esters produced. Both mixed and single fermentation will yield different kinds of esters, creating various profiles in the wine. Acyl-CoA will also affect several attributes in the wine profile. As both fermentation processes will result in a yield of Acyl-CoA, the profile of the wine will also be affected due to the culture used (Liu et al., 2019).

Thereafter, with the good compounds produced and bad compounds reduced in mixed fermentation, the end quality may bring a new taste benefiting the winemakers. People are not afraid of exploring a new, higher wine quality with respect to its taste, flavor, aroma, and appearance. In fact, the *S. pombe/S. cerevisiae* mixed fermentation might become a unique wine production due to the combination of compounds and the interaction of the yeasts. Reasonably, a well-researched mixed culture fermentation may result in a richer wine profile compared to a single culture fermentation. Furthermore, as different cultures utilize different compounds, performing mixed fermentation can also result in a higher alcohol compound, richer esters, and higher alcohol content. Hence, the mixed culture fermentation technique has the potential in creating a very different wine profile such as flavor, aroma, smoothness, and roundness in comparison to the wines produced from the single culture fermentation technique.

Health aspects of the mixed culture fermentation

Mixed culture fermentation of *S. pombe* has been studied and resulted in the reduction of histamines, other biogenic amines, ethyl carbamate, urea reduction in the wine (Benito *et al*., 2016). Thus, by utilizing *S. pombe*, the health-associated problem can be hindered or avoided (Loira *et al*., 2018). In addition, studies reported that the *S. pombe* had a lower histamine concentration than wine undergoing malolactic fermentation (Benito et al., 2015). *S. pombe* is also effective in reducing the risk of biogenic amines formation due to the fact that *S. pombe* had removed all the sugar and malic acid in the wine fermentation that can be used for the LAB to generate biogenic amines (Benito, 2019).

Histamine is the most toxic biogenic amine, and its high contents in wine are found to contribute to eliciting adverse reactions with various allergy symptoms (Durak Dados et al., 2020; Esposito et al., 2019; Konakovsky et al., 2011). Moreover, the symptoms that may appear are headaches, blushing, heart palpitation, skin irritation, tachycardia, nausea, and many more that will appear shortly after the food intake (Durak Dados et al., 2020; Ladero et al., 2010; Martuscelli et al., 2013). However, the tolerance of bioamine concentration is diverse between individuals (Durak Dados et al., 2020). The high concentration of amine correlated with poor winemaking. These compounds are found depending on various factors such as the crop's condition, varieties, and fermentation process.

Prevention of acute diseases such as diabetes has gained a lot of interest in the younger generation. One of the potential Chemo-preventive natural (phenolic compound) agents, along with low cost and toxicity, can be found in tea, red wine, and cocoa (Schini Kerth et al., 2010).

Polyphenolic compounds in red wine have been shown to be associated with beneficial health effects. The polyphenols had been studied to inhibit; prevent platelet aggregation, cardiovascular, osteoporosis, several types of cancer and diabetes, expanded lifespan, etc. with the consumption of moderate dosage (Arranz et al., 2012; Artero et al., 2015; Li & Förstermann, 2012). Resveratrol, one of the polyphenols that can be found in the wine, had varieties of pharmacological properties such as lowering cholesterol (LDL) in mice model (Chang et al., 2015), anti-cancer (Zulueta, 2015), and diabetic angiopathy prevention (Peng et al., 2014).

Current clinical by Chiva-Blanch et al. (2012) conducted the study with dealcoholized wine consumption daily reported the decrease of both systolic and diastolic blood pressure in the participants. Other clinical studies by Gepner et al. (2015) with diabetes mellitus type 2 (T2DM) that carried out for two years reported the increase of HDL or good cholesterol along with glycemic control. Similar results also reported by several studies report the reduced risk of T2DM in women, not men (Beulens et al., 2012 & Cullmann, Hilding & Östenson, 2012). In contrast, Rasouli et al., 2013 reported the other way around. The results showed the reduction of T2DM in men only.

Results may vary due to the region or wine sample that was used for the clinical testing. But the majority showed health-promoting effects after consuming the red wine in the moderate dosage. Moreover, the amount of polyphenol in red wine is dependent on its variety, geographical condition, maturity, environment, etc. (Cavallini et al., 2015).

CONCLUSION

In recent years, the effort to improve wine taste and quality has made the wine industry experiment with different yeast cultures. Currently, *Saccharomyces cerevisiae* is still considered the standard yeast starter culture in wine fermentation. Fermentation solely using *S. cerevisiae* may also result in high acidity taste of wine since they could not efficiently degrade malic acid in wine fermentation. This limitation also contributes to the formation of biogenic amines in the wine end product due to the growth of lactic acid bacteria. On the other hand, a single starter culture of *Schizosaccharomyces pombe* showed a high acetic acid production in the wine, giving rise to the off-flavor in wine. With the research question initially asking what makes the mixed culture of *S. cerevisiae* and non-saccharomyces yeast popular in the wine fermentation industry, the review hypothesized that the mixed culture *S. cerevisiae/S. pombe* fermentation will enhance wine quality. This hypothesis is found to be correct due to the compounds uniquely produced by the two yeast species, as deliberated in this systematic review. The mix culture fermentation using *S. cerevisiae* and *S. pombe* in the winemaking industry is proven to be effective and promote a higher wine product's end quality, especially studied by Romani et al. (2010). Both yeasts work sequentially; *S. cerevisiae* converts the sugar while *S. pombe* yeast improves wine quality through malic acid degradation and production of quality improving compounds, such as pyruvic acid, polysaccharide, pyranoanthocyanin, and glycerol. Moreover, the reduction of acetic acid due to mixed fermentation may result in a sweeter wine. This review provides solutions to reduce the undesirable compounds—acetic acid, acetaldehyde, and acetoin—produced by *S. pombe* through strains selection, mixed Fermentation, and fed-batch Fermentation. Thus, through this systematic review, it is highly suggested for future studies to involve fed-batch fermentation technique and proper strains selection of *Schizosaccharomyces pombe*. With this, the technique of S*chizosaccharomyces pombe/Saccharomyces cerevisiae* mixed Fermentation can be beneficial for wine industries in exploring new wine tastes and quality.

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