Review Article

Grape Wine Beyond the Glass Covering Benefits, Bioactive Components and Fermentative Microbes

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ABSTRACT

Grape wine is a popular alcoholic beverage, with its quality influenced by grape composition, fermentation conditions, and storage methods. Factors such as Saccharomyces cerevisiae activity, microbial interactions, acid and tannin levels, terroir, potassium content, nitrogen and urea levels, yeast strains, and fermentation temperature all play important roles. While moderate wine consumption may offer health benefits due to antioxidants like resveratrol and quercetin, it can also contain harmful substances such as mycotoxins and pesticide residues. Emerging technologies like nanotechnology are improving wine quality and safety. In production, mature grapes are selected, destemmed, crushed, and placed in containers with sugar and preservatives, adjusting pH to 3.5–4.0. After resting, yeasts is inoculated, and fermentation occurs over three days, followed by filtration, secondary fermentation, racking, and maturation for 6–8 months. The wine is then bottled, sealed, and pasteurized at 80 °C for 2 minutes. Microbes were isolated from decayed grapes using serial dilution, spread and streak plating techniques on YEMA medium. Fermentation was conducted in stainless steel tanks at 10–18 °C for 7–14 days, followed by aging and storage in glass bottles protected from light. Moderate red wine consumption has been associated with cardiovascular benefits, such as improved heart function, better lipid profiles, and reduced risk of ischemic heart disease. These effects are largely attributed to polyphenols, which contribute to anti-inflammatory and antioxidant activity and are central to the "French Paradox." When paired with a Mediterranean diet, red wine may further enhance heart health and vascular function.

Keywords: Grapewine, Saccharomyces cerevisiae, Fermentation, Heart health.

INTRODUCTION

Grape wine is arguably one of the most commonly and widely produced alcoholic beverage obtained from fruit juice. Due to its significant commercial value, the industrial production process has received extensive research attention[1]. "Wine-making usually consists of multiple crucial phases, including grape crushing to release the juice, bulk storage and aging in cellars, clarification of the wine, and ultimately packaging the end product.", and ultimately packaging the final product. While the procedure appears straightforward, maintaining product quality requires controlled fermentation conditions to ensure a premium final product[2]. The unique taste of grape wine stems from both the grapes themselves and the processing techniques applied. Grapes contribute minute amounts of aromatic compounds—mainly terpenes—that provide the wine's characteristic fruity aroma. They also contain non-volatile compounds such as tartaric and Malic acids, which influence taste, along with tannin responsible for bitterness and astringency. These tannin are more prevalent in red wines as they are primarily located in the grape skins[3] Although yeasts are the main organisms in the fermentation process, other microorganisms such as filamentous fungi, lactic acid bacteria, acetic acid bacteria, and additional bacterial species also contribute to the production of alcoholic fruit beverages. Fresh grapes naturally host a variety of microbes, but the desirable fermentative yeast *Saccharomyces cerevisiae* is usually in the minority. Lactic and acetic acid bacteria are also commonly present, and their populations are influenced by factors such as temperature, humidity, grape maturity, mechanical damage during harvest, and fungicide use. Promoting the growth of beneficial microbes while suppressing undesirable ones is crucial, typically achieved by creating favorable fermentation conditions for Saccharomyces species[4]. Fermentation can begin by inoculating the juice with a starter culture of *Saccharomyces cerevisiae* at concentrations of 10^6 to 10^7 cfu/ml. This controlled method ensures consistency in taste and quality. Alternatively, spontaneous fermentation, relying on natural yeast populations on grape skins, may be used. While less predictable, this method can result in wines with diverse flavor profiles and is commonly used in traditional or home wine-making. "Several yeast species are involved and become active during the initial 2–3 days of natural fermentation.". However, as ethanol accumulates, these less tolerant species die off, allowing *Saccharomyces cerevisiae*—which can withstand up to 15% alcohol or more—to complete the fermentation process[5]. Due to its high ethanol tolerance, *Saccharomyces cerevisiae* dominates the fermentation process and has become the standard species used in commercial yeast starter cultures[6]. Proteins found in grapes may lead to haze formation in white wine during storage. The amount and composition of soluble proteins change during and after alcoholic fermentation, influencing their thermal stability in the final

product. Since grape-derived proteins make up a large part of the total protein content, they play a key role in haze development. However, yeast-derived poly Saccharide can enhance the thermal stability of wine proteins, even though they may increase the proportion of less stable protein components. Thus, protein haze potential in white wine is influenced by changes in its macro-molecular makeup early in the wine-making process[7]. The flavonoid content of grapes influences wine's sensory attributes. Different sunlight exposure levels—managed through canopy control—alter flavonoid profiles in grape berries. Grapes grown under shaded conditions showed reduced levels of anthocyanin and skin tannin, while seed tannin remained largely unaffected. The concentrations and ratios of anthocyanin, skin, and seed tannin are closely linked to the formation of pigmented polymers and tannin in wine. Higher quality wines are often associated with higher anthocyanin and skin tannin content, and lower seed tannin levels.

FACTORS RESPONSIBLE FOR GOOD WINE

1.Potassium's Role in Wine Quality

Potassium is vital for the growth of grapes-vine and the development of their fruits. However, when present in excess within grape berries, it can negatively affect wine quality by reducing free tartaric acid, thereby increasing the pH of grape juice, must, and wine. This often necessitates pH correction during wine-making and the addition of tartaric acid. High potassium content can also result in substantial loss of added tartaric acid due to its precipitation as potassium bitartrate, making pH regulation more complex and costly. Maintaining low natural potassium levels in grapes can help minimize input costs and waste management in wineries. Vineyard strategies to control potassium accumulation in berries include careful selection of root-stock and scion combinations, as well as canopy and irrigation management. Nonetheless, optimizing these practices requires precise calibration of production conditions to maintain ideal potassium levels for desirable grape juice and wine quality[8].

2.Impact of Bottle Closures and Storage Conditions on Wine

The composition, color, and aroma of wine are greatly affected by the type of closure used and the conditions under which it is stored. Wines were sealed using screw caps, two types of natural corks, a synthetic stopper, and glass ampule. Storage orientation was also considered. Under regulated temperature and humidity, several analyses were performed, including measurements of sulfur dioxide and ascorbic acid levels, sensory evaluations, and spectral assessments. Wines sealed with synthetic closures tended to be more oxidized, exhibited a browner coloration and contained reduced level of sulfur dioxide. Screw cap and ampoule-sealed wines exhibited a flinty or rubbery aroma, while those sealed with natural corks showed minimal such characteristics. Bottle positioning had little influence on the wine's chemistry or sensory properties in this study[9].

3.Malolactic Fermentation and Nitrogen Levels

Saccharomyces yeasts may inhibit malolactic fermentation during alcoholic fermentation, specially when nitrogen level are either too low or too high. This was studied by fermenting synthetic grape juice or wine using various commercial *Saccharomyces cerevisiae* strains. The malolactic bacterium *Oenococcus oeni* was usually inhibited when wines contained high levels of sulfur dioxide, and certain yeast strains only hindered bacterial growth under nitrogen-rich conditions. Yeast tended to generate more sulfur dioxide under these conditions, suggesting nitrogen indirectly affected malolactic fermentation by influencing sulfur dioxide production. However, sulfur dioxide alone did not fully account for the inhibition of *Oenococcus oeni,* indicating other inhibitory mechanisms were also at play[10].

4.Urea and Ethyl Carbamate Concerns in Wine

While urea levels are not routinely measured in wine labs, they are important in understanding yeast metabolism and the formation of ethyl carbamate, a known carcinogen. Efforts have been made to decrease urea concentrations to minimize the production of ethyl carbamate in alcoholic beverages. The techniques used to detect urea in wine are generally grouped into three main types: colorimetric methods, enzymatic degradation, and chromatographic analysis. The two most widely used techniques over the past 15 years include the ammonia test kit and a method involving the reaction of urea with 1-phenyl-1,2-propanedione-2-oxime. Both methods are labor-intensive and time-consuming, but they follow well-established procedures[11].

5.Yeast Strain Influence on Wine Color and Pigment Profile

The coloration and pigment composition of wines fermented with *Saccharomyces cerevisiae* and *Saccharomyces bayanus* were examined at 8 and 387 days after inoculation. *Saccharomyces bayanus* wines had lower grape anthocyanin levels but showed higher color intensity. There was no clear relationship found between the anthocyanin concentration and the color density of the wine. At 387 days, *Saccharomyces bayanus* wines had increased levels of pigmented polymers and sulfur dioxide-resistant pigments, suggesting enhanced formation of stable pigments like pyranoanthocyanins. Electrospray mass spectrometry was used to examine pigment composition, showing higher production of acetaldehyde-related pigments in *Saccharomyces bayanus* wines. The differences in pigment profiles were largely attributed to this increased pigment formation[12].

6. Flavonol Content in Wines

High-performance liquid chromatography was used to assess flavonol content in red and white wines. These compounds and their glycosides affect a wine's color, flavor, and potential health benefits. Red wines primarily contains quercetin and myricetin-N ,with quercetin-3-

glucuronide being the most prevalent glycosidic form. Flavonol levels in white wines were considerably lower, frequently falling below detectable limits.. Contrary to earlier studies, rutin was not detected in any sample, and when added, it quickly degraded to quercetin. Additionally, the order of elution for certain quercetin glycosides changed based on the type of acid present in the mobile phase[13].

7.Temperature Variation During Red Wine Fermentation

Temperature variations occur during red wine fermentation, especially during cap management. Fermentation are not uniform in density or temperature, as grape solids separate from liquids. "Cap management is carried out by winemakers to extract color and flavor, as well as to ensure an even temperature throughout the fermenting must." Temperature sensors recorded a 12°C difference between the cap center and liquid phase in fermentation, both large and small. A 1600-liter tank equipped with internal sensors revealed that effective pump-overs significantly reduced these differences to below 5°C. These temperature gradients can influence yeast activity, compound extraction, and the final wine quality[14].

8.Effect of Indigenous Yeasts on Wine Flavor

Wines made with native or "wild" yeast often exhibit more complex flavor profiles, though the chemical reasons remain unclear. In a study, paired wines were fermented from the same must under similar conditions—one with a *Saccharomyces cerevisiae* starter, the other with indigenous yeast. The main differences between the wines were in yeast-derived volatile compounds. Wines produced using commercial yeast strains were abundant in esters such as ethyl hexanoate and 3-methylbutyl acetate. Conversely, wild yeast wines had more variable aroma compounds and higher levels of volatiles like 2-methylpropanol and ethyl dodecanoate, which may significantly impact aroma[15]. Pprimarily glucose and fructose—serve as the energy source for yeasts during fermentation, converting into alcohol. Grapes are usually picked at optimal ripeness, when their sugar level ranges between 20%-30%. Acids, particularly tartaric and malic, are essential not only for flavor but also for preventing the growth of unwanted microbes during fermentation. Tannins and other phenolic compounds add to the wine's body and astringency. Pigments in grape skins also influence astringency and, more importantly, color—red grape skins contain anthocyanins, while white grapes are rich in flavanols. Each grape variety carries a unique aroma profile, though many aromatic compounds are chemically bound and are released during fermentation through enzymatic activity. Hence, wine is a complex beverage, and enhancing its beneficial compounds while eliminating harmful substances requires strict regulation of its physicochemical and biochemical properties. The wine making process is deeply interrelated with other fields, including viticulture (which ensures high-quality grape production), biochemistry (supporting the transformation processes), and microbiology (for identifying and managing the microbial populations involved in fermentation and storage). An emerging innovation in this field is the integration of nanotechnology, which enhances wine monitoring and analysis during production. It also helps improve wine quality by reducing spoilage, lowering volatile acidity, and increasing the content of beneficial compounds such as resveratrol, anthocyanins, and other valuable substances.

MICROBIOLOGY OF WINE PREPARATION

Selection of mature grapes → Destemming and crushing of berries → Filling containers up to three-quarters full → Incorporation of sugar → Adjustment of pH to 3.5–4.0 → Incorporation of preservative → One-hour resting period → Introduction of wine yeast for inoculation→ Primary fermentation for 3 days → First filtration followed by secondary fermentation → Racking (siphoning off the clear liquid) → Maturation (aging) for 6–8 months → Final bottling and Sealing with crown corks → Pasteurization at 80 °C for 2 minutes

Microorganism Used: Saccharomyces cerevisiae

Sample Collection: Decayed grape samples were procured from the local marketplace.

Isolation of Microbial Strains: "One milliliter of juice from spoiled grapes was combined with 9 milliliters of sterile distilled water in a test tube to isolate the microorganisms present.

Isolation Procedures:

The microbial isolation was performed using three main techniques:

1. Serial Dilution: From the primary test tube, 1 ml of the mixture was added to 9 ml of sterile normal saline (0.85%), creating a dilution. "The step was consecutively repeated until a dilution factor of 10^{-6} was achieved.". Then, 0.1 ml from the 10^{-2}, 10^{-4}, and 10^{-6} dilutions was plated on Yeast Extract Mannitol Agar (YEMA) medium for further analysis.

2. Spread Plate Technique: Using a sterilized glass rod, 0.1 ml of each chosen dilution was uniformly distributed across the surface of the Yeast Extract Mannitol Agar (YEMA) plates. The plates were incubated at 21°C for 72 hours. Colony morphology was then observed both visually and using a magnifying lens. "The key traits analyzed included colony color (creamy or whitish), elevation (flat, raised, or wavy), and transparency (opaque or partially transparent)."

3.Streak Plate Technique: From the colonies observed on the agar plates, individual colonies were picked and streaked onto fresh YEMA slants to obtain pure isolates.

ALCOHOL AND WINE CONSUMPTION AND THEIR INFLUENCE ON HUMAN HEALTH

Elmadhun et al.[16] conducted an animal-based experiment using pigs and found that moderate ethanol intake directly stimulated new blood vessel formation in healthy myocardial tissue. Platisa et al.[17] investigated how moderate consumption of red wine affects cardiovascular function. Both red wine and a control alcoholic beverage (13.5% alcohol, 0.2 L each) temporarily increased systolic and diastolic blood pressure, but within ten minutes, levels normalized. Long-term, only red wine exhibited a beneficial effect by lowering blood pressure and reducing cardiac interval variability. "According to Matsumoto et al.[18], moderate alcohol intake in adults—such as 0.15 liters of wine, 0.33 liters of beer, or 0.03 liters of spirits—was found to lower the likelihood of ischemic heart disease, cardiomyopathy, and overall mortality.". Toth et al.[19] studied the impact of consuming (0.2l) of red wine daily for three weeks in healthy,non smoking individuals between ages of 18 to 40. The findings showed reduced red blood cell aggregation and improved cell flexibility under stress, highlighting red wine's positive impact on blood parameters related to heart disease. "Elmadhun et al.[20] developed a pig model of chronic myocardial ischemia to investigate the physiological impact of moderate alcohol intake." They reported that moderate ethanol levels could suppress cell death and enhance cell survival in both ischemic and healthy cardiac tissues, pointing to red wine as an ideal source of ethanol with added heart-protective compounds. Droste et al.[21] conducted a dietary intervention on 108 patients with carotid artery disease, some of whom consumed red wine daily (0.1 L for women, 0.2 L for men). Results indicated improvements in LDL/HDL ratios, independent of statin use, attributed to wine, dietary changes, and physical activity. Chu et al.[22] compared the effects of red wine (0.375 L) and vodka (0.1 L) on cardiovascular health in hypercholesterolemic pigs. Both alcoholic beverages improved blood supply to ischemic heart tissue, suggesting reduced cardiovascular risk. Yoo et al.[23] analyzed consumer perceptions in Australia and Korea, finding that red wine was favored for its perceived health benefits, particularly its role in preventing heart attack, hypertension, and cardiovascular diseases.Djoussé et al.[24] tracked alcohol consumption and cardiovascular outcomes over 12 years in women. "Consuming 5–15 grams of alcohol daily, primarily from red wine, was associated with a 26% decrease in cardiovascular disease, a 35% reduction in overall mortality, and a 51% lower risk of death due to cardiovascular causes compared to those who abstained."

A Mediterranean cohort study[25] involving over 20,000 graduates found that moderate wine intake (20 g/day for men, 10 g/day for women), when paired with polyphenol-rich diets and healthier lifestyles, correlated with significantly lower cardiovascular events and all-cause mortality compared to consumption of other alcoholic beverages. O'Keefe et al.[26] J.J. examined alcohol consumption in 27,000 adults. The findings showed that consuming small to moderate amounts of alcohol daily—namely, up to 0.2 liters of wine for men and 0.1 liters for women—was associated with a reduced risk of cardiovascular diseases, with red wine identified as the most advantageous type. Red wine was highlighted as the most beneficial alcohol type, while excessive drinking was linked to adverse effects such as hypertension and atrial fibrillation. Yamagata et al.[27] studied over 129,000 individuals and reported that wine drinkers had a lower risk of death from cardiovascular diseases. Polyphenols are believed to enhance endothelial function, which may explain the 'French Paradox'—the phenomenon of low incidence of heart disease despite the consumption of high-fat diets[28]." further explored the French Paradox, noting reduced coronary artery disease in wine drinkers (three glasses daily) compared to those consuming beer or other alcohols. Regular red wine intake was also associated with reduced blood pressure and heart attack risk, particularly in men over 65.

Medina-Inojosa et al.[29] analyzed the "Hispanic Paradox," suggesting that moderate red wine consumption might explain the lower rates of cardiovascular diseases among Hispanic groups in Europe, the USA, and South America, despite shared environments with higher-risk populations. Vilahur and Badimon[30] highlighted the cardiovascular advantages of the Mediterranean diet, emphasizing that daily red wine (0.15 L for women and 0.45 L for men) reduced inflammation, improved lipid profiles, and supported endothelial health. "Cioni et al.[31] conducted a study on 95 men and women at risk of cardiovascular disease and found that those adhering to a Mediterranean diet combined with moderate wine consumption exhibited enhanced endothelial function, as reflected by increased reactive hyperemia index values.Giacosa et al.[32] supported the cardiovascular benefits of the Mediterranean diet, demonstrating its effectiveness in preventing heart disease and reducing mortality, even outside Mediterranean populations.Tognon et al.[33] conducted a study which involves 1849 adults. Those following Mediterranean dietary practices, including wine consumption, showed reduced risks of coronary heart disease and cardiovascular mortality, though stroke incidence remained unaffected.

Enhances Memory and Mental Performance

Red wine contains high levels of polyphenols—natural antioxidants that contribute to the body's defense against illnesses. One such polyphenol, resveratrol, found in red wine, is thought to support short-term memory enhancement[34]. Research indicates that resveratrol may protect the brain from cognitive issues linked to sleep deprivation, such as forgetfulness, while also enhancing learning and mental agility[35]. "Another cognitive advantage of red wine is its possible role in lowering the risk of dementia, a group of conditions that generally impair memory in elderly individuals." Write in another wordings but meaning should be same "Evidence suggests that moderate red wine intake may decrease the likelihood of developing dementia, a leading factor in Alzheimer's disease," says dietitian Sally Stevens[36].

Supports Vision Health

Thanks to resveratrol, red wine may also promote better eye health. Its ability to enhance blood flow in tiny capillaries around the eyes can contribute to preventing vision-related issues. This improved microcirculation may help reduce the risk of age-related macular degeneration, glaucoma, and diabetic retinopathy—all of which can lead to gradual vision loss[37].

Contributes to Cardiovascular Wellness

Among the most well-known advantages of red wine is its association with better heart health. Diets enriched with polyphenols, such as those found in red wine, have long been connected to a reduced risk of cardiovascular conditions.Red wine may help maintain healthy blood vessel linings and raise levels of high-density lipoprotein (HDL), often known as 'good' cholesterol. HDL assists in clearing low-density lipoprotein (LDL), or "bad" cholesterol, from the body, which if elevated, can lead to cardiovascular diseases and stroke. "Wine may support a reduction in LDL cholesterol," explains Stevens. "High levels of LDL can damage arteries"[38].

Promotes Oral Health

Scientific research has suggested that red wine might benefit oral hygiene. The polyphenols in red wine can inhibit harmful bacteria in the mouth that contribute to cavities and gum inflammation. These antioxidants prevent bacteria from adhering to the surfaces of teeth and gums, thereby lowering the chances of plaque buildup and tooth decay[39].

May Decrease Cancer Risk

Some evidence suggests that red wine could potentially help in lowering the risk of certain cancers by restricting the spread of cancer cells, particularly in breast and esophageal cancers[40]. This is primarily due to resveratrol, a compound in grape skins that has been shown to destroy cancerous cells. Polyphenols act by blocking the production of a protein that supports tumor growth. "Resveratrol, an effective antioxidant found in grapes and grape juice, helps neutralize free radicals that could harm the body's tissues," Stevens explains[41].

Helps Manage Blood Sugar

Though it naturally contains sugars, red wine may aid in stabilizing blood glucose levels. A single serving has been shown to lower blood sugar for up to 24 hours, especially beneficial for individuals with elevated glucose[42]. However, red wine should not replace prescribed treatment for diabetes, but can be considered a supportive option if consumed responsibly.

Boosts Immune Defense Against Viruses

Maintaining a strong immune system is essential, and red wine may play a role due to its antiviral properties. Thanks to its antioxidant compounds—specifically flavonoids—red wine may assist the body in fending off viral infections. "Flavonoids in wine serve as antioxidants that may help fight viruses," Stevens notes[43].

Aids in Blood Pressure Regulation

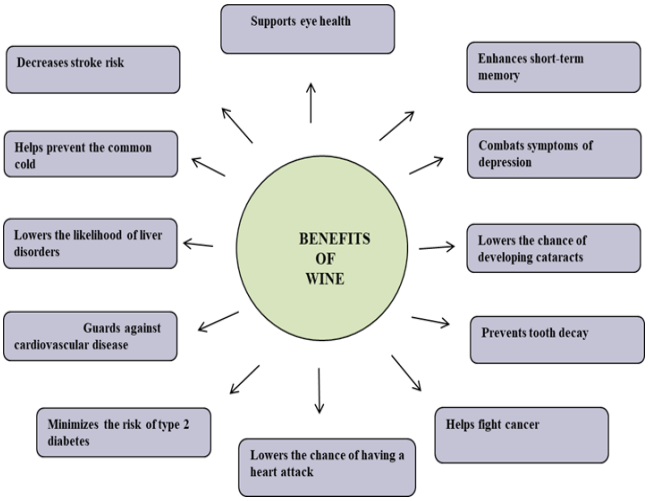
The polyphenols found in red wine may also help control blood pressure levels. Studies have shown that even red wine without alcohol can lead to reductions in both systolic and diastolic pressure[44]. Additionally, grape-based wine extracts rich in polyphenols have been shown to produce similar benefits, independent of alcohol content[45].

Promotes Gut Health

Red wine may positively influence the balance of gut bacteria due to its polyphenol content. A healthy and balanced gut microbiota is essential for supporting immune function and protecting against disease. An imbalanced microbiome, on the other hand, can weaken immunity and raise disease susceptibility[46].

Supports Bone Strength

Emerging research suggests that resveratrol in red wine may help enhance bone density and fortify bone structure[47]. This could reduce the chances of osteoporosis, a condition marked by fragile bones and higher risk of fractures. However, excessive alcohol intake has the opposite effect and can harm bone integrity over time.



CONCLUSION

Grape wine production begins with the selection of mature grapes, followed by fermentation using specific yeasts such as Saccharomyces cerevisiae. The wine is then aged to develop its flavor and improve quality. Key factors influencing the final product include pH, temperature, type of yeast, and storage conditions. Microbiological techniques like streak and spread plating are employed to isolate important microorganisms involved in fermentation. Moderate consumption of red wine, which contains polyphenols like resveratrol, has been associated with benefits to heart health, brain function, and the immune system. Nonetheless, excessive intake may result in negative health consequences. Recent advancements, including the use of nanotechnology, are enhancing the

monitoring and assurance of safety and quality in winemaking.

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