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Research article

EVALUATION OF WINE PARAMETERS DURING THE MATURATION PROCESS IN DIFFERENT TYPES OF WOOD

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Abstract

This study investigates the influence of various oak types on the microbiological, chemical, and physical properties of wines during maturation. Eight samples were analysed, including Cabernet Sauvignon matured in American, French, Hungarian, and Balkan oak barrels, as well as a young Cabernet Sauvignon, a Vranç matured in Balkan oak, and grape juice. Parameters assessed included microbiological content, sugar levels, alcohol content, glycerol content, pH, acidity, turbidity, and sulfur dioxide (SO_2) levels. Results showed that samples matured in Hungarian oak exhibited higher stability compared to other wood types, while significant discrepancies were observed between declared and measured alcohol levels. Microbiological analyses revealed the presence of *Acetobacter,* yeasts, and molds in several samples, except for the young wine and Vranç, which were microorganism-free. Additionally, turbidity values exceeded acceptable standards across all samples. These findings highlight the influence of wood type and maturation conditions on wine quality, suggesting strategies for optimizing aging processes to ensure product stability and optimal sensory attributes.

1. Introduction

Wine is an alcoholic beverage produced by the fermentation of fresh grapes or grape must (Kelebek et al., 2009). Different grape varieties possess unique vinification characteristics and distinct styles. Additionally, the style of wine is influenced by the geographical environment (Wang et al., 2022). Today, the winemaking industry and global wine consumption represent significant socio-economic markets, with production ranging from 250 to 300 million hectoliters per year over the past two decades (Martin et al., 2020). Wine is primarily composed of 86% water, 12% ethanol, and 1% glycerol and sugars, with 0.4% organic acids. Key components include polyphenols, anthocyanins, tannins, minerals, volatile compounds, and vitamins. Organic acids like tartaric and malic acids come from grapes, while acetic and succinic acids are fermentation byproducts. Sulfites, produced during fermentation, should not exceed 350 mg/L.

Nitrogen compounds, absorbed from nitrate, ammonia, or urea during cultivation, are converted into amino acids such as proline and arginine. These amino acids can form higher alcohols that affect the wine's aroma. Terpenes, although present in small amounts, significantly influence the sensory properties of wine. Polyphenols, especially flavonoids, contribute to wine's color, taste, and bitterness, with tannins affecting bitterness levels. Essential vitamins in wine include vitamins B and C, as well as folic acid. The overall composition and quality of wine depend on the grape variety, production techniques, and interactions between ingredients (Markoski et al., 2016; Evers et al., 2021; Nemzer et al., 2022).

Maturation is a historical practice that has been used for thousands of years by many civilizations. Two fundamental parameters in the maturation process are the quality of the wood barrel and the duration of maturation. Wooden barrels, which are used for 6 to 18 months, incur significant economic costs. The maturation process varies with the type of wine. Red wine aging has two phases: the wood (oxidation) phase and the bottle (reduction) phase. During maturation, wine undergoes numerous physical and chemical changes. Compounds such as phenolic, lactones, aldehydes, and furfuryl compounds are transferred from the wood to the wine. The type and quantity of these substances depend on maturation time, wood type, wood origin, and wood usage. Other changes include the evaporation of volatile components and exposure to natural oxygen, resulting in condensation reactions and the polymerization of flavonoid compounds. These reactions affect the phenolic composition, color, and bitterness of the wine. Compounds may remain in the wood or lees, causing continuous changes during maturation (Alamo-Sanza and Nevares, 2018; Carpena et al., 2020; Pfahl et al., 2021; Jordão and Cosme, 2022).

Given the high consumption of wine today and the significant impact aging has on its characteristics, this study aims to investigate wine parameters, including microbiological and chemical content, and compare these parameters and stability during the maturation process in different types of oak wood. The research aims to investigate the impact of oak wood on wine quality, with a particular emphasis on the crucial role of the type of oak used in the maturation process.

2. Materials and methods

2.1. Materials

2.1.1. Wine samples

This research was conducted in the Faculty of Food Technology laboratory at the University "Isa Boletini" in Mitrovica. For the study, samples were collected from various wineries in the Rahovec region, Kosovo, which we will not disclose by name for confidentiality reasons. Codification and characteristics of the wine samples are detailed in Table 1.

Table 1. Codification and wine characteristics

Samples	Grape variety	Wooden oak	Time of maturation	Alcohol percentage labelling
S1	Cabernet Sauvignon	"American oak"	8 months	14.1%
S2	Cabernet Sauvignon	"French oak"	8 months	14.1%
S3	Pinot Noire	"Balkan oak"	16 months	ND#
S4	Cabernet Sauvignon	"Hungarian oak"	8 months	14.1%

S5	Cabernet Sauvignon	"Hungarian oak"	16 months	16%
S6	Cabernet Sauvignon	"Young wine "	NA*	16%
S7	Vranç	"Balkan Oak"	14 months	ND#
S8	Grape Juice	NA*	NA*	NF^

* NA- Not aged, NF^ - Not Fermented, ND# - Not Declared

2.1.2. Chemicals and reagents

Plate Count Agar (PCA) (#COT090219502) was purchased from Liofilchem. Glucose yeast extract $CaCO_3$ medium (GYC) (#40545) was purchased from Sigma-Aldrich. Yeast Glucose-Chloramphenicol agar (YGC agar) (#620070) was purchased from Liofilchem (Conda, Spain). MRS agar (#69964) was purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) (#3034) was purchased from Lachner, Czech Republic, and other chemicals were of analytical reagent grade.

2.2. Methods

2.2.1. Microbiological analysis

For microbiological analysis, the wine samples were processed immediately after collection. The total counts of bacteria (TBC), number of *Acetobacter* cells (A), *Lactobacillus* cells (L), and yeast and molds (Y/M) were assessed using an optical microscope (N-400M microscope with an achromatic objective, 100X for bacteria and 40X for molds, respectively). The plate dilution method was applied for the quantitative determination of CFU (Colony Forming Units) counts for respective groups of microorganisms in 1 mL of wine. Petri dishes of gelatinous nutritive substrate were inoculated with 1 mL of wine samples (TBC, A, L, and Y/M) in duplicate. For the identification of TCB, PCA was used, and the samples were incubated aerobically at 30°C for 72 hours. *Acetobacter* cells were cultivated on GYC medium and incubated at 30°C aerobically for 48 hours. *Lactobacillus* species were grown on MRS agar and incubated at 37°C for 72 hours in anaerobic conditions. Finally, yeast and molds were cultivated on YGC agar and incubated at 25°C for 5 days. After incubation, the results were collected and expressed in CFU/mL (Kántor *et al.,* 2014).

2.2.2. Sample preparation for chemical characterization

All wine samples were filtered and decanted into laboratory bottles for the analysis of sugar levels, alcohol content, glycerol content, pH, acidity, turbidity, free SO2, total SO2, and stability.

2.2.3. Chromatographic analysis of sugars, glycerol, and alcohol

The sugar content in wine samples was measured using HPLC (LC Shimadzu, Model: RID-20A, Duisburg, Germany) with a Shim-Pack SPR-Ca, 250 x 7.8 mm column, and a refractive index detector at a working flow rate of 0.65 mL/min and 80°C, using distilled water as the mobile phase. The samples were filtered through a 0.45 µm PTFE membrane filter. Each sample was injected twice with a 20 µL injection volume, and the peak was detected. Different concentrations (8, 4, 2, 1, 0.5, 0.25 g/L) of sucrose, glucose, fructose, and glycerol, respectively, were used for standard curve construction, where different concentrations (18, 9, 4.5, 2.25, 1.125, 0.562 g/L) of alcohol were used for standard curve construction. The remaining concentrations of sucrose, glucose, fructose, and glycerol in the samples were determined by comparing their peak areas with those of standard curves. The retention times for sucrose, glucose, fructose, and glycerol were 7.66, 9.17, 11.12, and 13.28 minutes, respectively. For ethanol, the retention time was 13.7 minutes (Figure 1).

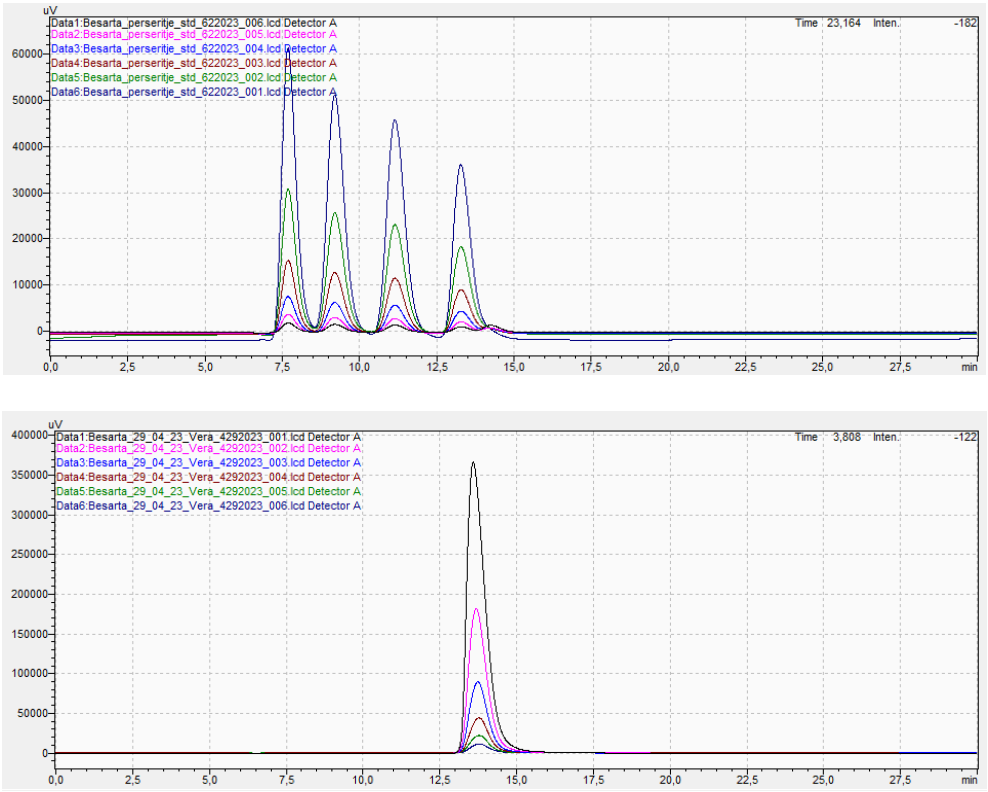


Figure 1. HPLC chromatograms of standards. a) Sucrose, glucose, fructose, and glycerol; b) Ethanol

2.2.4. Measurement of turbidity

Turbidity in wine, measured using a turbidity meter (#ORIONAQ4500, Singapore), is quantified in Nephelometric turbidity units (NTU). This measurement indicates the clarity of the wine, with higher NTU values corresponding to greater turbidity (cloudiness) due to suspended particles.

2.2.5. Measurement of wine protein stability

The stability of the wine was assessed through a series of tests designed to evaluate its resistance to common instabilities based on (Protein Stability in Wine, Virginia Tech, Enology and Fermentation Science protocol). First, a 55% trichloroacetic acid (TCA) solution was prepared. Following this preparation, 8 test tubes were each filled with 10 mL of wine, and 1 mL of the 55% TCA solution was added to each test tube. These test tubes were then mixed in an evaporator. For comparison, another set of 8 test tubes was prepared by adding 10 mL of wine and 1 mL of distilled water to each, and these were also mixed in the evaporator. Both sets of test tubes were placed in a water bath maintained at 80°C for 2 minutes. After 2 minutes, the test tubes were removed from the water bath, and turbidity measurements were conducted on each sample.

2.2.6. pH measurement

The pH was determined using a pH meter (HI2002-01 HANNA Instruments pH meter, USA/Romania) based on the Vinmetrica protocol for wine pH measurement. Firstly, the pH meter was calibrated using a set of pH buffer solutions to ensure accuracy. After calibration, the pH probe was inserted into the wine samples and slowly stirred until the reading on the meter stabilized.

2.2.7. Measuring TA (titratable acidity)

Titratable acidity is determined by titrating a sample with the TA Titrant, which has a standard known concentration (0.133 N) of NaOH according to the Vinmetrica protocol for wine acidity measurement. We add the TA Titrant slowly with stirring until the pH meter reaches the target pH of 7. From the volume of titrant used, the TA value was calculated following the formula:

TA (g/tartaric acid/L) =(V*0.133*75)/S (1)

where V = mL of Titrant needed to reach the endpoint; 0.133 = normality of the Titrant, S = mL sample. The value 75 is the equivalent weight of tartaric acid.

2.2.8. Measurement of total SO_2

Total SO_2 was determined using the Ripper method (Thermo Scientific notes, No. T5 method) and as described in the article by Guerra, M, and Cantos-Villar, E. (2015). Twenty milliliters of wine were transferred into a 250-milliliter Erlenmeyer flask, and 10 milliliters of 1.0 N NaOH were added. After a 10-minute reaction period at a high pH, 5 mL of 1% starch solution and 10 mL of 25% sulfuric acid were added. The sample was then titrated with a 0.01 N iodine solution until a purple color (VI) appeared. The volume of iodine solution spent was recorded. This procedure was repeated for all samples to determine the total SO_2 content. The total SO_2 content was calculated by using the following formula:

SO_2 (mg/L) = VI × NI × 1280 (2)

where, VI = Volume of iodine titrant used at the endpoint of the titration (mL), NI = Normality of the iodine titrant (certified or standardized value), 1280 = (32g SO_2/equivalent × 1000 mg/g)/25 mL wine.

2.2.9. Measurement of free SO_2

Free SO_2 was determined using the Ripper method (Thermo Scientific Notes No. T5 method, as described in the article by Guerra, M., and Cantos-Villar, E. (2015). Twenty milliliters of wine were transferred into a 250 mL Erlenmeyer flask, and 5 milliliters of a 1% starch solution and 5 milliliters of a 25% sulfuric acid solution were added. The mixture was titrated with 0.01 N iodine solution until a purple color appeared (VI). The same formula used for determining total SO_2 was applied to calculate the concentration of free SO_2

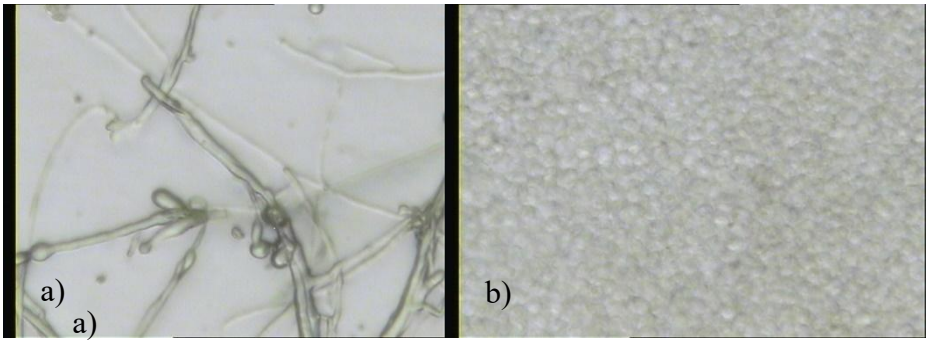
2.3. Statistical analysis

Most parameters were measured in duplicate, with each value representing the mean of two readings. Statistical analysis was performed to evaluate the relationships between key variables using the Pearson correlation coefficient. This coefficient measures the strength and direction of the linear association between two variables, with values ranging from -1 to +1.

3. Results and discussions

3.1. Microbiological analysis of wine samples

The microbiological performance of wine samples shown in Table 2 reveals that all wine samples were free of total mesophilic bacteria. *Acetobacter* was detected in samples S1, S2, S3, and S4. However, no *Acetobacter* were found in samples S5, S6, S7, and S8. To no surprise, yeasts were detected in several samples, as shown in Table 2. However, no yeasts were found in samples S6, S7, and S8. Molds were detected in samples S2, S4, and S8. We characterized the molds found in samples S2, S4, and S8 under the microscope, identifying them as *Penicillium*, *Penicillium spp*., *Trichoderma*, and *Penicillium cyclopium* (Figure 2). In contrast, no molds were found in samples S1, S3, S5, S6, and S7.



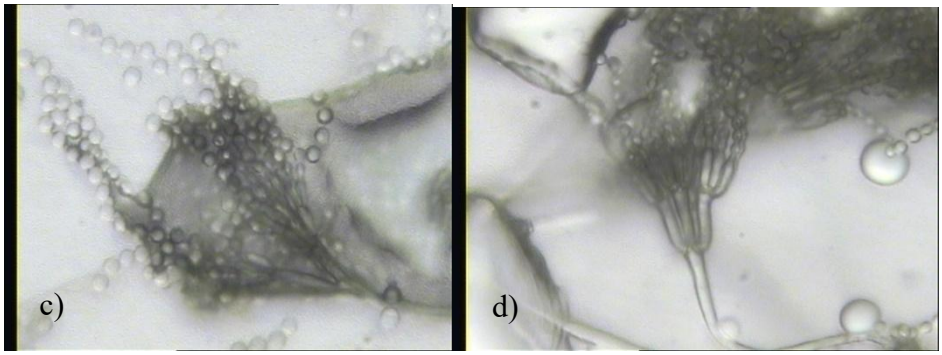


Figure 2. Microscopic examination of Molds in wine samples. a) *Trichoderma*; b) *Penicillium*; c) *Penicillium spp*; d) *Penicillium cyclopium*

All wine samples free of total mesophilic bacteria can be attributed to the acidic nature of wine, with a pH ranging from 3.1 to 3.9, which inhibits the growth of most human pathogenic microorganisms. The combination of high acidity and alcohol content in wine creates a strong antimicrobial environment.

According to literature, a wine composition containing 0.6% tartaric acid, 15% ethanol, and a pH of 3.0 exerts a potent bactericidal effect (Azevedo et al., 2016). The physical and chemical analyses further support these findings, indicating that the majority of wine samples had a pH range of 3.1 to 3.4.

Additionally, the titratable acidity, expressed as tartaric acid, averaged between 0.5% and 0.7% (5-7 g/L tartaric acid). These parameters align with those reported in scientific literature, which likely accounts for the absence of bacteria and Lactobacillus in the wine samples. These bacteria are ubiquitous, adapting well to environments with high levels of sugars and alcohol. Being aerobic and mesophilic, *Acetobacter* can spoil wine, necessitating careful monitoring by winemakers during both production and storage. They are capable of converting ethanol into acetaldehyde and acetic acid, which can lead to wine spoilage (Zgardan et al., 2022). The presence of *Acetobacter* in samples S1, S2, S3, and S4 could be due to exposure to inappropriate temperatures and oxygen during production. High sugar levels after fermentation, combined with the presence of oxygen, may also promote the growth of these bacteria. During fermentation, high concentrations of SO_2 are typically added, which, along with other factors, increases stress on the yeast. This stress arises from anaerobic conditions, reduced levels of nitrogen, lipids, and vitamins, increased acidity, ethanol concentration, and temperature. Despite these challenges, some yeasts can survive the stresses of the fermentation process (Romano et al., 2022). Perpetuini et al. (2021) show that specific yeasts found in wine environments are resistant to potassium metabisulfite and even to cleaning agents used in wine cellars. Based on this, the yeasts present in samples S1, S2, S3, S4, and S5 may be those that have successfully withstood the rigorous conditions of the wine production process.

Molds are ubiquitous and can be found on various surfaces, including the air, the surfaces of grapes, and the wood used for maturation. Common mold genera include *Aspergillus*, *Botrytis*, *Penicillium*, and, to a lesser extent, *Phytophthora*, *Alternaria*, and *Cladosporium*. The presence of mold has a significant impact on the physical, chemical, and sensory properties of wine. Uncontrolled mold proliferation on grapes before harvest can lead to secondary contamination by bacteria and yeasts, potentially causing a condition known as rot. Moreover, mold on grapes can facilitate the spread of infections.

Beyond aesthetic concerns, molds can produce potent sensory metabolites, which play a crucial role in determining wine quality. *Penicillium* is a diverse genus with over 200 known species, many of which grow at low temperatures, below 5°C, earning them the

moniker "cold-weather mold." The primary concern with the presence of molds in grapes or wine is the potential production of mycotoxins. Fugelsang and Edwards (2015) show that Penicillium spp. can produce one or more mycotoxins in grape must. *Trichoderma* spp. are known to cause green mold disease, which can lead to reduced yield without fortification and cause malformation of fruiting bodies. Such malformations negatively impact all parameters of product quality (Zorić et al., 2023). Each isolate of *Penicillium* cyclopium is known to produce a strong musty odor. This species thrives at low temperatures and is likely responsible for food spoilage, even in refrigerators (Mislivec, 1981).

Given the above, the presence of molds in samples S2, S4, and S8 may be attributed to their initial presence on the grapes, which then spread to the surfaces where the wine was produced. Additionally, the conditions in the production environment may have been conducive to mold growth. The molds identified in these samples could negatively impact the quality of the wine and pose potential health risks.

Table 2. Microbiological analysis of wine samples

Sample	*Total bacterial count* CFU/mL	*Lactobacillus* CFU/mL	*Acetobacter* CFU/mL	*Yeasts* CFU/mL	*Molds* CFU/mL
S1	0	0	$4x10^2$	$7x10^2$	0
S2	0	0	$3x10^2$	$2x10^2$	$1x10^2$
S3	0	0	$6x10^2$	$7x10^2$	0
S4	0	0	$10x10^2$	$17x10^2$	$2x10^2$
S5	0	0	0	$2x10^2$	0
S6	0	0	0	0	0
S7	0	0	0	0	0
S8	0	0	0	0	$1x10^2$

3.2. Sugars, glycerol, and alcohol analysis of wine samples

The sugar content measurements are presented in Table 3. According to Law No. 02/L-8 on wines, which classifies wines based on their sugar content, the categories are as follows: a) dry wine: containing no more than 4 g/L of unfermented reducing sugar, and b) semi-dry wine: containing more than 4 g/L but not exceeding 12 g/L of unfermented reducing sugar (Republic of Kosovo. Law No. 02/L-8 on wines). Based on this classification and the sample results, S1, S3, and S7 are categorized as dry wines, while samples S2, S4, S5, and S6 are classified as semi-dry wines. Sample S8, with 103.43 g/L of reducing sugar, is identified as grape juice. Sucrose levels were also measured in the laboratory, showing that all samples, except for S8, have similar sucrose levels. This means they applied the chaptalization process, which involves adding sugar to grape must before fermentation to increase the wine's alcohol content. This practice is often used when the climate is too poor for the grapes to develop sufficient sugar content naturally (Martin, 1990). Figure 3 represents an HPLC chromatogram showing the peaks of sucrose, glucose, fructose, glycerol, and ethanol in wine samples.

Table 3. Measurement of sugars, glycerol, and alcohol in wine samples

Sample	Sucrose (g/L)	Glucose (g/L)	Fructose (g/L)	Glycerol (g/L)	Alcohol (%)
S1	5.75	0	1.51	10.31	13.70
S2	5.79	5.68	1.58	16.35	11.78
S3	4.02	0	1.87	25.91	11.15
S4	5.30	4.05	1.56	15.72	11.86
S5	3.28	3.79	1.37	14.43	13.89
S6	5.08	0	5.00	8.65	13.59
S7	6.94	2.61	1.34	11.14	12.90
S8	0	49.71	53.72	0	0

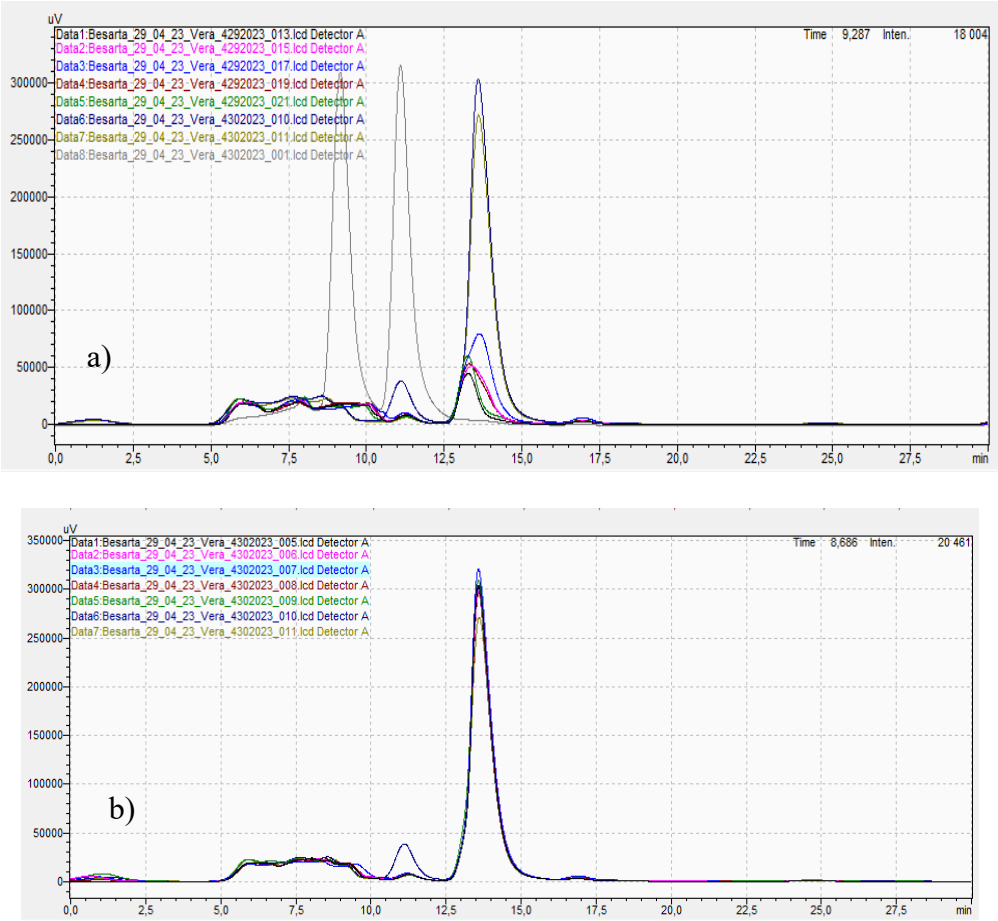


Figure 3. HPLC chromatograms of sugars and ethanol in wine samples. a) HPLC chromatogram of sucrose, glucose, fructose, and glycerol; b) HPLC chromatogram of ethanol.

Glycerol is a sugar alcohol that is the most significant quantitative product in wine after ethanol and carbon dioxide, typically found in concentrations ranging from 1 to 15 g/L. Table

3 shows the results of the alcohol and glycerol content in all samples. Sample S8 does not contain glycerol. The alcohol content ranged from 11% to 13%. Based on the information provided and the results shown in Table 3, it is evident that only sample M6 has a normal glycerol level. In contrast, samples S1, S2, S3, S4, S5, and S7 exhibit higher-than-normal glycerol levels, while sample S8 contains no glycerol. The two key roles of glycerol production in yeast are maintaining redox balance and aiding in the response to hyperosmotic stress. Glycerol production in wine is influenced by several factors, including higher temperatures (20–25°C) that increase glycerol content, especially in red wines compared to white wines. On the other hand, limited oxygen promotes glycerol production, though results vary by yeast strain. Some non-*Saccharomyces* yeasts produce more glycerol under low oxygen conditions. High sugar levels in grape juice, especially from late harvests, lead to increased glycerol as yeast cells accumulate it to balance osmotic pressure. Limited nitrogen in the must increases glycerol production, as does higher sulfur dioxide content. Non-Saccharomyces yeasts, such as *Metschnikowia pulcherrima* and *Lachancea thermotolerans*, often produce more glycerol than *Saccharomyces cerevisiae* and can reduce the ethanol content. These factors can be managed to enhance glycerol production, which affects the wine's texture and sweetness (Scanes et al., 1998). Sample S8 does not contain alcohol, as it is grape juice and has not undergone fermentation. According to Administrative Instruction No. 15/2009 of the Republic of Kosovo, which sets the parameters for the physical-chemical analysis of wine, the minimum natural alcohol content for quality wines with a designated geographical origin is 10.5 vol.% (Administrative Instruction No. 15/2009 of the Republic of Kosovo). Based on this instruction and the sample results, none of the wines have an alcohol content below the required minimum. This confirms that the analyzed wines have the desired strength, ranking them among high-quality wines. It is important to note that most wine samples had their alcohol content declared on the label, as shown in Table 3. However, none of the declared alcohol levels matched the laboratory-measured results. In all cases, the measured alcohol levels were lower than those stated on the labels. A positive Pearson correlation coefficient of 0.55 was observed between glycerol and ethanol, indicating a moderate association between these two variables. A strong positive correlation was observed between sucrose and ethanol, with a coefficient of 0.82, indicating a significant and robust relationship between these two variables.

3.3. Turbidity analysis of wine samples

Turbidity is a crucial parameter in wine production, influencing both the final composition and the aroma of the wine. It measures the total suspended particles in the liquid, which in this case, reflects the clarity and potential quality of the wine. According to the Australian Wine Research Institute, the maximum acceptable turbidity in red wine after filtration is 10 NTU (Duarte et al., 2015). Based on these standards and the results presented in Table 4, it is evident that none of the wine samples exhibit turbidity values within the normal range.

Table 4. Measurement of turbidity in wine samples.

Sample	Turbidity (NTU)
S1	25.2
S2	127
S3	19.5
S4	120
S5	104
S6	33.4
S7	33.0

Table 5. Measurement of wine protein stability in samples

Sample	Samples with TCA (NTU) ±SD	Blank Sample (NTU)±SD
S1	1.85 ±0.056	2.38 ±0.098
S2	9.97± 0.106	10.49± 0.579
S3	2.05 ±0.162	1.53 ±0.388
S4	4.79 ±0.077	9.35 ±0.169
S5	0.91 ±0.042	0.67±0.077
S6	2.83±0.862	1.3 ±0.367
S7	1.66 ±0.325	1.39 ±0.070
S8	2.17±0.021	3.46 ±0.056

3.4. Protein stability analysis of wine samples

The results of protein stability in wine are shown in Table 5. Wine, like many other products, contains proteins primarily derived from grapes. However, proteins can also originate from yeast. Yeast influences the protein composition of wine in two ways: a) by transferring proteins during the yeast autolysis process, and b) by hydrolyzing must proteins through the extracellular protease present in the yeast (Tian and Harrison, 2012). The proteins present in wine are responsible for colloidal instability, leading to the formation of amorphous sediments and haze. Protein haze can develop at high temperatures during storage or transport due to protein self-aggregation. Several factors, including storage or aging temperature, pH, wine protein composition, organic acids, ethanol, phenolic compounds, and sulphate content, influence the formation of this haze. Protein instability can also occur through the mixing (stirring) of otherwise stable wines. While the formation of haze or deposits in wine bottles does not pose a health risk to consumers and does not affect the wine's aroma or taste, it significantly impacts the wine's commercial appeal. This is particularly true for white wines, where visual clarity is crucial for consumer acceptance (Cosme et al., 2020). When the turbidity values are higher for the TCA-treated samples than for the Blank samples, it indicates a potential for instability due to the presence of proteins. Samples S3 and S6 exhibit potential instability. These samples may be unstable due to the factors mentioned above. Other studies suggest that protein instability is not strongly linked to the total protein content in wine. Instead, the molecular characteristics of individual proteins determine their propensity to precipitate. However, the exact proteins responsible for wine turbidity remain uncertain, with contradictory findings in the literature regarding which proteins cause haze and sediment formation. Some researchers have identified a fraction or specific group of proteins that contribute to this instability (Mesquita et al., 2001).

3.5. Chemical analysis of wine samples

The results of the total SO_2 analysis reveal a variation in SO_2 content among the samples, with S1 exhibiting the highest SO_2 concentration and S8 showing the lowest (Table 6). According to Administrative Instruction No. 15/2009 of the Republic of Kosovo, which outlines the wine parameters for physical-chemical analyses, the total SO_2 content should not exceed 160 mg/L (Administrative Instruction No. 15/2009 of the Republic of Kosovo). Based on this standard and the results presented in Table 6, it is evident that all the wine samples have permissible levels of total SO_2, staying well within the established limit.

Regarding the free SO_2 content, sample S4 has the highest concentration at 24.8 mg/L, while samples S3 and S8 have the lowest at 5.6 mg/L (Table 6). According to the same Administrative Instruction No. 15/2009, the maximum allowable free SO_2 content is 30 mg/L (Administrative Instruction No. 15/2009

of the Republic of Kosovo). Based on this standard and the results, we conclude that all the analyzed samples have free SO_2 levels within the permissible range.

A strong positive correlation was also identified between total SO_2 and free SO_2, with a Pearson coefficient of 0.89, implying that as one variable increases, the other tends to increase proportionally.

The results of the pH measurements on the wine samples indicated that there are no significant differences in pH values among the analyzed wine samples (Table 6). As previously mentioned, the typical pH range for wine is between 3.1 and 3.9 (Azevedo et al., 2016). Based on the results, we can confirm that all the wine samples fall within the normal pH range.

Table 6. Chemical analysis of wine samples

Sample	Free SO_2, mg/L ±SD	Total SO_2, mg/L ±SD	pH ±SD	Acidity g/L ±SD
S1	17.6 ± 2.26	52 ± 1.13	3.4 ± 0.098	7.63 ± 2.44
S2	19.2 ± 6.78	40.8 ± 10.18	3.335 ± 0.049	7.2 ± 1.83
S3	5.6 ± 1.13	11.2 ± 0	3.73 ± 0.028	5.6 ± 0.14
S4	24.8 ± 7.9	41.6 ± 4.52	3.575± 0.063	5.6 ± 0
S5	8.8 ± 3.39	12.8 ± 2.26	3.375 ± 0.021	5.85 ± 0.07
S6	11.2 ± 6.78	15.2 ± 5.65	3.345 ± 0.134	5.9 ± 0.14
S7	7.2 ± 3.39	12.8 ± 9.05	3.29 ± 0.070	5.85 ± 0.07
S8	5.6 ± 3.39	7.2 ± 1.13	3.375 ± 0.007	5.15 ± 0.07

The acidity of the wine samples, expressed as tartaric acid, is presented in Table 6. Samples S1 and S2 have higher acidity values, which correlate with lower pH levels, while minor differences in acidity are reported for the remaining samples. According to Law No. 04/-L for wines, the total acidity content expressed as tartaric acid should not be less than 3.5 g/L (Republic of Kosovo, Law No. 02/L-8 on wines). Rajkovic and Sredovic (2009) indicated that the titratable acidity of red wine, expressed in tartaric acid, typically ranges from 4.0 to 8.0 g/L.

A weak negative correlation was found between pH and acidity, with a Pearson coefficient of -0.287, indicating an inverse relationship between these two variables.

4. Conclusions

In conclusion, the microbiological and chemical analyses of the wine samples provide significant insights into the factors that influence wine quality and stability. The absence of total mesophilic bacteria, Lactobacillus, and the presence of *Acetobacter* in specific samples underscore the importance of controlling oxygen exposure and temperature during production to prevent spoilage. The identification of yeasts and molds in some samples highlights the resilience of microorganisms under challenging fermentation conditions, as well as the potential for mold contamination during grape harvesting and wine processing. Chemical analyses, including measurements of pH, acidity, alcohol, glycerol, sugars, and turbidity, indicate that the wine samples largely meet acceptable regulatory standards, thereby ensuring their overall quality. However, discrepancies between the declared and measured alcohol levels, along with the presence of protein instability in some samples, suggest areas where further optimization is needed. Ultimately, this study underscores the importance of rigorous monitoring and control during wine production to ensure its microbiological safety and sensory properties while maintaining adherence to quality standards.

The statistical findings highlight both significant positive and negative associations among the studied parameters, providing insights into their interdependencies.

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