Clinical and Experimental Health Sciences

Research on the Determination of the Physicochemical and Sensory Characteristics of the Wine Produced by Malolactic Fermentation from *Sauvignon Blanc, Merlot* and *Kalecik Karası* Grapes

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ABSTRACT

Objective: The aim of this study is to examine how malolactic fermentation and classical fermentation affect the physicochemical and sensory properties of wines made from red and white grapes.

Methods: In our research, we opted for Sauvignon blanc variety in white grapes and locally produced Kalecik karası and French Merlot variety in red grapes all of which are recognised as important grape varieties both in our country and around the world. To preserve the natural aroma of the grape, start the fermentation rapidly and ensure formation of a balanced amount of glycerol, the use of Saccharomyces cerevisiae strain among vine yeasts deemed suitable. Oenoccus oeni MBR®UVAFERM®BETA (2x1011CFU/g) (Lallemand Inc., France) strain was used for malolactic fermentation. Sensory analyzes of the produced wines, chromatographic analysis of organic acids, and pysico-chemical analyses of products which are formed as a result of the processing of the fruits, were also made. For this purpose, the panelists evaluated the wines according to seven different criteria and the evaluation was made on a nine-point hedonic scale, and the most liked sample was given 9 points and the least liked one was given 1 point. Chemical and sensory properties of the produced wines were evaluated statistically.

Results: As a result of our study, it has been determined that the amount of ash in red wines is higher which indicates higher amount of grape extracts obtained from Kalecik karası and Merlot grapes.When compared according to fermentation types, the amount of lactic acid increased in wines produced by malolactic fermentation whereas the amount of phenolic compounds was higher in ethyl alcohol concentrations, and these values decreased with malolactic fermentation. Additionally, the accommodation of two foreign origin grapes one of which is red and the other one white, country's geography has been revealed in this study with the characteristics of the wine produced.

Conclusion: The harmony of two foreign grapes, one red and one white grape, to the geography of our country was revealed with the characteristics of the wine produced. As a result of the thesis study, it was found that malolactic fermentation improved the quality of the wine, making it more pleasurable. In this context, the results of the research has the quality and attributes that will shed light on winemakers.
Keywords: Malolactic fermentation, phenolic compounds, wine, maceration.

1. INTRODUCTION

Vine is the resource material of wine and belongs to the *Vitis* genus of the *Vitaceae* Family. *Vitis* genus includes two species namely *V. vinifera* and *V. muscadinia* (1). Archeological excavations show that Anatolian peninsula is the homeland of the first vine tree, and it is known that wine has been produced in different ways since 4000 BC, spreading to the Hittites, Lydians and other civilizations (2). Fresh grapes can be divided into three groups according to their consumption patterns; table grapes, dried grapes, wine grapes (3). *Papazkarası, Öküzgözü, Boğazkere, Kalecik Karası, Pinot Noir, Gamay* and *Merlot* can be named among the grape varieties that give quality red wine. *Kalecik Karası* initially produced in Ankara later started to be produced in Thrace region as well. In our country *Kalecik Karası* and *Merlot* varieties attract increasing interest and *Kalecik Karası* and *Merlot* wines are sold at the highest prices in the markets (4). The main factors affecting the ripening of grapes and the composition of the wine are; grape variety, maturity state, ecological factors, nurturing, diseases and presence of damage. Too high or too low air temperature during ripening, excessive or insufficient rainfall, or excessive irrigation reduce the synthesis of phenol compounds. In addition, the amount of phenol components in red wines is 20-25 times more than white wines. The reason why red wines are rich in phenol components is the pulp fermentation applied during the production process (5). Generally, alcohol fermentation, which is a biological process like all other fermentations, is used in winemaking. During fermentation, besides ethyl alcohol and carbon dioxide, low amounts of glycerin, acetic acid, acetaldehyde, high alcohols and many substances that affect the aroma of the wine produced are formed (6,7). Another fermentation applied to wines is malolactic fermentation, which is generally preferred for red wines; however it can also be applied in white wines with high acidity. During malolactic fermentation, malic acid (2-10 g/L) is reduced entirely and this causes the pH of the wine to rise and the taste to change, giving way to increased sensory properties and permanence. In addition, acetaldehyde, diacetyl and high alcohol contents also change, contributing to the aroma of the wine (8,9).

The aim of this study is to investigate the effects of wine production methods by classical and malolactic fermentation on the sensory and physicochemical properties of the samples obtained, and to determine the physico-chemical composition and sensory properties of the samples obtained by both methods. In addition, the study is aimed to reveal the accommodation of two foreign origin grapes, one of which is red and the other white, to the geography of our country, by the characteristics of the wine produced.

2. METHODS

2.1. Collection of research material and processing into wine

Sauvignon blanc from white grape varieties, *Kalecik karası* of native origin and *Merlot* of France origin from red grape varieties were used as research material. The research materials were harvested on 10.10.2012 from vineyards of Tekirdağ Viticulture Research Institute (40° 58′ 25′ ′N – 27° 28′ 53′′ E, Altitude: 575m). Brix, acid, pH and density analyzes of the grapes were made prior to harvesting. At the end of the analysis, maturity indices were calculated and to proceed the harvest was decided. The harvest of the grapes, whose maturity was determined by performing raw material analyzes, was carried out in a controlled manner, and the samples were carefully collected from beginning, middle and end of the vineyard and each furrow. Raw material analyzes were carried out in the laboratory of Tekirdağ Viticulture Research Institute. Following the harvest of approximately 60 kg of each grape variety, the grapes were brought to a private wine establishment where the selection and removal process of bruised and dented grapes was initially carried out. Later, the stems were separated from the grain using a grape mill and the mash was prepared by cracking the grain. The following processes were applied for red and white grapes as shown in the wine production flow charts. White wine production flow chart is shown in Supplementary Figure 1. and the red wine production flow chart is shown in Supplementary Figure 2. After the grain cracking process, 25 mg/L potassium metabisulfite was added to the mash as a preservative before pressing and the mash was mixed again. To preserve the natural aroma of the grape, start the fermentation rapidly and ensure formation of a balanced amount of glycerol, the use of *Saccharomyces cerevisiae* strain among vine yeasts deemed suitable (10). In alcohol fermentation, *S.cerevisiae* N JB3 (Selection CIVAM cor 8) was used for white grapes, *S.cerevisiae* N 7303 (INRA NARBONNE) was used for red grapes, and *Oenoccus oeni* MBR®UVAFERM®BETA ($2x10^{11}$CFU / g)(Lallemand Inc., France) was used for yeast and malolactic fermentation. 5L, 10L and 15L glass and pet demijohns were used for the must fermentation. Viusal inspections of the fermentation process were monitored from fermentation heads attached to demijohns, and demijohns were preserved in temperature controlled rooms. 75 cL brown wine bottles were used as packaging material for the final product.

2.1.1. Fermentation of white grape

Hydraulic basket presses were used in pressing. In fermentation, the "first wort" flowing spontaneously without any applied pressure was combined with the "second must" obtained after the first pressing and the amount of must obtained was measured as approximately 35L. YEPD broth was used for activation of the culture, the culture was transferred to the broth under aseptic conditions and incubated for 48 hours under 28°C after being mixed. At the end of this period, the culture was grown up to the volume that will be used in fermentation in the same medium, then separated from the liquid part using centrifugation, re-suspended in 0.85% saline before inoculation and added to the vessels, at the rate of 0.25% (V/V), where the fermentation will be carried out. The number of microorganisms in the added culture was determined to be $1.1x10^9$, in YEPD agar after 48 hours in 28°C. The obtained must was transferred to a demijohn and fermentation caps were attached, after the mouths were sealed with paraffin, it was left to fermentation at cellar temperature (15±2°C). Density analyzes were performed between the 0th and 21st days of fermentation, taking care to make measurements at the same time every day or every other day and the decision to end the fermentation process was taken according to the densitometry measurements.

2.1.2. Fermentation of red grape

Dry yeast *S.cerevisiae* N 7303 INRA NARBONNE which will be used for alcohol fermentation was activated as made with white grapes, the number of microorganisms in the culture at the end of activation was determinedas $1.2 x10^9$. The active culture was added to the must 0.25% (V / V). Red grapes were left to the grain fermentation at 20±2°C for a week (11). At the end of the grain fermentation, the red grapes were pressed using hydraulic basket presses. In fermentation, the "first must" flowing spontaneously without applying pressure was combined with the "second must" obtained after the first pressing, and 34 and 35L must obtained from *Merlot* and *Kalecik karası* grapes respectively, were transferred to demijohns. Demijohns were then left to fermentation at cellar temperature (15±2°C). Density analyzes were performed between the 0th and 21st days of fermentation, taking care to make measurements at the same time every day or every other day and the decision to end the fermentation process was taken accordingly.

2.1.3. Transfer and resting in red and white wine

The first transfer process was applied to the white wines left to fermentation at the end of December 2012, the inactive microorganisms settled at the bottom of the container were separated from the must. While transferring helps the wine to mature, over-transfer and contact with air will cause excessive oxidation of the wine and flattening of the taste and aroma, so the transfer was done carefully. The transfer process was repeated at the beginning of February and density measurements were made. Since the density measurement results for white wine of *S.blanc* variety the were detected as 990 fermentation was deemd as complete. The amount of must at the end of the fermentation was determined as 33 liters. The measurement results for red wine of *Kalecik karası* variety were 990, and *Merlot* were determined as 995 therefore it was decided that the fermentation was completed. The amount of must at the end of the fermentation has been measured as approximately 32L for both *Kalecik karası* and *Merlot* varieties.

2.1.4. Malolactic fermentation

MRS Broth was used in the activation of the lyophilized culture, the culture was transferred to the liquid medium under aseptic conditions, after stirring, it was left to incubation at 37°C under microaerophilic conditions for 48 hours. At the end of the period, the culture was grown up to the volume that will be used in fermentation in the same medium, then separated from the liquid part using centrifugation, re-suspended in 0.85% saline before inoculation and added at 5% (V / V) to the containers where the fermentation will be carried out. The number of microorganisms in the added culture was determined to be $1.1x10^9$, in MRS agar medium after 48 hours in 37°C. Sulfurization was avoided during malolactic fermentation, as excess sulfur would harm the bacteria. Demijohns with fermentation caps sealed were kept at 18–20°C. After approximately 2 weeks, completion of malolactic fermentation was observed and the wines were left to rest.

2.1.5. Bottling and aging

After the end of malolactic fermentation, the samples obtained were filtered with plate filter using 8 plates (Pall SeitzSchenk Filtersystems GmbH, Germany) .Samples were filled into 70 cL glass bottles after filtration, and the bottles were closed with corks and labeled. No collage (clarification) process has been applied, as the phenol component of wines will be determined later.

2.2. Physico-chemical analyzes on wines

The physico-chemical analyzes of the product obtained from the processing of fruits were carried out in the Food Analysis Laboratory of Tekirdağ Viticulture Research Institute, and the chromatographic analysis of organic acids were carried out in the Liquid Chromatography (HPLC) Laboratory of Bursa Food and Feed Control Center Research Institute. Citric acid, oxalic acid, malic acid, tartaric acid and lactic acid standards used in the determination of organic acids by HPLC were obtained from SigmaAldrich Co. LLC. HPLC (Agilent 1100, Agilent Technologies, USA), C8 HPLC column (ACE 150X4.6 mm), 0.45 µm membrane filters (Agilent, screw tap 5182-0716), shaking water bath (julabo SW22, JULABO Labortechnik GmbH, Seelbach, Germany), ultrasonic water bath (VWR Ultrasonic cleaner, VWR International GmbH, Darmstadt, Germany), centrifuge (Heraeus Megafuge 40R Centrifuge, Thermo Fisher Scientific Inc. USA), spectrophotometer (Optizen 322OUV, Optizen Labs LLC, Warsaw, Poland) has been used for Organic acid determination.

2.3. Sensory Analyzes of the wines

The sensory analyzes of the wines in the thesis were carried out by 35 panelists aged between 25-40 who had no experience in wine tasting. During the analysis, attention was paid to the basic rules such as a bright environment, the absence of foreign odor, the selection of the appropriate glass and the tasting time. The form used in sensory analysis is shown in Appendix 1.The panelists evaluated the wines according to seven different criteria and the evaluation was made on a nine-point hedonic scale, where the most liked sample was given 9 points and the least liked one was given 1 point. The analysis form for the sensory evaluation is given in the Appendix. Panelists scored in a bright and closed environment without being affected by each other. Evaluation was made by taking the average of the points given by each panelist for each criteria group (12,13).

2.4. Statistical Analyses

The chemical properties of the wines produced were evaluated statistically. The data obtained as a result of chemical analysis of 6 groups of wines, two varieties from each of the three grape types used, were evaluated using the variance analysis with the Version 8 (SAS Institute Inc. 2008) statistical program.

3. RESULTS

Results of the raw materials chemical analyses and physicochemical and sensory analyses of the wines produced in our study are given together with the variance analyses.

3.1. Raw material analysis results.

Table 1. *Analysis results of raw materials before fermentation.*

Grape Varieties	pH	Brix% (Dry matter)	Acid (g / L)	Maturity Index	Density (density)
Sauvignonblanc	3.20	24.6	9.0	25.4	1108
Kalecik karası	3.41	22.6	11.06	17.6	1095
Merlot	3.50	23	7.2	29.2	1100

The density values were 1.108 for *S.blanc*, 1.095 for *Kalecik karası*, and 1100 for *Merlot*. According to the raw material analysis obtained, pH values were; 3.20 for *S.blanc*, 3.41 in *Kalecik karası*, 3.50 in *Merlot*, and brix intervals and acid values are in parallel with the literature studies. Brix values were found as 24.6% in *S.blanc* variety, 22.6% in *Kalecik karası* and 23% in *Merlot* variety. The acid content of the raw materials used in the study was determined as 9.0 g/L in *S.blanc* variety, 11.06 g/L in *Kalecik karası*, and 7.2 g/L in *Merlot* variety. Raw material maturity indices were determined as 25.4 in *S.blanc* variety, 17.6 in *Kalecik karası* and 29.2 in *Merlot* variety.

3.2. Chemical analysis results of wine samples

The chemical analysis results obtained in the study are given in Table 2. At the end of our study, the lowest density values of the wines produced were determined as 0.9901 for *S.blanc* malolactic wine, and the highest as 0.9952 for *Merlot* malolactic wine. In a study conducted in our country, the density of wines was determined between 0.9934-0.9970 (14). In a study using different yeast species, it was stated in the experiments that the specific gravity of wines varied between 0.9260 and 0.9940 (15). In a study conducted with wine density, the density of black grape wine was found to be 0.9864 and the density of white wine to be 0.9867 (16). According to a study conducted with red wines, the density in red wines is between 0.9917-0.9927 (17).

The dry matter value of *S.blanc* classic wine was 20.90 g/L, the dry matter value of malolactic *S.blanc* wine was 21.1g/L. The dry matter value of the *Kalecik karası* classic wine was 26.0 g/L. The dry matter value of the malolactic wine of *Kalecik karası* was 27.6 g/L. The dry matter value of *Merlot* classic wine was 29.5 g/L and the dry matter value of *Merlot*'s malolactic wine was found as 25.5 g/L.

Table 2. *Chemical analysis results of wine samples.*

Wine Types	Density 20/20°C	Alcohol%, V/V	Dry matter g/L	Ash g/L	Ash thickness	Total Acid g/L	Acid g/L	pH	Total SO_2 mg/L	Free SO_2 mg/L	Sugar g/L
***S. blanc* Classic**	0.9902 ± 0.1^{a}	12.5 ± 0.17^{a}	20.9 ± 0.26^{d}	1.61 ± 0.35^{c}	23.6 ±0.94^{c}	6.38 ± 0.11^{c}	0.38 ± 0.06^{a}	3.33 ± 0.0^{c}	25 ± 0.69^{d}	8 ± 0.64^{at}	0.97 ± 0.10
***S. blanc* ML**	0.9901 ± 0.05^{a}	12.5 ± 0.0^{a}	21.1 ± 0.05^{d}	1.52 ± 0.15^{c}	20.8 ± 0.25^{d}	6.38 ± 0.16^{c}	0.42 ± 0.04^{a}	3.29 ± 0.05^{c}	25 ± 1.52^{d}	7 ± 1.15^{e}	1.27 ± 0.18^{e}
***Kalecik karası* Classic**	0.9943 ± 0.50^{a}	12.3 ± 0.28^{a}	26.0 ± 1.38^{c}	2.51 ± 0.04^{ab}	35.6 ± 0.81^{a}	6.68 ±0.11^{bc}	0.40 ± 0.05^{a}	3.71 ± 0.02^{a}	37 ± 0.83^{b}	15 ± 0.66^{b}	2.07 ± 0.03^{d}
***Kalecik karası* ML**	0.9929 ± 0.06^{a}	12.3 ± 0.35^{a}	27.6 ± 1.11^{b}	2.39 ± 0.07^{ab}	32.8 ± 0.20^{b}	7.13 ± 0.06^{a}	0.34 ± 0.07^{a}	3.71 ± 0.01^{a}	45 ± 3.52^{a}	19 ± 1.05^{a}	3.42 ± 0.01^{a}
***Merlot* Classic**	0.9952 ± 0.51^{a}	12.1 ± 0.35^{a}	29.5 ± 0.65^{a}	2.87 ± 0.16^{a}	32.8 ± 1.38^{b}	6.90 ±0.15^{ab}	0.35 ± 0.03^{a}	3.65 ± 0.21^{b}	33 ± 1.05^{bc}	12 ± 0.62^{c}	2.52 ± 0.36^{b}
***Merlot* ML**	0.9941 ± 0.58	12.5 ± 0.32	25.5 ± 1.45^{c}	2.86 ± 0.05^{a}	32.8 ± 0.62^{b}	7.05 ±0.07^{ab}	0.36 ± 0.03^{a}	3.65 ± 0.08^{b}	30 ± 1.73^{cd}	9 ± 0. 79^{d}	2.21 ± 0.23^{c}

P≤0.05; There is a statistically significant difference between the averages shown in different letters in the LSD test.
Classic: Control groups produced by classical fermentation.
ML: Experimental groups produced by malolactic fermentation

In a study conducted on red and white wines, the amount of dry matter was determined as 22.2-24.0 g/L in white wines and 25.8-31.5 g/L in red wines (14). When we look at the dry matter results of the wines obtained in our study, it is compatible with the dry matter amounts given in the previously mentioned studies. When we look at the studies on the alcohol content of wines, it has been seen that the values vary between 8.8% and 14% (17-20). Ash contents were determined as 1.61 g/L for *S.blanc* classic wine, 1.52 g/L for *S.blanc* malolactic wine, 2.51g/L for *Kalecik karası* classic wine, and 2.39g/L. for its malolactic wine. The ash content of *Merlot* classic wine is 2.87g/L and that of malolactic wine is 2.86g/L. Ash thickness values were measured as lowest in malolactic wine of *S.blanc* variety with 20.8 g/L and highest in classical wine of *Kalecik karası* variety with a value of 35.6 g/L. Ash is the sum of non-flammable substances in wine. Although its amount in wine is less than that of must, it varies according to the processes applied to the wine (21). Kubilay (1996) states that there is 1.4-1.6 g/L ash in white wine and 1.9-2.3 g/L ash in red wine. In another study, ash amounts in red wines were found between 2.5 and 2.9 g/L. Ash thickness on the other hand is between 26.4 and 29.2 (17).

PH values of wines were found as; 3.33 in S.blanc classical wine, 3.29 in malolactic wine, 3.71 in both types of Kalecik karası wines, and 3.65 in both types of Merlot wines. During fermentation, organic acids, together with their salts, remain stable in the wine and the pH of the wine is kept constant in the range of 2.90-4.00, ensuring a healthy fermentation (22). Total SO_2 amounts were determined as 25 mg/L in both wine types of *S.blanc* variety; 37 mg/L in *Kalecik karası* classical wine and 45 mg/L in its malolactic wine; 33 mg/L in *Merlot* classical wine and 30 mg/L in malolactic wine. Free SO_2 amounts were found as; 8 mg/L in *S.blanc* classic wine, 7 mg/L in its malolactic wine; 15mg/L in *Kalecik karası* classical wine and 19 mg/L in malolactic wine; 12 mg/L in *Merlot* classical wine and 9 mg/L in malolactic wine. Total acid contents were the lowest in two ypes of *S.blanc* wines with 6.38 g/L and the highest in *Kalecik karası* malolactic wine with 7.13 g/L. The amount of volatile acid was found to be the least amount in *Kalecik karası* malolactic wine with 0.34g/L and the most in *S.blanc* malolactic wine with 0.42g/L.

The amount of volatile acid in Turkish wines is between 0.2-0.8 g/L. According to Turkish wine regulations, acid should not exceed 1.8 g/L. According to the EC wine regulations, the maximum amount of volatile acid is 1.1 g/L for white wines and 1.2 g/L for red wines (23). According to this information, the volatile acid amounts in of our study are in accordance with the Turkish wine charter.

Sugar content of wines were detected as in *S.blanc* malolactic wine, 1.27 g/L in *Kalecik karası* classic wine, 3.42 g/L in *Kalecik karası* malolactic wine, 2.52 g/L in *Merlot* classic wine and 2.21 g/L in its malolactic type.

3.3. Phenolic contents of wine samples.

The contents of phenolic substances found and detected in the wines produced in our study are given in Table 3.

Table 3. *Phenolic contents of the obtained wines*

Grape variety	Phenolic Compounds (Catechins) mg/L	Tannin (tannic acid) g/L	Anthocyanin mg/L
***S.blanc* Classic**	311 ± 2.96^{d}	0:32 ± 0.15^{d}	ND
***S.blanc* ML**	291 ± 1.45^{d}	0.30 ± 0.03^{d}	ND
***K. karası* Classic**	2743 ± 33.42^{a}	2.83 ± 0.20^{a}	54.2 ± 2.36^{c}
***K. karası* ML**	2288 ± 25.05^{b}	2.21 ± 0.05^{c}	48.5 ± 3.10^{d}
***Merlot* Classic**	2093 ± 17.15^{c}	2.29 ± 0.12^{b}	136.9 ± 6.17^{a}
***Merlot* ML**	2053 ± 11.20^{c}	2.14 ± 0.04^{c}	98.5 ± 4.01^{b}

P≤0.05; In the LSD test, there is a statistically significant difference between the averages shown with different letters
ND: Not Detected

Phenolic compounds detected in wines; catechin amount were measured as 311 mg/L in *S.blanc* classic, 291 mg/L in *S.blanc* malolactic; 2743 mg/L for *Kalecik karası* classic, 2288 mg/L for *Kalecik karası* malolactic; 2093 mg/L for *Merlot* classic and 2053 mg/L for *Merlot* malolactic wines. Tannin amounts were detected as 0.32 g/L in *S.blanc* classic wine, 0.30 g/L in *S.blanc* malolactic wine; 2.83 g/L in *Kalecik karası* classic wine, 2.21 g/L in *Kalecik karası* malolactic wine; 2.29 g/L in *Merlot* classical wine and 2.14 g/L in *Merlot* malolactic wine. The amount of anthocyanin was determined as 54.2 mg/L in *Kalecik karası* classic wine, 48.5 mg /L in *Kalecik karası* malolactic wine; 136.9 mg/L in *Merlot* classic wine and 98.5 mg/L in *Merlot* malolactic wine.

3.4. Analysis results of organic acids in wines

The amount of organic acids detected in the study is given in Table 4. In addition, the HPLC chromatogram and calibration curves of the analyzed organic acids are given in Figures 1-5.

Table 4. *The amount of organic acids in the obtained wines.*

	Lactic Acid ng/µL	Oxalic Acid mg/L	Tartaric Acid mg/L	Malic Acid mg/L	Citric Acid mg/L	T/M	M/L
***S.blanc* Classic**	1.71±0.08[ab]	0.085±0.01[a]	4.02±0.05[a]	2.25±0.02[d]	Trace	1.78±0.04[b]	1.31±0.07[c]
***S.blanc* ML**	2.06±0.10[a]	0.065±0.01[a]	2.96±0.08[b]	1.24±0.02[e]	Trace	2.38±0.03[a]	0.60±0.04[c]
***Kalecik karası* Classic**	1.28±0.04[b]	0.075±0.01[a]	3.06±0.06[b]	3.03±0.04[a]	Trace	1.01±0.00[c]	2.36±0.05[a]
***Kalecik karası* ML**	1.53±0.53[ab]	0.060±0.02[a]	1.79±0.14[d]	2.76±0.08[b]	Trace	0.65±0.04[e]	1.92±0.71[ab]
***Merlot* Classic**	1.59±0.06[ab]	0.075±0.01[a]	2.62±0.02[c]	3.10±0.01[a]	Trace	0.84±0.01[d]	1.95±0.07[ab]
***Merlot* ML**	1.69±0.07[ab]	0.055±0.00[a]	1.59±0.10[d]	2.36±0.02[c]	Trace	0.67±0.04[e]	1.40±0.07[b]

P≤0.05; In the LSD test, there is a statistically significant difference between the averages shown with different letters.
Trace: Below the working range.

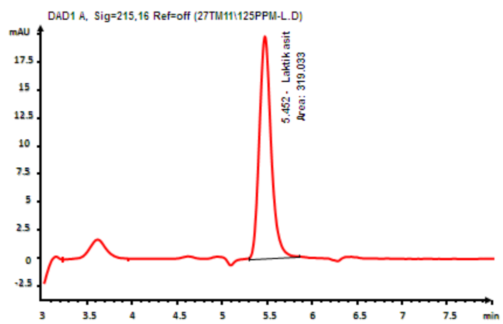

Figure 1.A-HPLC *chromatogram of Lactic acid standard.*

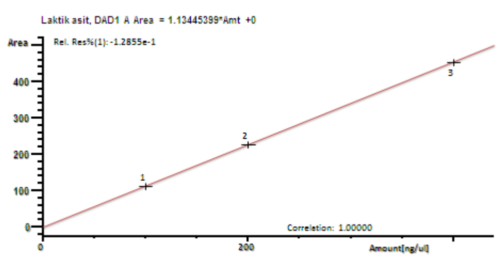

Figure 1.B *– Calibration curve for lactic acid.*

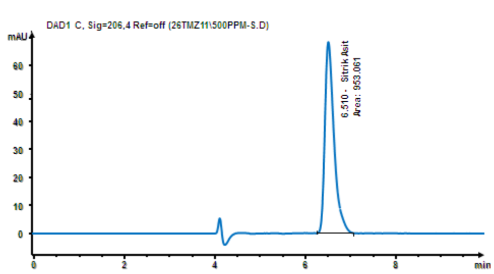

Figure 2.A *– HPLC chromatogram of citric acid standard.*

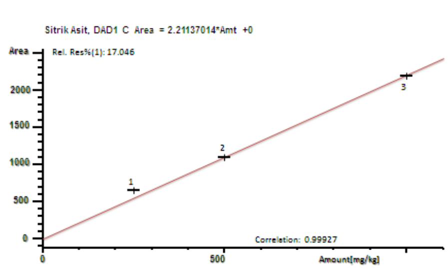

Figure 2.B *– Calibration curve for citric acid.*

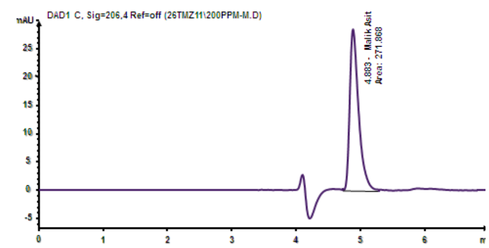

Figure 3. *A – HPLC chromatogram of malic acid standard.*

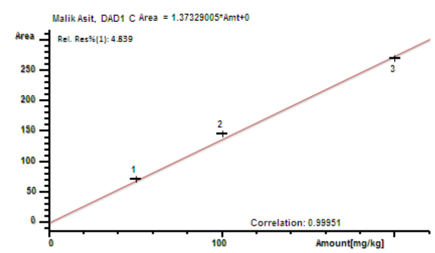

Figure 3. *B – Calibration curve for malic acid.*

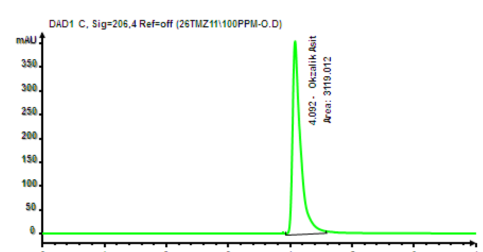

Figure 4. *A – HPLC chromatogram of oxalic acid standard.*

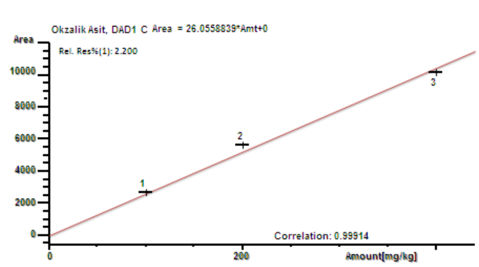

Figure 4.B *– Calibration curve for oxalic acid*

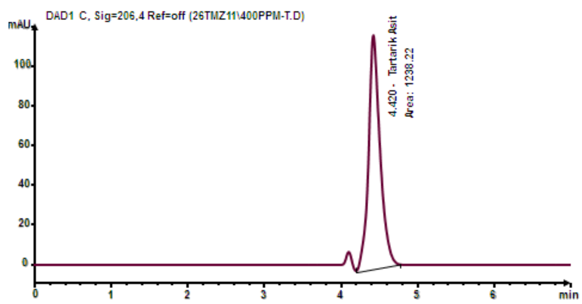

Figure 5. *A – HPLC chromatogram of tartaric acid standard.*

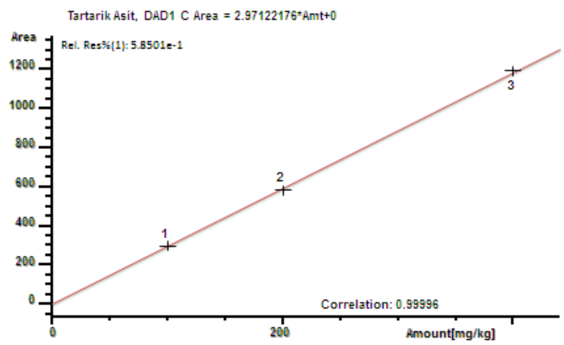

Figure 5. *B – Calibration curve for tartaric acid.*

Table 5. *Wavelengths and accuracies of researched organic acids.*

Compound	Wavelength (nm)	RT (d)	Accuracy R^2
Lactic Acid	360	5:45	1.00000
Malic Acid	280	4.88	0.99951
Oxalic Acid	360	4:09	0.99914
Tartaric Acid	320	4:42	0.99996
Citric Acid	320	6.510	0.99927

RT: *Retention time*

The wavelengths, retention times and accuracies of the samples analyzed at three wavelengths, 280, 320 and 360, are given in Table 5. The accuracy of the compounds range from 0.99914-1.00000. As seen in table 4, Lactic acid contents, among the organic acids studied in the produced wines, for wines produced with classical fermentation was determined between 1.28–1.71 ng/mL and for wines produced by malolactic fermentation, it was found between 1.53–2.06 ng/mL. The amount of oxalic acid for wines produced by classical fermentation was between 0.075-0.085 mg/L, while for wines produced by malolactic fermentation, the value is between 0.055-0.065 mg/L. The determined amount of tartaric acid for wines produced by classical fermentation was 2.62–4.02 mg/L whereas it was found between 1.59-2.96 mg/L for wines produced by malolactic fermentation. The amount of malic acid is between 2.25-3.10 mg/L for wines produced by classical fermentation, and between 1.24-2.76 mg/L for wines produced by malolactic fermentation.

3.5. Sensory analysis results

Sensory analysis results of the produced wines are given in Table 6. Wines were scored out of 9 while performing sensory analysis.

It was observed that the color scores of the samples were between 7.85-8.42, clarity scores 7.28-8.42, bouquets points 6.42-7.57, fullness scores 6.48-7.54, fragrance scores 6.62-7.54, taste points 6.57-8.01 and general taste points 7.00-8.14. When we look at the sensory analysis results in terms of taste; *S.blanc*'s wine produced by traditional fermentation scored 6.57 points, and wine produced by malolactic fermentation scored 7.57 points. While traditional wine of *Kalecik karası* scored 7.57 points, malolactic type was appreciated more and got 8.01 points. The traditional wine of *Merlot* scored 7.01 points, and the malolactic wine 7.57 points.

Table 6.The *scores of the wines obtained as a result of the sensory analysis.*

Varieties	Color	Clarity	Bouquets	Fullness	Fragrance	Taste	General Likes	Group Average
SC	7.85±0.69	7.28±1.38	6.42 ± 1.51	6.48±1.45	6.62 ± 1.32	6.57± 1.81	7.00±1.63	6.88±1.39
SM	8.28 ± 0.95	7.85± 1.21	7.57 ± 0.97	7.53± 0.95	7.58 ± 0.81	7.57± 0.97	7.85± 1.06	7.74± 0.98
Mean	8.06 ± 0.82	7.56± 1.29	6.99 ± 1.24	7.0 ± 1.2	7.1 ± 1.06	7.07± 1.39	7.42± 1.34	7.31± 1.19
KC	8.42 ± 0.53	7.85± 0.69	7.02 ± 1.21	7.42± 1.20	7.12 ± 1.20	7.57± 1.25	7.71± 1.27	7.58± 1.05
KM	8.42 ± 0.53	8.42± 0.53	7.28 ± 1.25	7.54± 1.18	7.50 ± 0.82	8.01± 0.81	8.14± 0.69	7.90± 0.83
Mean	8.42 ± 0.53	8.13± 0.61	7.15 ± 1.23	7.48± 1.19	7.31 ± 1.01	7.79± 1.03	7.92± 0.98	7.74± 0.94
MC	8.01 ± 0.53	8.03± 0.81	7.28 ± 0.95	7.25± 0.93	7.22 ± 1.31	7.01± 1.41	7.71± 1.11	7.50± 1.00
MM	8.14 ± 0.48	8.14± 0.69	7.57 ± 0.97	7.52± 0.90	7.54 ± 1.22	7.57± 0.97	8.01± 0.81	7.78± 0.86
Mean	8.07 ± 0.50	8.08± 0.75	7.42 ± 0.96	7.38± 0.91	7.38 ± 1.26	7.29±1.19	7.86± 0.96	7.64± 0.93
Minimum	7.85	7.28	6.42	6.48	6.62	6.57	7.00	6.88
Maximum	8.42	8.42	7.57	7.54	7.58	8.01	8.14	7.90
Average	8.19	7.93	7.20	7.30	7.26	7.38	7.74	7.56

Abbreviations: SC: S. blanc Classic, SM: S.blanc, Malolactic KC: Kalecik karası Classic, KM: Kalecik karası Malolactic MC: Merlot Classic MM: Merlot Malolactic

3.6. Statistical Analysis

Results of the variance analysis of the wines obtained at the end of our study are given in Appendix Table 2. According to the results of variance analysis, while the yeast used did not have a statistically significant effect on the density, alcohol and pH values of the wines, it had a significant effect on the dry matter, ash, ash thickness, free and total sulfur, total acid and volatile acid values (*P <0.01*).

Lactic acid culture does not seem to have a statistically significant effect on density, alcohol, total sulfur, volatile acid and pH values of wines, however it has a significant effect on dry matter, ash, ash thickness, total acid, free sulfur and sugar values (*P <0.01*).

When the culture and yeast interaction is examined; It has no significant effect on density, alcohol, ash, volatile acid and pH values, whereas a significant effect was determined on dry matter, total and free sulfur, sugar, ash content and total acid values (*P <0.01*). In addition, the groups have a significant effect on anthocyanin, catechin and tannin values (*P*<0.01).

According to the results of variance analysis, there is no statistically significant effect of the yeast used on the organic acids of the wines (*P*<0.01). Variance analysis also revealed that the yeast used had a statistically significant effect on the groups in terms of clarity, bouquets and fullness in the sensory analysis test of the wines (*P <0.01*).

While no significant effect was observed in classical varieties in terms of fragrance parameter, a significant effect was found in malolactic varieties (*P* <0.01). It was determined that the yeast and lactic acid bacteria used had a significant effect on the groups in terms of taste values (*P*<0.01).

Culture and yeast interaction had a significant effect on fullness, odor and bouquets values, although no significant effect was observed on taste, color, clarity and general taste parameters (*P <0.01)*.

4. DISCUSSION

The density results of the wine samples obtained in our study were in parallel with the studies carried out and it was determined that they were within normal limits. Dry matter amount in wines fluctuates within a wide range however it is desired to be between 13-45 gram per liter. According to the Turkish food codex wine regulations, dry matter should be at least 18g in white wines (23). When we look at the dry matter results of the wines obtained in our study, it is compatible with the dry matter amounts given in the previously mentioned studies. The density is low in wines rich in alcohol, and the density increases as the amount of dry matter increases (11). A parallel result was found in our study. When we look at the wine samples, *Merlot,* which has a higher density than the others has a high amount of dry matter. A striking point in the study is that the density of *Kalecik karası* wine made with malolactic fermentation decreased more than the others.

As a result of our study, it was determined that the values of the wines obtained did not change much. Although in the *Merlot* variety it is observed that the alcohol value of the wine produced by malolactic fermentation increases compared to other wines, according to the results in the literature and Turkish standards, the alcohol amounts at the end of our study are within the normal values. Although the ash and ash thickness of the wines obtained as a result of our study is parallel to other studies, it has been determined that the amount of ash in red wines is higher. This result shows us that the extract amouts *of Kalecik karası* and *Merlot* grapes that we studied are higher. It is not preferred that the pH of the wine be higher than 3.50. This is because; As the pH of the wine increases; it becomes susceptible to oxidation reactions, unwanted color changes, protein instability and bacterial fermentation. Moreover the effectiveness of SO_2 on the wine also decreases (5). The pH values obtained in our study are in accordance with the specified values. Sulfur dioxide inactivates the oxidase enzyme in the structure of the product to be processed into wine, prevents oxidation by binding oxygen, and has an antiseptic effect on microorganisms (24). The highest and lowest amounts of sulfur added to prevent oxidation in our research were found in *Kalecik karası* and *Sauvignon blanc* varieties respectively.

Total acidity is determined by titration and gives the amount of free mineral and organic acids in the wine It is stated that the organic acid content of the wine and the changes in the acid content give information about the ripening or contamination of the wine. For example, an increase in the ratio of acetic acid and lactic acid gives information about the ripening of the wine (25). According to the Turkish Food Codex Alcoholic Beverages Regulation, wines must contain at least 4.5 g/L total acid (23). In red wines, higher diacetyl content occurs depending on the wine production technique and malolactic fermentation time (9). In another study, the total acidity of red wines ranges between 6-7 g/L (17). As a result of our study, when we look at the total acid amounts, the acid values of malolactic fermentation were higher in red wines, but remained the same in white wines.

Less volatile acid formation is observed in high acid wines, and therefore it can be thought that the presence of high acid reduces volatile acid formation (16).

In a study conducted, it was reported that the reducing sugar ratios in the samples obtained ranged from 0.35% to 0.86% and the average sugar content was stated as 0.58% (26). According to the results of another study, the amount of sugar in red wines was determined between 2.4-4.9 g/L. (17). According to the results of our research, the amount of sugar in wines is compatible with the studies.

As the shell contact time increases, the total nitrogen content increases (27). It has been shown in studies that total phenolic compound extraction increases at high fermentation temperatures (28,29). In a different study, it has been shown that the effect of temperature on total phenolic compounds was in the first few days of mash fermentation (3-4 days). And its effect decreases in the following days (30). In addition, the extraction of phenolic compounds is faster in the presence of ethyl alcohol (31). As a result of our study, the amount of phenolic compounds was higher in ethyl alcohol concentrations, while these values decreased with malolactic fermentation.

Phenolic compound content values showed great differences in many studies. In a study using 18 different wine samples, it was stated that the total phenolic compound content of wines was between 2.5 and 3.6 g/L (32). There are studies that draw attention to the fact that the total phenolic compound content in red wine in terms of gallic acid is between 1000-4000 mg/L and this value is found as 6500 mg/L in some samples (33,34). According to a study, it was stated that the content of phenolic compounds in wines obtained from the *S.blanc* variety ranged from 50-2000 mg/L in gallic acid (34). However, as it is known, the amount of phenolic substance can vary according to factors such as grape variety, soil and climate. In another study, it was reported that the phenolic content in red wines was between 1882 mg/L (17). The values we found in our study are consistent with the results of the mentioned study.

According to some researchers, “cold” and “enzyme-applied cold maceration” processes generally yield high total anthocyanin amounts in wines (35), while according to some, the total amount of anthocyanins is higher in wines produced by classical maceration (36).

In the study conducted with the wines obtained from Sangiovese grapes of Tuscany region, the total anthocyanin amount was determined as 98 mg/L and 154 mg/L in wines obtained by cold maceration application (under nitrogen gas at 5ºC) (37). These results are consistent with the values obtained in our study.

In another study, the catechin and epicatechin amounts in enriched red wines were determined as 41.34 mg/L and 14.89 mg/L, respectively. These values were determined as 29.41 mg/L and 12.14 mg/L in enriched white wines. The total phenol and antioxidant capacity averages were determined to be 2155.26 mg/L, 414.36 mg/L respectively for red and white wines. Researchers explain the differences in phenol level and antioxidant capacity between red and white wines with the differences in grape variety, climatic conditions and the fermentation methods applied, especially emphasizing the beneficial effect of red wine on health (38).

Tannin amounts should be 1,5 g/L in normal red wines and between 2-2,5 g/L in dark and heavy red wines. The amount of tannins is not desired to be high, as excess tannins will give the wine a bitter taste (18). Although the values obtained from our study are in accordance with the previous studies, amount of tannins in the *Kalecik karası* wine was found higher, however this did not affect the results obtained in the sensory analysis. When the organic acid concentrations determined by HPLC-DAD technique were examined in all varieties, it was seen that the amount of tartaric acid was the highest in general. According to a study, it was found that the highest amount of acid in grapes is tartaric acid (39).

While monitoring the amount of tartaric acid and malic acid, it was determined that the amount of oxalic acid was the least. When compared according to fermentation types, the amount of lactic acid increased in wines produced by malolactic fermentation, while the amounts of tartaric acid, malic acid and oxalic acid were found to be higher in wines produced by classical fermentation. When we look at the sensory evaluation results; In terms of color, two types of *Kalecik Karası* wine take the first place, followed by *Merlot*. In terms of clarity, the malolactic type of *Kalecik Karası* takes the first place. Clarity average was determined as 7.92 in all wines. Although the shades of the colors varies according to the type of grapes, white wines that appear brown and brownish are generally oxidized. In red wines with higher amout of acids, the color becomes bright red (11).

In terms of Bouquets, the malolactic variety of *Merlot* grape got the highest score. Bouquets average was determined as 7.19 in all wines. This is the aroma found in grapes and transferred to wine which is formed during fermentation and resting. These aromatic substances in structure of esther, aldehyde, alcohol and ketones, which are numerious, affect the overall quality of the wine (11). Looking at the fullness results, the malolactic varieties of red wines got the highest score. These results were found in parallel with the amount of acid and other properties we found in the chemical results. Wines that are not full are thin, juicy and without bitterness. Full wines on the other hand, leave a greasy character in the mouth (40). In terms of taste scores, malolactic type *Kalecik karası* wine ranked first. The taste average of all wines was determined to be 7.38. Low acidity creates a, flat and insipid wine. A bitter taste is sometimes seen in red wines. Sweetness is an important criterion for sweet table wines (11). When we evaluate the sensory analysis results in total, the total point average of all wines was found to be 7.73. According to the results of the total evaluation, the most admired variety is the wine produced by malolactic fermentation of *Kalecik karası,* followed by the malolactic wine of the *Merlot* variety.

5. CONCLUSION

In this research, malolactic fermentation involving lactic acid bacteria was carried out following the yeast fermentation in the production of classical wine with two red and one white grape varieties. Afterwards, the effects of malolactic fermentation carried out under controlled conditions on the physico-chemical and sensory qualities of the young wines produced were evaluated. The wine samples obtained were examined in terms of organic acid changes, phenolic compositions and sensory properties.

The obtained results showed higher amounts of phenolic compounds in ethyl alcohol concentrations, however these values decreased with malolactic fermentation. While the amount of lactic acid increased in wines produced by malolactic fermentation, the levels of tartaric acid, malic acid and oxalic acid were found to be elevated in wines produced by classical fermentation. Tartaric acid amounts were found to be the highest among all organic acids in general. Wines produced by malolactic fermentation were more appreciated than the control group consisting of traditional wines. This difference was not considered to be significant in terms of color, however it was regarded important in terms of other sensory criteria (*P*<0.01). Subsequently, it was determined that red grape varieties responded more positively to MLF in terms of wine production technology than white grape varieties and these differences were found to be significant (*P*<0.01). As a result of sensory analysis, the most popular wine type is *Kalecik karası* wine produced by malolactic fermentation. This was followed by the malolactic wine of the *Merlot* variety. It has been observed that malolactic fermentation also reduces the amount of tannins, nevertheless affects the flavors of the produced malolactic wines favorably. In addition, the harmony of two foreign grapes, one red and one white grape, to the geography of our country was revealed with the characteristics of the wine produced. As a result of the thesis study, it was found that malolactic fermentation improved the quality of the wine, making it more pleasurable. In this context, the results of the research has the quality and attributes that will shed light on winemakers.

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Drafting the manuscript: AGA, ZS, GK, AY

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APPENDIX 1

Sensory Evaluation Analysis Form

<table>
<tr><td rowspan="3">Please give your score between 1-9 in the relevant box..
Age :.......
Gender : M □ W □
Do you smoke?
M □ W □</td><td>1awful</td><td>4 not bad</td><td>7 good</td></tr>
<tr><td>2 very bad</td><td>5 I neither liked nor disliked</td><td>8 very good</td></tr>
<tr><td>3 bad</td><td>6 tolerable</td><td>9 excellent</td></tr>
</table>

varieties	Color	Clarity	Bouquets	Saturity	Fragrance	Taste	General Appreciation	Group Average
Savignon blanc								
Savignon blanc+ML								
average value								
Kalecik Karası								
Kalecik Karası+ML								
average value								
Merlot								
Merlot+ML								
average value								

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