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M.Sc. in Food Engineering

İŞİL AYSEL AÇIKGÖZ

**PUBLIC OF TURKEY
GAZİANTEP UNIVERSITY
GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES**

**RESEARCH ON THE CHARACTERISTICS OF RED WINES
PRODUCED FROM GAZİANTEP RED GRAPES**

**M.Sc. THESIS
IN
FOOD ENGINEERING**

**BY
İŞİL AYSEL AÇIKGÖZ
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PRODUCED FROM GAZİANTEP RED GRAPES**

**M.Sc. Thesis
in
Food Engineering
Gaziantep University**

**Supervisor
Prof. Dr. Osman ERKMEN**

**by
Işıl Aysel AÇIKGÖZ
November 2019**



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REPUBLIC OF TURKEY
GAZİANTEP UNIVERSITY
GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES
FOOD ENGINEERING

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Name of the Student : Işıl Aysel AÇIKGÖZ

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Approval of the Graduate School of Natural and Applied Sciences

Prof. Dr. A. Necmeddin YAZICI
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of
Master of Science.

Prof. Dr. Medeni MASKAN
Head of Department

This is to certify that we have read this thesis and that in our consensus/majority
opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master
of Science.

Prof. Dr. Osman ERKMEN
Supervisor

Examining Committee Members:

Signature

Prof. Dr. Haşim KELEBEK

.....

Prof. Dr. Mustafa BAYRAM

.....

Prof. Dr. Osman ERKMEN

.....

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Işıl Aysel AÇIKGÖZ

ABSTRACT

RESEARCH ON THE CHARACTERISTICS OF RED WINES PRODUCED FROM GAZİANTEP RED GRAPES

AÇIKGÖZ, Işıl Aysel

M.Sc. in Food Engineering Department

Supervisor: Prof. Dr. Osman ERKMEN

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In this study, the suitability in wine production of Hönüsü and Horoz Karası grape varieties grown in Gaziantep in different concentrations were investigated. The wines were obtained by mixing two grapes in the following percent; wine-1: 100% Hönüsü grape must, wine-2: 85% Hönüsü grape must + 15% Horoz Karası grape must and wine-3: 70% Hönüsü grape must + 30% Horoz Karası grape must. The obtained wines were analyzed during the fermentation (1, 2, 3, 4 and 5 days), after resting with oak chips (7 days), after maturation (45 days) and after storage in bottle (90 days). Then physical, chemical, sensory, phenolic compound, sugar and organic acid analyzes were performed. HPLC was used to determine sugar, organic acid and phenolic compounds of wine. The amount of alcohol in the wines ranged between 12.58 and 12.66%, the total acidity amount between 6.11 and 6.51 g/L, the brix amount between 6.10 and 6.94, the amount of volatile acid between 0.50 and 0.57 g/L. In terms of phenolic compounds, the highest content was found in wine-3, while the lowest content was found in wine-1. The highest amount of fructose examined in this study was determined in wine-3, while the lowest content was determined in wine-1. In sucrose content, the highest content was determined in wine-1, while the lowest content was determined in wine-3. Tartaric, malic, citric and succinic acids examined in this study were highest in wine-1. At the end of 147 days production process of the wines, according to sensory analysis results, it was found that wine-3 gives better quality wine in terms of taste and appearance than other varieties.

Key Words: Red Wine, Hönüsü Grape, Horoz Karası Grape, Phenolic Compound, Fermentation

ÖZET
GAZİANTEP SİYAH ÜZÜMLERİNDEN ÜRETİLECEK KIRMIZI
ŞARAPLARIN ÖZELLİKLERİNİN ARAŞTIRILMASI

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Bu çalışmada, Gaziantep'te yetiştirilen Hönüsü ve Horoz Karası üzüm çeşitlerinin farklı konsantrasyonlarında elde edilen şarap üretimine elverişlilik durumları araştırılmıştır. Şarap türlerinin yüzde oranları; şarap-1: %100 Hönüsü üzüm şırası, şarap-2: %85 Hönüsü üzüm şırası + %15 Horoz Karası üzüm şırası, şarap tipi-3: %70 Hönüsü üzüm şırası + %30 Horoz Karası üzüm şırası. Elde edilen şaraplar, fermentasyon boyunca (1, 2, 3, 4 ve 5 gün), meşe yongasıyla dinlendikten sonra (7 gün), olgunlaşmadan sonra (45 gün) ve şişede depolamadan sonra (90 gün) analiz edilmiştir. Sonra alınan örneklerle fiziksel, kimyasal, duyuşal, fenolik bileşik, şeker ve organik asit analizleri yapılmıştır. Şarapların şeker, organik asit ve fenolik bileşiklerini belirlemek için HPLC kullanılmıştır. Şaraplardaki alkol miktarı %12.58 ve 12.66 arasında, toplam asit miktarı 6.11 ve 6.51 g/L arasında, brix miktarı 6.10 ve 6.94 arasında, uçar asit miktarı 0.50 ve 0.57 g/L arasında değişmiştir. Fenolik bileşikler açısından en yüksek içerik şarap-3'de saptanırken en düşük içerik şarap-1'de saptanmıştır. Çalışmada incelenen fruktoz miktarı en yüksek içerik şarap-3'de belirlenirken, en düşük içerik şarap-1'de belirlenmiştir. Sükroz miktarında ise en yüksek içerik şarap-1'de belirlenirken, en düşük içerik şarap-3'de belirlenmiştir. Çalışmada incelenen tartarik, malik, sitrik ve süksinik asitler wine-1'de en yüksek düzeyde saptanmıştır. Şarapların 147 günlük üretim işlemi sonunda duyuşal analiz sonuçlarına göre, şarap-3'ün diğer çeşitlere göre tat ve görünüş bakımından daha kaliteli şarap verdiği saptanmıştır.

Anahtar Kelimeler: Kırmızı Şarap, Hönüsü Üzüümü, Horoz Karası Üzüümü, Fenolik Bileşik, Fermentasyon

“Dedicated to my lovely mom,

Ayşe DEMİREL”

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LIST OF ABBREVIATIONS

hL	Hectoliter
Cfu	Colony forming unit
HPLC	High Performance Liquid Chromotograph
KHC₈H₄O₄	Potassium hydrogen phthalate
NaOH	Sodium hyrdoxide
mhl	Million hectoliter
kha	Thousand of hectares
H₂SO₄	Sulphuric acid
min	Minute
<i>S.cerevisiae</i>	<i>Sacchromyces cerevisiae</i>
PDA	Potato Dextrose Agar
h	Hours
SO₂	Sulphure dioxide
N	Normality
rpm	Revolution per minute
mm	Millimeter
nm	Nanometer
FAO	Food and Agriculture Organization
OIV	International organisation of vine and wine
TSE	Turkish Standardization Institute
TFC	Turkish Food Codex
w/w	Weight/weight
v/v	Volume/volume

LIST OF SYMBOLS

g/L	Gram/Liter
g/mL	Gram/Milliliter
mg/L	Milligram/Liter
°C	Degree of Celcius
μm	Micrometer
μL	Microliter

CHAPTER I

INTRODUCTION

The quality of the wine depend on the grape variety, the composition of the grapes, the processes applied in wine making and the conditions of resting and aging after fermentation (Ribéreau-Gayon et al., 2000; Jackson, 2000b).

The composition of the grape is under the influence of irreversible factors such as climatic conditions, soil structure and geography of the vineyard region; and modifiable factors such as grape variety, rootstock, viticulture technique and vintage (Amerine et al., 1972; Canbaş, 2006). Another factor determining the composition of grapes is the maturity of grapes. Grapes ripen on the vine in a certain time. The most important substances in the composition of grapes are sugars, organic acids, phenolic compounds (anthocyanins, tannins, etc.), flavoring agents, pectic substances, nitrogenous substances, mineral substances, vitamins and enzymes (Farkas, 1988; Ribéreau-Gayon et al., 2000).

One of the most important factors affecting the quality of the composition of wines is phenolic compounds. Phenolic compounds play an important role on the color and sensory properties of black grapes and the wines obtained from these grapes. The color and taste of red wines such as fullness and astringency are due to phenol compounds. The amount of phenolic compounds varies according to the grape variety, the maturity of the grapes, the irrigation and fertilization of the soil and viticulture, and the climatic conditions of the region (Kelebek, 2009). The quality of the wine depends primarily on the raw material and its composition; The grape type is related to the climatic conditions in which grapes are grown and cultivation technique. The wine value of a grape grown in a certain region and in climatic conditions can be determined to some extent by the analysis of grapes. However, the wine value of grapes is best determined by processing grapes into wine. Apart from the variety, another factor affecting the quality is the processing technique. How to

process any grape grown in a region has emerged as a result of many years of technological research (Farkas, 1988, Freitas et al., 1998).

Gaziantep province is located between 36° 28' and 38° 01' east longitudes and 36° 38' and 37° 32' northern latitudes in South East Anatolia Region. The surface area is 6222 km². The southern parts of the province, which is located at the transition point of the Mediterranean and continental climate, are influenced by the Mediterranean climate. In general, summers are hot and dry and winters are cold and rainy. There is more rainfall in spring and winter season. Table, dryness and wine grapes grow on the fertile soil of Gaziantep. Gaziantep province is suitable for growing all kinds of agricultural products. The most important red grape varieties of Gaziantep are Hönüsü and Horoz Karası is cultivated as wine grapes.

The aim of study, three mixtures of Hönüsü and Horoz Karası red grape varieties grown in Gaziantep province were prepared. Examples of mixtures are; wine-1; 100% Hönüsü, wine-2; 85% Hönüsü + 15% Horoz Karası and wine-3; 70% Hönüsü + 30% Horoz Karası. The sugar of grapes were converted to ethyl alcohol by fermentation with *S. cerevisia* yeast. The wine samples were fermented for 5 days and then flavored with oak chips for 7 days. In addition, the wine samples were matured in glass demijohns for 45 days and finally bottled and stored for 90 days, after which the red wine process was completed. Must and wine samples were for pH, total acidity, volatile acidity, Brix, density, color, SO₂, phenolic compound, sugar and organic acids. In addition to the must analysis, wine samples were analyzed for the alcohol and sensory analyzes were implemented. HPLC was used to analyze phenolic compound, sugar and organic acid contents in wine. This research has been carried out in order to indicate red wine production from Gaziantep grapes, to reveal the importance of grapes in the red wine production, to indicate availability of grapes for wine production with specified characteristics, and to provide contribution to the Gaziantep economy and the country with added value products.

CHAPTER II

LITERATURE REVIEW

2.1 Wine

“Wine” is an alcoholic beverage obtained only by fermentation of fresh grape must. There is a close relationship between the composition of grapes and wine quality (Amerine et al., 1972; Ribéreau-Gayon, 1982; Canbaş et al., 1995). Wine can be made from other sugar-containing fruits. According to TS 521 (Turkish Standards Institute), wine is defined as "alcoholic drink obtained from fermentation method only from fresh grapes or must" (Aktan and Kalkan, 2000; Kılıç, 1990; TS, 521). The compositions of grapes varies depend on variety and the geography where grapes grow. The uses of grapes can be grouped under three groups: table, dried and must wine varieties. However, the distinction between these groups is not very clear (Jackson, 2000).

Although the beginning of wine making is not known, its history is estimated to date back to 7000 years. There is also some scientific evidence that proves that the first wine was not produced consciously, but spontaneously. The archaeological excavations show that the first vine is the homeland of Iran, Georgia and Turkey; It is known that wine was made in B.C. 4000, then wine production was spread in Hittites, Lydians and other civilizations (Çelik et al., 2000).

2.2 Grape Properties

Climate and soil structure is one of the factors that determine the composition of grapes, the composition of grapes is one of the factors that determine the product quality. Grapes ripen on the vine in a certain time. The composition of the grapes changes during ripening. After the color transformation of grapes, maturity checks are started and vintage is done when they reach the most suitable composition according to the type of wine to be obtained. It is very important to make vintage timely in terms of the quality of the wine to be obtained (Gomez et al., 1995;

Deryaoglu, 1997). Grapes are healthy fruits with high nutritional value. Nutritional values of grapes are shown in Table 2.1.

Table 2.1 Nutritional data of grapes (FAO and OIV., 2016)

Compositions	For 100g grapes
Carbohydrates (g)	17
Calories (kcal)	65
Glycaemic Index (GI)	51
Potassium (% RDAs)	11%
Vitamin B6 (% RDAs)	3%
Vitamin C (% RDAs)	3%
Vitamin B1 – Thiamin (% RDAs)	6%
Vitamin E (% RDAs)	2%
Manganese	3%

(RDAs): Recommended Daily Allowances

2.3 The Composition of Grape Must

The composition of grapes includes water, sugar, carbohydrate, organic acid, protein, phenol compounds and minerals. The amount of water in grape must vary between 70-80% depending on the maturity of grape (Borazan, 2008). Carbohydrates are the most abundant compounds. The carbohydrates in the grapes include glucose, fructose, galactose, sorbitol and a small amount of sucrose, arabinose, rhamnose, xylose, raffinose, galactose and galacturonic acid (İlter et al., 1997). The main sugars are glucose and fructose. Glucose and fructose dissolve easily in water and alcohol and are easily fermented by yeasts (Borazan, 2008). Carbohydrates form monomers of polymeric carbohydrates (Hidalgo-Togores, 2002). Grape mainly contains tartaric acid, malic acid, citric acid, oxalic acid and salicylic acid (Keskin, 1981). While the acid content of the grapes on the north-facing side of the vineyard is about 6 g/L, the amount of acid in the grapes on the south-facing side can drop to 2-3 g/L at the end of ripening, while grape clusters continue to grow at high temperatures. Tartaric acid is a very strong acid and gives the acidity to wine and increases its pH to 3.0-3.5 during malolactic fermentation (Ribéreau-Gayon et al., 2006). During the ripening period, the amount of tartaric acid in grapes usually does not change. However, decreases in the amount of malic acid occur (Jackson, 2003). The amount of malic acid increases continuously in the grain until the grapes ripen and increases to 15-20 g/L. However, when the grain starts to mature, it falls continuously and remains in

the mature grain. Since the grapes live full ripening in our country, the amount of malic acid is very low. Malic acid is found in grapes as partially free, partly as potassium, calcium and magnesium salts. Malic acid is broken down by lactic acid bacteria to lactic acid (Borazan, 2008). There are twenty one most common amino acids in grapes. 85% of the amino acids in the grape are glutamic acid, arginine, threonine and proline (Canbaş, 2003). The main minerals in grapes are potassium, calcium, phosphorus, sodium, iron and magnesium. Mineral substances contain 2-3% of the bark of grapes and 1-2% of fruit meat (Canbaş, 2003). The minerals in the grape were taken from the soil by the vine and transferred to plants and fruits. The amount of minerals depend on the grape variety, degree of maturity, type of soil, fertilization and climate conditions. Generally in dry climatic conditions and in dry years, the amount of minerals is lower. Although the amount of mineral substances is affected by soil conditions, atmospheric conditions and pesticides used against plant diseases and pests. The amount of iron in the grape is directly affected by the iron content of the vineyard soil. Although the amount of copper in grapes is very small, it shows a small increase in cases where certain pesticides are used (Considine and Considine, 1982; Fidan and Yavaş, 1986). Phenolic compounds are most important compounds that give color to grape juice, and present upto 500-3000 mg/L. Phenols are identified by the $C_6-C_3-C_6$ carbon skeleton. Two aromatic rings are connected to each other by an aliphatic chain of three carbons (Can et al., 2005). The anthocyanidins (cyanidine, peonidine, delphinidine, petunidine and malvidine) responsible for the red, blue and purple color of the fruit peel. The molecule type varies depending on pH and the number of bound hydroxyl groups. As the number of hydroxyl (-OH) groups in anthocyanidin molecule increases, the color turns to blue and the increase in the number of methoxyl (-OCH₃) groups causes the red tone to strengthen (Canbaş, 1983).

2.4 Types and Properties of Gaziantep Red Grapes

The cultivation of foreign origin grape varieties in our country, the 19th century ended and 20th Century early the (phylloxera) disease has been effective occurring in European and vineyards in Turkey has begun to establish vaccinated vineyards as in other countries. Thus, many wine grape varieties of foreign origin have entered our country. However, these vineyards, which were deteriorated due to neglect, were

regained by major innovations in the Republican period and the grape varieties that gave high quality wine in Europe started to be tried all over the country. As a result of these studies, new wine grape varieties with high quality foreign origin were also brought to our country (Aktan and Kalkan, 2000).

The grapes are divided into table, dried and wine-grapes according to their consumptions. Grapes are important with respect to human health. Turkey, due to the motherland of the vine, still retains a rich heritage of viticulture. The old varieties, which have preserved their genetic content very well, are still cultivated and have made a wide difference. When Turkey's viticulture is evaluated across regions; The main reason for the selection of this province was the fact that the Southeastern Anatolia Region has a larger variety potential compared to other regions and Gaziantep province has a greater variety potential compared to other provinces in this region. Despite the importance of Gaziantep in the production and export of grapes, vineyard areas and production amount in this region is declining.

Hönüsü grape variety is large, long, translucent, ellipsoid and partially in the form of long eggs and 100 of them weigh 465 g. Its shell is thin, hardly crushed in the mouth and easily separated from the grain. The inside of the grain is yellowish in color, full, loose flesh, thin-edged and slightly juicy, with a small number of seed and the seeds are easily separated from the grain. The core is Vinifera type, medium sized, the back is slightly upward, its neck is medium length, the ends are cut to the abdominal side and are double ended. Shalaz shape is small according to the core, ellipse-shaped, surrounded by a pit and a high ring in the middle. It is not sandy and only the edge ring is sandy. The bark is a color between red and brown. This type of grape, which receives 4-5 kg of product from each vine stock, it is generally used as table grape (BahceBitkileri.org, 2010).

The grain shape of Horoz Karası variety is very large, white misty, equal long egg and acorn grains. The color of this grape variety is slightly hazy, dark purplish-black. One of them is very large and the average weight of 100 grain is 585 g. The shell is thick, hard, wire-structured, low-grain, difficult to crush in the mouth and easily separated from the flesh. The grain is full, fleshy and medium juicy. The number of seeds is small and the seeds are easily separated from the grain flesh. The taste of

Horoz Karası grape is succulent, medium juicy and sweet. The core is Vinifera type. The core shape is large, plump, long necked, pointed, and the ends are inclined towards the side of the abdomen, cut and double-ended. An average of 8-15 kg of product is taken from each vine stock and used as dryness and wine (BahceBitkileri.org, 2010).

Sergi Karası grape varieties are cone shaped, slightly white misty and dark purplish-black color. The average weight of 100 grains weighed 455 g. Its shell is medium-thick, multi-grained, fleshy, crushed in the mouth and difficult to separate from the grain. The grain is full and succulent, the number of seeds is small and the seeds are difficult to separate from the grain. This grape variety has a astringent taste. Core type is Vinifera type, plump, broad, flat short and thin neck, with the ends bent towards the side of the cut and double-ended. It has narrow, thin and long veins and extends from the core end to the middle of the ridge. 8 kg product is taken from each vine stock and is generally consumed as dryness and wine (BahceBitkileri.org, 2010).

2.5 Composition of Wine

There are more than 500 compounds in wine and 160 of them are esters. It contains 0.8-1.2 g of aromatic compounds per liter. The majority of these aromatic compounds are fusel alcohols, volatile acids and fatty acid esters. Fusel alcohols make up 50% of volatile components in wines. Carbonyls, phenols, lactones, terpenes, acetals, hydrocarbons are compounds that affect wine quality and wine taste, although very little in wine. The concentration of water, ethanol, organic acid, sugar and glycerol in wine is more than 100 mg per liter and forms the unique taste of wine (Bozoğlu, 2006). The components and amounts of wine are given in Table 2.2.

Table 2.2 Components and quantities of wine (Jackisch, 1985)

Components	Amounts in Wine (%)
Water	80-90
Alcohols	8-15
Organic acids	0,3-1,1
Sugars	0,1-0,09
Phenolic compounds	0,05-0,35
Nitrogen compounds	0,01-0,09

Ethyl alcohol (C_2H_5OH) is a colorless, fluid, pleasantly smelling and caustic liquid in the composition. It can be mixed easily in any ratio in water and in many organic liquids. All kinds of liquids that have been fermented by alcohol in nature contain ethyl alcohol. Ethyl alcohol is an important component of wine's color and sensory properties. Many chemical compounds found in grapes and extracted into must during production are dissolved in ethyl alcohol (Aktan and Kalkan, 2000).

The acids in wine are tartaric, malic, citric, acetic, lactic, sulphurous and sulfuric acids. Some of these acids are grape-derived (tartaric and malic) and some are fermentation-based (lactic and acetic) acids. There are also oxalic acid and succinic acid and succinic acid occurs after yeast metabolism, so it is not found in grape wine (Anonymous, 2009). The acids that enter the wine during the sulphurization process are sulphurose and sulfuric acids. The highest amount of organic acid in wine is tartaric acid (dioxisuccinic acid). Tartaric acid is also the strongest acid in wine. Tartaric acid, a grape-derived acid, is characteristic for wine. Tartaric acids in wine play a role in the formation of wine stone (potassium tartrate). It is known that organic acids in wine are important components affecting wine quality (Aktan and Kalkan, 2000).

Sugars are carbohydrates containing several hydroxyl groups and a ketone or aldehyde group. The main grape sugars are glucose and fructose. Ripe grapes, equal proportions; the amount of fructose at the end of the term is higher than glucose. Sucrose, which is added before fermentation, or slightly amount found naturally in grapes; it is enzymatically broken down into glucose and fructose during fermentation. The content of grape sugar depends upon the type, maturity and health of the fruit. The sugars in grapes are of great significance for yeast growth and metabolism. The yeast (*S. cerevisiae*) is mainly capable of metabolizing glucose and fructose. Moreover, non-fermented sugars that are pentose (arabinose, rhamnose, xylose and ribose) are residual sugars (Jackson, 2000).

Glycerol (glycerin) is considered as a measure of the amount of glycerin in providing information about the aging status of wines. Especially in wine stored in barrels, the amount of water and alcohol decreases with time and the amount of glycerin increases (Güven, 2008).

The major aroma components present in wine are compounds such as terpenes, volatile acids, higher alcohols and volatile phenols. The aroma components found in grapes and wine can be found in free or bound state. There are four main sources of aroma compounds in wine. These are grape origin flavors, grape aromas during the processing of grapes, ethyl alcohol and malolactic fermentation and aroma (bouquets) that occurs during the ripening of the wine. The characteristic aromas of the wines are grape origin. These are the flavors that vary according to each grape variety and are characteristic for that grape variety. It is known that the characteristic aromas of wines obtained from *Vitis vinifera* L. grapes originate from terpenes (Salinas et al., 2005).

The higher alcohols consist of amino acids. Wine yeasts break down to amino acids with their amidase enzymes to make their plasma, the majority of which is composed of proteins, while higher alcohols are formed. The highest portion of the higher alcohols is amylalcohol followed by propyl and butylalcohols. It is reported that high alcohols play a role in the formation of bouquet substances in wines (Güven, 2008).

2.6 Wine in the World

According to OIV (2018) data, Italy is the leader in world wine production. Italy is followed by France and Spain. When we look at the wine consumption data of 2018 in the world, the USA is the highest consuming country by 32.6 mhl. The USA is followed by France and Italy. Spain, Italy and France are the leading countries in wine exports (OIV, 2018). According to OIV (2019), Spain is the world leader in wine grape production with 968 kha vineyard area. China, France, Italy and Turkey follow to Spain. Turkey with 448 kha vineyard area after an ongoing decline since 2003, it has been shown to stabilize in 2018. In most European countries, wine production is experiencing a significant increase compared to 2017. In 2018, wine production increased by 54.8 mhl in Italy, 49.1 mhl in France and 44.4 mhl in Spain compared to 2017. Countries recorded an increase of 12.3 mhl, 12.7 mhl and 11.9 mhl compared to the decline in 2017 due to bad weather conditions. When we look at the wine consumption data since 2011, the country with the highest consumption is the The United State with 33.0 million hectoliters. France and Italy follow The United State. Spain, Italy and France with a volume of 54.8 mhl in 2018, covering 50.7% of wine exports in 2018. The leading countries in wine exports, representing

more than half of total imports, Germany, the United Kingdom, the United States, France and China, respectively. In 2018, most importing countries remained in balance with slight increases in the market, excluding China and Russia (OIV, 2019).

2.7 Wine Production in Turkey

Turkey located in the World's most efficient climate has a very old and well-established culture of viticulture covering all of *Vitis vinifera* grape varieties. It is estimated to be 1100-1200 grape varieties are grown in Turkey. However, 34 of these are wine grapes. Of these 34 kinds of wine grapes, 12 are foreign and 22 are local. In wine production, grapes are used either as monocepage (wine produced from single grape variety) or as coupling (blend) (Özay et al., 2005). According to 2017 data; in terms of the vineyards and grape production, Turkey is one of the world's leading countries. Turkey has 448,000 hectares of vineyards. Turkey takes place in 5th after Spain, China, France and Italy in vineyard, while in terms of production takes place in 6th after China, Italy, USA, France and Spain with 4.2 million tons of fresh grape production. Most of the grapes produced in the world are used in wine making. In Turkey, 57% of the grapes are used as table grapes, 40% are used as dryness and only 2% are allocated to wine production (OIV, 2018). Vineyard area and grape production (tonnes) in Turkey is shown in Table 2.3. According to TÜİK data, total consumption expenditures increased by 7% in the first half of 2010 and remained at 1.7% in food, alcoholic beverages and tobacco (DPT, 2014).

Table 2.3 Vineyard area and grape production (tonnes) in Turkey (TÜİK, 2018)

Years	Area (Decare)	Total	For table	Raisins	For wine use
2008	4 827 887	3 918 442	1 970 686	1 477 471	470 285
2009	4 790 239	4 264 720	2 256 845	1 531 987	475 888
2010	4 777 856	4 255 000	2 249 530	1 543 962	461 508
2011	4 725 454	4 296 351	2 268 967	1 562 064	465 320
2012	4 622 959	4 234 305	2 219 813	1 613 833	400 659
2013	4 687 922	4 011 409	2 132 602	1 423 578	455 229
2014	4 670 929	4 175 356	2 166 749	1 563 480	445 127
2015	4 619 557	3 650 000	1 891 910	1 334 563	423 527
2016	4 352 269	4 000 000	1 990 604	1 536 862	472 534
2017	4 169 068	4 200 000	2 109 000	1 603 000	488 000
2018	4 170 410	3 933 000	1 945 262	1 524 091	463 647

2.8 Factors Affecting Wine Quality

The quality of the wine varies depending on the type of grape, the soil in which it is grown, the location, the climate of the region and the processes applied during the production stage.

2.8.1 Diversity

The characteristics of the grapes and wine grapes which determine the quality of the wine are that they have smaller grains and clusters, and they are thin shelled and abundant must. In order for the high quality to be of wine, it is important that the must is rich in some aromatic substances and the acid content is high (Çelik et al., 1998). When wine grapes ripen, sugar and acid levels in must must be balanced and color changes should not be sharp after processing (Özen et al., 1998).

2.8.2 Structure of the Land

Vine's use of the sun is an important element. Except for very hot regions, it is preferable that the vineyards be in the south, southwest, southeast, west or east directions, respectively. In very hot regions, the northern side should be preferred in order not to burn the grapes from the sun. 5-10% in vineyards in temperate areas and 10-15% in cold regions are suitable for viticulture. In more inclined places, surely terrace should be made, the soil should be considered to be processed perpendicular to the slope (Gücüyen, 2007).

2.8.3 Impact of Climate

The annual average temperature of the regions where wine grapes will be grown should be 14-15°C, above 19°C in summer and the average annual precipitation should be around 650-700 mm (Borazan, 2008).

2.9 Production of Wine

Starting from the harvesting of the grape, which is the raw material in wine production, all the processes applied determine the wine formation and quality. Grapes should be processed within 1-2 hours after harvest. The stages from grape harvest to wine production are extremely important.

2.9.1 Harvesting

Accurate determination of harvest time is an important factor for winemaking. Harvest should be done by hand and the grapes sour and moldy should be separated. For the correct determination of the harvest time, which is important for the quality of the wine, the amount of acid and sugar of the grapes should be measured and since the substance which forms the alcohol is the sugar of the grape, the sugar in the grapes should reach a certain level.

2.9.2 Handle Separation and Crushing

Since bunches of grapes will add unwanted bitterness to the must, they must be separated from the grains in the first stage of wine making. In addition to the must of grapes in wine making, the shell and seeds are valuable. For this reason, grape grains are separated from their clusters without being damaged and are lightly crushed to get the must. After the clustering separation and crushing process is finished, white wine and red wine production process is done in different ways.

2.9.3 Maceration

The color and astringent tannins contained in the bark of the grape obtained in the crushing stage are kept in closed containers for a while to pass into the grape juice. According to the type of grape and the type of wine to be made during the maceration, must gains from the shell of grapes color, smell and flavor as well as tannin and fullness. During maceration, must must turn red. This process does not apply to white wine production. Rosé wine production should be kept for 8 to 48 hours according to the desired color tone. The maceration process is held in constant temperature controlled tanks in the range of 26-28°C for 5-6 days. During maceration, there is also a partial fermentation (caliber fermentation) due to the effect of natural microflora, depending on the temperature. At the end of the process, the must is separated from the bark (Taylan, 1972; Tosun, 2005).

2.9.4 Pressing

Pressing is the process of squeezing grapes in press machines to obtain must. The crushed or partially must taken mash is squeezed in presses and must is taken. Various types of presses such as hand presses, hydraulic presses, air pressure presses,

mechanical pressing regular presses and continuous work screw presses can be used. The must which flows spontaneously without pressure is called the “first must”. The first pressing produces “second must” and the second pressing produces “third must”. These musts are different in taste and composition. The first must and the first press must are taste-soft and richer in sugar and acid. The musts obtained later are acrid and bitter as more tannins and other substances pass through the shells. Depending on the grape variety, one hundred kilograms of grape yields eighty liters of must (Shahidi and Naczki, 1995). At this stage, grape juice is sulfurized in order to prevent the development of unwanted microorganisms and to prevent oxidation (Ribereau-Gayon et al., 2006). Thus, the negative effects of microorganisms on crushed grapes during fermentation are eliminated. The must must be desulfurized without allowing much contact with air. Wines are subjected to filtration several times at various stages of the production process. The first filtration process is carried out in order to remove the residues and solids obtained from the mixture after pressing (Tosun., 2005).

2.9.5 Fermentation

It is the process of converting must into wine. The sugar naturally found in the grape is converted into alcohol (ethyl alcohol) and carbon dioxide gas by the wine yeasts and thus the grape must becomes wine. The yeast culture is added to the must and the fermentation process is started. The fermentation process of the wines is between 5 and 15 days. In order to preserve the aromas and vigor in the wine during the fermentation of white wines, a temperature range of 13-14°C and 26-28°C is preferred for red wines.

Alcohol fermentation is defined as the breakdown of sugars by yeasts in the absence of oxygen to ethyl alcohol and carbon dioxide. Yeasts come to mind when it comes to wine fermentation, and even if alcohol fermentation is understood, lactic acid bacteria are also involved and malic acid is converted to lactic acid and carbon dioxide by lactic acid bacteria when conditions are appropriate. The degradation of malic acid to lactic acid is only required if the total amount of acid in the medium is high (Troost, 1986).

The fermentation temperature is desired to be between 14-26°C, depending on the type of wine. Although the temperature is higher, it reduces the fermentation time, but also leads to the loss of volatile aromatic substances due to the effect of carbon dioxide gas. Preserving the aroma at lower temperatures, slowing down the growth of unwanted bacteria and making wine clearer more easily can prevent the yeast from working efficiently, causing death and stopping fermentation. Fermentation is a long process that lasts between 10-30 days. The yeast added tank is kept under control by daily temperature and brix measurements and the course of fermentation is monitored. The fermentation, which starts at approximately 24 °Brix levels, decreases as the sugar is used by the yeast as alcohol progresses. As °Brix approaches 0, the amount of sugar that can be fermented decreases to around 2 g/L. Wine yeasts use sugar as a nutrient and when there is no sugar in environment, the activity of yeast stops. Fermentation monitoring can also be followed by density measurement. Initially, the density is around 1,090-1,100, which decreases as the fermentation progresses. A density of around 0.995 means that there is no sugar left to ferment. Towards the end of the fermentation, the yeast becomes unable to work comfortably under the influence of the alcohol it produces. Its activity slows down and dies. Except some specially developed yeast species, yeasts can usually withstand up to 13.5-14% alcohol (Bozaran and Bozan, 2006).

2.9.6 Transfer

Transfer the wine to another container to separate it from its sediment. The wine, which has received a small amount of air during the aeration process, easily matures and the undesirable odors are removed. However, it should be remembered that excessive ventilation will also damage the wine. The process is repeated at regular intervals. Excess transfer may also lead to excessive oxidation of wine, negative consequences such as deterioration of taste and aroma. Clean wine separated from the sediment by air transfer is cooled. During cooling period of 10-15 min stirring should be applied. Cooled wine is kept in insulated tanks for 5-7 days. After waiting the wine is filtered in the filters (Bozoglu, 2006).

2.9.7 Filtration and Decanting

This is the first stage of the preparation of the wines for bottling. The product image from the fermentation is blurry. The turbidity needs to be clarified by removing it from the wine and the organic substances having negative effects on taste should be precipitated and separated from the wine. During the maturity period, the wines gradually become clear. It is intervened externally to accelerate the clarification process. Gelatin, bentonite, fish glue, activated charcoal, kaolin and casein are used to clarify the wines (Boulton et al., 1999). The goal in wine filtration is not only to clarify the wine, but also to preserve the aroma and taste profile of the product (Neilman, 2013).

2.9.8 Maturation

This stage, also called aging, is the ripening of the wine in the tank or barrel between the end of the alcohol fermentation and the bottling phase. The period of ripening is usually as short as 1-2 months at 0-14°C for light wines, and 12 months for wines with a slightly more developed majority, which require more ripening before the market. Strong wines can last up to 36 months (Neilman, 2013). It adds vanilla and creamy flavors to the wine according to the type of tree selected during ripening in barrels. Aging in stainless steel tanks does not contribute to wine. The amount of phenolic compounds depends on the aging period, the type of oak used, the size of the barrel and whether it has been used before (Shahidi and Naczki, 1995). The aging process in oak barrels improves the wine quality and changes the taste, smell and texture of the wine (Vichi et al., 2007). However, the fact that oak barrels are expensive and that the barrel aging process takes a long time has led to various alternatives. Today, oak chips, which are more economical, are used to accelerate the ripening process instead of wooden (oak) barrels for quality wines (Bozanglo et al., 2007).

2.9.9 Bottling

Bottles for wines can be of various sizes. The timing of bottling, which constitutes the final stage in wine production, is extremely important. Premature bottling adversely affects the development of red wine characteristics, and a late bottling may cause the light wine to lose fruit flavor. Bottling should be sterile and wine

temperature in bottling should be at an average of 19 °C and not below 16 °C. The shape and color of the bottle used in winery are determined according to the type and regional characteristics of the wine. In white wines, cylindrical "Rhine Type" skewers are generally used which narrow the shape towards the throat, while red wines use green, wide-bodied, narrow cylinder short-necked "Bordeaux (Bordeaux)" or "Burgundy" bottles. Bottoms of bottles are pitted inward and the surface is enlarged. After filling the bottles are closed with a cork. One of the most important points in bottling, the final stage of wine production, is the quality of the cork. There should be approximately 1-1.5 cm gap between wine and cork. Wine should be stored in a slightly damp, low-temperature, dark room. It is recommended to keep 8-12 degrees for white wines and 12-18 degrees for red wines. Higher temperatures cause premature aging. Color and flavor defects are seen (Neilman, 2013). If the wine is kept in very cold weather it will shrink in volume and cause air to leak into the bottle (Nowak, 2005). The environment is humid enough to prevent the moisture in the bottle from escaping from the cork and entering the air. Wine, thanks to the cork breathing, there should be a distinct smell in the cellar (Anlı, 2010). Bottled wines should always be stored horizontally and the cork should always remain wet in the bottle. If the cork does not come into contact with wine, it will dry out over time and leak more air into the bottle, causing both alcohol loss and oxidation. After bottling, the wines, which complete the aging period, are ready to be served.

2.10 Organic Acids in Grapes and Wines

There are two main acids originating from grapes: tartaric and malic acid. In addition, trace amounts of citric, lactic, succinic, fumaric, pyruvic, α -oxoglutaric, glyceric, glucolic, dimethyl-succinic, cycimic, quinic, mandelic, cis and trans acotinic, maleic and isocytic acids are present in grapes (Zatou et al., 2004). Tartaric acid is a grape-specific acid. It is synthesized in green grapes and is transported from young leaves to the grain. It is the most powerful acid in grapes. However, like all organic acids is a weak acid (Canbaş, 2006). The amount of acids in immature grapes can reach up to 15 grams per liter (Ribéreau-Gayon et al., 2006). While the acid content of the grape must on the north-facing side of the vineyard is about 6 g/L, the amount of acid in the grapes on the south-facing side can drop to 2-3 g/L at the end of ripening, while grape clusters continue to grow at high temperatures. Tartaric acid is a very strong acid and gives the acidity that increases its pH to 3.0-3.5 (Ribéreau-

Gayon et al., 2006). Malic acid is an acid found in all fruits. Malic acid has a more crude taste and, unlike tartaric acid, does not match with tannin. In mature grapes in regions with a warm climate, malic acid is low. In red wine with abundant tannins, malic acid is removed by means of malolactic fermentation, which is usually carried out after alcohol fermentation, ie converted to lactic acid. In the wines in Turkey, because it is low in malic acid is not referred to this method. During the maturity of grapes in the northern regions of the vineyards, 4.0-6.5 g/L of malic acid, although the musts obtained from the southern regions only 1.0-2.0 g/L malic acid (Ribéreau-Gayon et al., 2006). Citric acid is considerably reduced during fermentation. Citric acid is a tri-acid and is very common in nature. The concentration of this acid in must and wine is between 0.5-1.0 g/L (Ribéreau-Gayon et al., 2006).

The main organic acids in wine; tartaric, malic, citric, succinic, lactic, acetic and fumaric acids. Non-volatile acids diminish as the wine ages and thus soften the wine. Tartaric acid combines with other basic materials to form precipitated, insoluble crystals. Tartaric and malic acids are the basic acids that determine the acidity of the wine and determine the pH. Tartaric acid is important for wine stability and malic acid is important for malolactic fermentation. Most of the organic acids in wine are tartaric acid. It is the basic acid that determines the acidity of the wine and ensures that the pH of the wine is between 3.0-3.5. The amount of tartaric acid in the wine can range from 1.5 g/L to 3.0 g/L (Usseglio-Tomasset, 1995).

After tartaric acid, the highest amount of acid in wine is malic acid. Malic acid forms the substrate of lactic acid bacteria, which leads to degradation in wine. Therefore, it is not desirable to have more malic acid in the wine and is converted to lactic acid by malolactic fermentation. Malic acid in excess; the microbiological, physical, biochemical stability of the wine and negatively affect the quality of the wine (Delcourt et al., 1995).

Citric acid is an acid capable of slowing yeast growth and ranges from 0.5-0.7 g/L in must or wine (Swiegers et al., 2005). Prolonged storage of wine with sediment reduces the effect of citric acid metabolism; it has a positive effect on the aroma of wine (Nielsen, 1996).

One of the acids formed as a result of fermentation in wine is succinic acid and is found in wine around 1g/L. Succinic acid, which is very resistant to bacteria, although it is a small amount, sour to salt; salty bitter to a variety of taste (Boulton et al., 1996). After the end of alcohol fermentation, the formation of succinic acid stops. Succinic acid, such as tartaric acid; because it is an acid that cannot be metabolized by bacteria; is a stable acid (Coulter et al. 2004).

Acetic acid constitutes approximately 90-95% of the volatile acidity in wine. As the amount of acetic acid in the wine increases, the volatile acidity of the wine will increase and a sharp and disturbing aroma will begin to form in the wine. As a result; the aroma quality and microbiological stability of the wine will be adversely affected. When the volatile acid exceeds the limit of 1 g / L (in acetic acid), the wine becomes unmarketable (Swieger et al., 2005).

Wine fermentation basically consists of two main stages: alcohol fermentation by yeasts and malolactic fermentation by lactic acid bacteria. During alcohol fermentation, the medium is unsuitable for growth of bacteria and yeast no longer grows when all of the reducing sugars are fermented to ethyl alcohol. However, after this stage, lactic acid bacteria begin to develop. The main substrates used in the metabolism of lactic acid bacteria are malic acid, citric acid and sugars (hexoses and pentoses) (Davis et al., 1985). The most important event in malolactic fermentation is the biological reduction of acidity as a result of the conversion of a molecule L-malic acid which is dicarboxylic acid by lactic acid bacteria into a molecule L-lactic acid which is monocarboxylic acid and 1 molecule CO₂. During malolactic fermentation, all of the malic acid (2-10 g/L) is reduced, which leads to an increase in the pH of the wine and a change in taste (Moreno-Arribas and Lonvaud-Funel, 2000).

Fumaric acid acts as buffer acid around pH 3. It gives lasting negative taste in environments under pH 4. It can be used to increase acidity in wine and lower pH. It prevents the second fermentation in the bottle after the bottling step in the wine. It prevents the formation of malolactic fermentation in wine due to its antibacterial properties (Boulton et al., 1996).

Escobal et al. (1997), the amount of organic acid in wine made from different grape varieties show some differences according to varieties of the most tartaric acid (2.65 g/L), malic acid (2.0 g/L), lactic acid (0.91 g/L), citric acid (0.56 g/L) and succinic acid (0.54 g/L). For the quality of the wine obtained from grapes, it is desirable that the must is rich with some aromatic substances and the organic acid content is high (Çelik et al., 1998).

2.11 Sugar Contents in Grape and Wine

A very small part is sucrose. Sucrose is synthesized in all the green parts of the vine and especially in the leaves, transported to the cells in the pulp by the delivery tissues, where it hydrolyses glucose and fructose and catalyzed by a group of enzymes. When the starch makes more photosynthesis, it is stored in the cell and, when necessary, hydrolyzed and disintegrated (Canbaş, 2006). It is broken down by yeast into anaerobic medium and converted into ethyl alcohol. They have reduction properties and can be oxidized. They are converted to gluconic acid as a result of oxidation. Quantities can be determined by using these properties (Canbaş, 2006). The most important among the disaccharides is sucrose. Its concentration is generally low and is between 2.0-5.0 g/L. Sometimes this amount can be even higher. The formation of sucrose in vine leaves occurs by photosynthesis. However, it is hydrolyzed into sugar, ie glucose and fructose. After conversion to sucrose, glucose and fructose, it is broken down by fermentation by the yeast (*S. cerevisiae*). For this reason, sucrose is not present in the wine if it is not subsequently added to the fermentation medium by illegal means. Sucrose is the sugars used to increase the potential alcohol levels of grapes due to their low purity and price (Canbaş, 2006). Grape fermentable sugars are broken down by alcohol by yeasts and, therefore, there is a strict relationship between sugar concentration and wine quality (Canbaş, 2006). Sugar content and quantity of grapes vary during ripening due to various factors such as environmental and viticulture management. In recent years, alcohol content in wines tends to increase due to various factors. One of these is the increase in the potential sugar content in must due to possible climate change (Jordão et al., 2015).

2.12 Phenolic Compounds in the Structure of Grapes and Wine

Some of the phenolic compounds are soluble only in organic solvents, while others are soluble in water by their carboxylic acids and glycosides. Some of the phenolic compounds have insoluble polymer structure (Taiz and Zeiger, 2008). The phenolic components that are important in terms of sensory properties and antioxidant effect of wine are substances containing a benzene ring and hydroxyl (-OH) group in their structure. The structure of the phenol ring is shown in Figure 2.1.

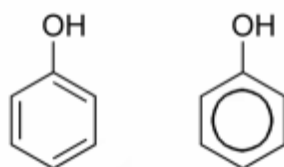


Figure 2.1 Phenol ring

Simple phenols are compounds which contain one or more hydroxyl groups containing one aromatic ring (Soleas et al., 1997). Polyphenols contain multiple phenol rings in their structure. The phenolic components found in wine are mainly divided into two groups as non-flavonoids (phenolic acids) and flavonoids. Non-flavonoid; hydroxycinnamic acids, benzoic acids, hydrolysable tannins (Vinyl phenol, guaiacol, syringol, etc.) and stilbens (resveratrol) which are derived from oak barrels during aging. Flavonoids can be classified as flavones, flavanols and anthocyanidins (Kerry and Abbey, 1997). Phenolic compounds are responsible for the differences between red and white wine, such as the color and aroma of red wine (Ribereau-Gayon et al., 2006). They are lower in white wines than in red wines. Its main sources are grape stems, seeds and bark. As they have anti-bacterial activity, their presence increases wine quality and hygiene (Uylaşer and İnce, 2008). Phenolic compounds, which are defined as secondary metabolism products of plants, are the most common components found in plants and are currently a chemically heterogeneous group of 10000 types of compounds (Taiz and Zeiger, 2008, Kafkas et al., 2006). Phenol compounds are found in the bark, fruit flesh and kernels of grapes. It is reported that 33.3% of total phenol compounds in black grapes are found in shells, 4.1% in fruit flesh and 62.6% in seeds (Deryaoğlu et al., 1997). The amount of phenol is less in the fleshy part of the grape, but its shell and kernel are important

sources of phenol. It is one of the rare fruits consumed with grapes, husks and seeds. In this respect, grapes are a very important source of phenol in human nutrition. During red wine production, phenol components in grape tissues dissolve in aqueous alcohol solutions and pass to wine. Thus, wine is a product rich in phenol components (Singleton and Esau, 1969; Ünsal, 2007). The quality and quantity of phenolic compounds of grapes depends primarily on the type of grape. While the ratio of the presence and amount of certain phenol compounds to each other depends on the genetically controlled variety and species characteristics, the total amount of phenol or the class of phenols in the composition depends on environmental factors (Singleton and Esau, 1969). The region in which grapes are grown, soil properties and agricultural activities also affect the amount of pigment in grapes and phenol composition of grapes (Shahidi and Naczsk, 1995). In general, simple phenols in grapes cause sourness, condensate phenols cause astringency (Cemeroğlu and Acar, 1986). The C₆-C₃-C₆ group is called flavonoid phenolic compounds and the C₁-C₆ and C₆-C₃ groups are called non-flavonoid phenolic compounds. These two classes of phenolic components are the main phenolic components in wines and fruits of many other plants (Margalit, 2004).

2.12.1 Phenolic Acids

Phenolic acids in wine are generally divided into two groups as hydroxycinnamic and hydroxybenzoic acids (Budic-Leto and Lovric, 2002). Cinnamic acids have the carbon structure C₆-C₃ and the most common cinnamic acids in plants are *p*-coumaric acid, *o*-coumaric acid, 5-hydroxiferulic acid, caffeic acid and ferulic acid.

Benzoic acids have C₆-C₁ carbon structure and are usually found in trace amounts in vegetable foods. The most common examples are; salicylic acid, *m*-hydroxybenzoic acid, gallic acid, vanillic acid. Benzoic acid and cinnamic acid are 100-200 mg/L in red wines and 10-20 mg/L in white wines, respectively. Phenol acids are colorless compounds. However, due to oxidation, it turns yellow compounds (Kelebek, 2009). Phenolic acids are colorless/yellowish, affecting the color of red wine. The most variable composition among the varieties is hydroxycinnamic acids, which are usually esterified with sugar, alcohol or organic acids (tartaric acid esters of caffeic, *p*-coumaric and ferulic acids are caftaric, coutaric and fertaric acids, respectively). The

amounts of hydroxybenzoic acid derivative are higher in wines aged in oak barrels (Şen, 2014).

Kılınç and Kalkan (2003) indicated that the content of various wines in the market on the content of phenol acids gallic acid is the most amount and this is followed by hydroxycinnamic acid, syringic acid and ferulic acid. 1-15 mg/L caffeic acid and 0.3-30 mg/L p-coumaric acid were found in the grape and wine (Ribereau-Gayon et al., 2006). Another group of more complex polyphenols is stilbenes. Of these compounds, the trans-isomeric resveratrol (3,5,4'-trihydroxystyrene) is thought to be formed as a result of the omnidirectional reaction to mold infection. Resveratrol is found in the shell of the grape and passes to the wine during fermentation. The amounts of resveratrol in wines vary between 1-3 mg/L (Ribereau-Gayon et al., 2000). White wine contains 0.0-0.5 mg/L gallic acid and red wine contains 12 mg/L gallic acid (Ribereau-Gayon et al., 2006).

Stilbenes consisting of *trans*-resveratrol, *cis*-resveratrol and resveratrol glucoside are also included in the non-flavonoid group (Chiou et al., 2007). Gallic acid and ellagic acid play an important role in the structure of tannin and the taste of wine as extracted from oak barrels during wine production stages (Margalit, 2004). Jeandet et al. (1993), in their study on Burgundy wines, resveratrol concentration in red varieties are higher than white varieties and also explained that the cultivation conditions affect these rates. These researchers found 0.06 mg/L resveratrol in Chardonnay and 0.4–2.0 mg/L resveratrol Pinot Noir. Adıgüzel (2007) examined the levels of resveratrol in the wine obtained from the vineyards in different regions of Turkey. Accordingly, Öküzgözü wine obtained from the vineyards of Eastern Anatolia Region has the highest value with 6.469 mg/L. The highest value in Cabernet Sauvignon was found in the wine produced in the Aegean region with a significant difference (2.257 mg/L), while the lowest resveratrol concentration was found in the Trakya Region with a value of 0.252 mg/L. Some other resveratrol values obtained in the study were: Syrah 1.790 mg/L, Musket 0.278 mg/L, Sauvignon Blanc 0.494 mg/L.

Tyrosol [2-(4-hydroxyphenyl) ethanol] is an mono-phenol compound that oil-soluble and produced during yeast fermentation (Di Benedetto et al., 2007). Tyrosol is

produced by *Saccharomyces cerevisiae* yeasts with oxidative decarboxylation from tyrosine amino acid during alcohol fermentation (Ribereau-Gayon et al., 2000). The results of a study on Spanish wines showed that tyrosol content is 20.38-44.46 mg/L (Pineiro et al., 2011).

2.12.2 Flavonoids

Flavonoids are the most important and common structures in polyphenols. Flavonoids have color pigments in plants. The carbon skeleton of flavonoids is a diphenylpropane ($C_6-C_3-C_6$) structure consisting of 15 carbon atoms consisting of the coupling of two phenyl rings with the propane chain. Flavonoids are present in the form of aglycones in red wine and their concentration is around 100 mg/L (Ribéreau-Gayon et al., 2006). The flavonoid group substances which are common in nature and which are important in wine production are flavonols. Flavonols are yellow pigments found in white and black grapes. Black grapes contain kaemferol, myricetin and quercetin and white grapes have kaemferol and quercetin pigments (Boulton et al. 1996; Jackson, 2000). Flavonols are found in the glycoside structure of grapes and are located in the skin portion of the grapes. It is known that the amount of quercetin from the flavonols in grape skin is high, quercetin, camferol and their glycosides cause bitterness in wines (Jackson, 2000). Since maceration is not applied in the production of white wine, the amount of white wine is less than that of red wine. Flavan-3-ols are found in the stems and seeds of grapes and gives an astringent and bitter taste to wine. Tannins [$(C_6C_3C_6)_n$] are polymers of flavonoids and are divided into gallic tannins and catechic tannins. Gallic tannins are formed during aging in wooden barrels. In addition, the stems and seeds of grapes contain catechic tannins (Jackson, 2000; Jaitz et al., 2010; Kelebek et al., 2010). Flavonols are hydrolyzed during fermentation and are therefore aglycated in wine (Ribéreau-Gayon and Glories, 1986). Flavonoids are flavanols, flavonols and anthocyanins in the wine (Özcan, 2006). Flavanols include catechin, epicatechin and their polymers and glucose and ester forms. These are colorless compounds that are effective in enzymatic coloration (Hulme, 1971). Anthocyanins are present as glycosides bound to sugars. Anthocyanins are categorized into different colors including cyanidine (red), paenodine (light red), delphinidine (dark blue), petunidine (blue-purple), malvidin (purple) and pelargonidine (orange) (Hulme, 1971). During maturation or storage, reactions between anthocyanins and other phenolic compounds such as

flavan-3-ols are bottomed on acetaldehyde intensification, co-pigmentation and self-association reactions (Castillo-Sánchez et al., 2008; Jackson, 2000). Copigmentation and polymerization reactions of anthocyanins during bottle storage of wines changed from bluish-red to orangish-red in color tones. Flavan-3-ols decreased with time owing to intensification with anthocyanins and turbidity-producing proteins. Flavonol concentrations may increase during maturation owing to hydrolysis of flavonol glycosides to aglycones (Marquez et al., 2014). The amounts of gallic and syringic acid increase during maturation due to their release from oak barrels (Lorenzo et al., 2005). These reactions affect the color and aroma of the wine. Vuorinen et al. (2000) determined that the amount of myricetin and quercetin in the wine is more than the amount of chemferol. They were determined between 2.5-22.0 mg/L and 1.6-17.7 mg/L respectively.

2.12.3 Anthocyanins

Anthocyanins are red colored pigments in the shell and flesh of grapes. In addition, the unique red, blue and purple shades of the wine that gives their own colors, water and wine in less and very soluble in alcohol are natural color substances (Mazza, 1995). The most important anthocyanidin pigments found in grapes are malvidin, peonidin, cyanidin, delphinidin and petunidin. While all grape varieties have essentially the same anthocyanidin structure, slight differences in their composition may be observed. Among anthocyanidins, malvidin of total wine anthocyanidins pigment is the most dominant in grape varieties. They range from 50% to 90%. Malvidin monoglycoside is the basis of red color in Shiraz grapes and red wines. The color of these pigments varies depending on pH, SO₂, molecular structure and ambient conditions (Ribéreau Gayon et al., 2006). Anthocyanidins are not free in nature, but are always esterified with one or several sugar molecules, that is, they are found as anthocyanins (Cemeroğlu, 2004). Malvidin is the most abundant amount of anthocyanidins in grapes and malvidin monoglycoside forms the basis of color in black grapes (Ribéreau-Gayon et al., 2000b). Mazza (1995) indicated that the total phenolic content of some grape varieties varies between 260 and 900 mg/100 g, while the total anthocyanin content is determined to be around 30 to 750 mg/100 g (Kelebek, 2009). The color of anthocyanins varies according to the pH and SO₂ content of the medium (Glories, 1999; Ribéreau-Gayon et al., 2000). In a study carried out on the grapes of Boğazkere and Öküzgözü grown in Elazığ region,

anthocyanin content was 83 mg/100 g and 96.5 mg/100 g, respectively (Deryaoğlu, 1997). It was reported that the total amount of anthocyanin (673 mg/L) was higher in the wines obtained from the Boğazkere variety grown in the same region compared to the anthocyanin amount of the wines obtained from Öküzgözü (average 445 mg/L) (Canbaş et al., 2001).

2.12.4 Tannins

Tannins, also known as proanthocyanidins, are compounds that can form compounds with other plant polymers such as polysaccharides and proteins (Ribéreau Gayon et al., 2006). The tannins in grape peels are important because they are the first compounds to dissolve in wine making. Tannins are better soluble in alcohol than water. As a result of maceration during fermentation, they pass from solid parts of grape to wine. If it is desired to obtain a wine rich in tannins, the fermentation period should not be less than 15 days and may even extend up to 3 weeks (Canbaş, 2003). Tannins are chemically divided into two groups as hydrolysable tannins and condensed tannins (Haslam, 1998). The tannins found in grape seeds and husks are defined as condensed tannins. Condensed tannins are compounds of complex structure formed by the polymerization of flavan-3-ol or catechins. The basic structure of condensate tannins is (+)-catechin and (-)-epicatechin. When condensed tannins are heated in acid medium, red cyanidines are formed. Therefore, condensed tannins are also called 'procyanidins' or 'leucoanthocyanidin' (Goldberg et al., 1998; Ribéreau-Gayon et al., 2000b). Flavan-3-ols, such as catechin and epicatechin, are components of proanthocyanidins (tannin) and gives flavor, aroma and astringency in wine. Grapes and wines include dimer (procyanidin B₁-B₈) and trimeric procyanidins which combinations of (-)-epicatechin and (+)-catechin (Goldberg et al., 1998; Ribéreau-Gayon et al., 2000b). Hydrolysable tannins; gallotannin and ellagic tannins (polymer of ellagic acid and glucose). These compounds decompose to gallic acid and ellagic acid as a result of acid hydrolysis. Hydrolysable tannins are not naturally present in grapes. The addition of these compounds as external wine additives is legally permitted. On the other hand, gallic acid in the shell and core passes directly to wine (Ribéreau-Gayon et al., 2006). Another important feature of tannins is that they give bitterness. The bitterness in wine is caused by low molecular weight tannins (monomeric catechins), while high-molecular polymers cause the astringent taste (Glories, 1999). Besides, (-)-epicatechin is more astringent and bitter than its

isomer (+) catechin (Noble, 1999). While the amount of tannins in red wine is 1.5-5 g per liter, it varies between 0-100 mg in white wines (Canbaş, 1983).

Peng et al. (2001) reported that the amount of procyanidin in grape seed varies between 33200-50700 mg/kg and that about half of the procyanidins in wines pass through the seed. Gómez-Plaza et al. (2001) investigated the effect of different fermentation time (4, 5 and 10 days) on the solubility of phenol compounds. The results indicated that with increasing duration of marc fermentation; catechin, procyanidin, anthocyanin compounds and color density is increased and content of polymeric compound is high in wine. In a study, some phenolic components (gallic acid, catechin, epicatechin, vanilic acid) of the wine obtained from Kalecik Karası, Gamay and Cabernet Sauvignon grapes harvested in the vineyards of Mürefte and Hosköy regions of Trakya region were determined by HPLC and the phenol components were determined by HPLC. In addition, basic chemical analyzes were carried out in the wine and according to all these data, the compatibility of the grapes to the region and the domestic and foreign wine varieties were compared. According to the results obtained; In both regions and in all three wine types, the most common phenolic component was gallic acid and the order of the other phenol components in the wine was found to change. For example; Gallic acid 70.2 g/L, epicatechin 61.8 g/L, catechin 24.3 g/L, vanilic acid 5.2 g/L for Cabernet Sauvignon wine in Hosköy, 52.6 g/L for Gamay grape wine, epicatechin 26.4 g/L, catechin 20.3 g/L, and vanillic acid 4.7 g/L. In the study, it was concluded that all three varieties are well adapted to the region, especially Cabernet Sauvignon gives rich and strong wine to the phenolics in the region (Ünsal, 2007). Canbaş (1978) determined that the amount of wine anthocyanin derived from black variety grown in the region of Turkey Nevşehir determined and the amount of wine anthocyanin in average 72 mg/L, respectively. At the same time, it was stated that Dimrit cultivar is a poor variety in terms of anthocyanin amount compared to other wine grapes. Rastija et al. (2009) indicated that the total phenolic compound in red wine were ranged from 1156 mg/L to 2619 mg/L (average 1665 mg/L). Goldberg et al. (1998) determined the contents of (+)-catechin and (-)-epicatechin in more than 800 red wines from different regions. They reported that the amounts of (+)-catechin and (-)-epicatechin were found most in Pinot noir and least in Shiraz wines and catechin/epicatechin ratio varies according to regions.

2.13 Factors Affecting Fermentation in Wine

The quality of wine is very important and the quality criteria are divided into three as sensory, physical-chemical and microbiological. Color, clarity, odor, taste and fullness affect sensory quality criteria. Physical and chemical quality criteria are alcohol percentage, alcohol grade, total acidity, constant acidity, volatile acidity ratios, total and free sulfur dioxide content, reducing sugars and sucrose content, ash content and basicity, density, heavy metal and foreign matter content. The fact that these values are more than acceptable amounts significantly affect the quality. Molds, yeasts and bacteria constitute microbiological quality criteria (Aktan and Kalkan, 2000).

Since yeast is a living organism that performs fermentation of alcohol in the production of alcohol, it is natural that, like other living things, it can be positively or negatively affected by temperature. At very high or very low fermentation temperatures, yeast goes into shock and this shows the importance of temperature in fermentation (Güven, 2003). Yeasts which alcohol fermentate usually perform best around 25°C. Low temperatures can interrupt fermentation, so that oxidation is possible, as well as wild yeast or molds can work. High temperatures can accelerate unwanted microbial activity, this development is adversely affecting wine (Parley, 1999). No air intake or contact is required during fermentation and ripening of the wine. If air enters, microorganisms (wild yeasts, acetic acid bacteria) develop which can operate in the presence of oxygen. However, in cases where the yeast does not reproduce to the desired extent, this is especially seen in sugar-rich musts. Because wine yeasts multiply in the presence of air, they tend to fermentation in an airless environment. Ventilation is unnecessary in normal composition musts. As mentioned before, the air in the environment is sufficient for the proliferation of yeasts (Güven, 2008). Sugar content in grape musts is no problem in fermentation up to 200 g/L levels. If the sugar content is up to 250 g/L, it is not a problem if strong yeast is used and meticulously fermented. However, when the sugar ratio increases to 250 g/L or more, the osmotic pressure, more accurately the osmotic absorption force of the sugar solution increases so much that the vital activity of the yeast becomes difficult (Aktan and Kalkan, 2000). Genuine wine yeasts are the most resistant to alcohol from various types of yeast capable of alcohol fermentation. Yeast strains with high fermentation power can produce alcohols up to 140-145 g/L (17.5-18.0%) if the

conditions are appropriate. However, the poisoning effect of alcohol increases with increasing fermentation temperature. The contribution of SO_2 is an indispensable stage in wine production. In some cases, sulfur dioxide up to 8-10 g/hl may be used, especially in order to precipitate the freshly pressed must. However, then fermentation begins late. For example, in a must with 10 g/hl sulfur dioxide fermentation begins after about 3 days (Güven, 2008).

Yeast needs nitrogenous substances to feed. Normally, nitrogenous substances such as proteins, amino acids and ammonium compounds are present in grape must in sufficient amounts and no external additives are required. However, the present nitrogenous substance should be capable of being used by yeast and amount (average 200 mg/L). If the amount of nitrogen is insufficient, fermentation cannot be completed in the wine, so it is necessary to add yeast food before fermentation. Yeast strain used in alcohol fermentation significantly affects the fermentation of the yeast. The yeast to be used must be capable of fermenting the sugars contained in the yeast and should also be able to finish the fermentation within the desired time. Yeast strain suitable for grape type and type of wine to be produced should not be used. Dry active pure yeast must be rehydrated according to the instructions before use. It is recommended by all researchers to give 2% pure yeast to fermentation. *Saccharomyces cerevisiae* is used as a yeast. Yeast strain affects the composition and type of wine (Aktan and Kalkan, 2000; Avcı, 2004).

In some cases, sulfur dioxide up to 8-10 g/hl may be used, especially in order to precipitate the freshly pressed must. The amount of acetaldehyde produced during fermentation is proportional to the amount of SO_2 added before fermentation. While the bound acetaldehyde is catabolized during malolactic fermentation, free SO_2 is released, but bound SO_2 oxidation leads to the formation of free acetaldehyde (Parley, 1999; Bakker, 1986; Bakker and Timberlake, 1986). The effect of acetaldehyde and SO_2 in red wine is linked and affects anthocyanin. Increased SO_2 content in wine slows down the polymerization of pigments and procyanidins (Bakker 1986; Dallas and Laureano 1994).

Acids in grape must and fruit juices are not inhibiting fermentation. Citric acid is sensitive to microorganisms and may decompose to form acetic acid. Tartaric acid or

malic acid more than 30 g/L adversely affects fermentation. Furthermore, 14 g/L lactic acid inhibits the growth of yeasts, and the amount of 30 g/L lactic acid completely stops. 2 g/L acetic acid slows fermentation, 8 g/L acetic acid completely stops (Güven, 2008).

2.14 Use of SO₂ in Wine

In the production of wine, SO₂ is added in various steps. However, alcohol is usually added to the must before wine fermentation or to wine during packaging in the cellar in later steps (Romano and Suzzi, 1993). SO₂ is also added to wine and wine to control unwanted yeasts and bacterial species causing degradation. Much attention should be paid to the SO₂ concentration used. The effectiveness of SO₂ as an antioxidant or antimicrobial agent in wine depends on the amount of molecular SO₂ in the solution (Ingram, 1948; Joslyn and Braverman, 1954). According to the World Health Organization (WHO), the amount of SO₂ that a person is allowed to take daily with food is 0.7 mg/kg body weight. Doses slightly above this dose cause a decrease in the specific alcohol dehydrogenase enzyme, causing discomfort (Stevenson and Simon, 1981). Yeast produces SO₂ during the metabolism of grape juice (Eschenbruch, 1974). Therefore, it is not possible to produce a SO₂-free wine. SO₂ production of *S.cerevisiae* is a characteristic of strains. Under comparable conditions, Suzzi et al., (1985) reported that the majority of *S.cerevisiae*'s 1700 strains (80%) produced less than 10 mg/L of SO₂ and only 4 strains produced more than 30 mg/L (Romano and Suzzi, 1993). In addition, *S. cerevisiae* strains produce low concentrations of SO₂ binding metabolites. Therefore, these strains are used to maintain fermentation or to reduce the total SO₂ content in wine (Romano et al., 1992).

CHAPTER III

MATERIAL AND METHODS

3.1 Materials

3.1.1 Raw Material

In this study, two types of grapes (“Hönüsü” and “Horoz Karası”) from *Vitis vinifera* ssp. *sativa* L. cultivated in Gaziantep were harvested in September 2018 at the appropriate maturity in 20 kg plastic crates and transported to the winery in the Food Engineering Department (Gaziantep University, Gaziantep, Turkey). The grain shape of Hönüsü grape variety is long, translucent and ellipsoid. Its skin is a color between red and brown, thin, hardly crushed in the mouth and easily separated from the grain. The inside of the grain is yellowish in color, full, loose flesh, thin-edged and slightly juicy, with a small number of seed and the seeds are easily separated from the grain. It is not sandy and only the edge ring is sandy. The image of Hönüsü grape used in this research is shown in Figure 3.1.



Figure 3.1 Hönüsü grape

The grain shape of Horoz Karası variety is very large, white misty, equal long egg and acorn grains. The color of this grape variety is slightly hazy, dark purplish-black.

The grain of them is very large and the average weight of 100 grain is 585 g. The shell is thick, hard, wire-structured, low-grain, difficult to crush in the mouth and easily separated from the flesh. The grain is full, fleshy and medium juicy. The number of seeds is poor and the seeds are easily separated from the grain flesh. The taste of Horoz Karası grape is succulent, medium juicy and sweet. The seed shape is broad, rotund, long necked, prickly and double-ended. The image of Horoz Karası grape from the red grape variety used in the research is shown in Figure 3.2.



Figure 3.2 Horoz karası grape

3.1.2 Material and Equipment

The materials and equipment used in this research were;

- High Performance Liquid Chromatography Shimadzu LC-20AB Prominence HPLC sistem (Shimadzu Corp., Kyoto, Japan)
- C18 analytic coloumn 4,6 mm x 250 mm x 5 μ m (GL Sciences, Kyoto, Japan)
- Computer (ZENON, China)
- Dry activated *Saccharomyces cerevisaea* yeast (Proform-W, Germany)
- Yeast nutrient (Vitastart, Germany)
- Potassium Bisulfite (BASF, Germany)
- Methanol, ethanol, acetic acid (Isolab, Germany)
- Disinfectant (Bioxeco-5, Germany)
- Otoclave (HMC Hirayama, Tokyo, Japan)
- Heater (Electromantle, United Kingdom)

- Potato dextrose agar (Biomark Laboratories, India)
- HPLC-grade methanol, acetic acid, tartaric acid and hydrochloric acid (Interlab, Adana, Turkey)
- Standards of gallic acid, (+)-catechin hydrate, routine hydrate, procyanidin B₂, *p*-coumaric acid, chlorogenic acid, resveratrol, tyrosol, myricetin and quercetin (Interlab, Adana, Turkey)
- H₂SO₄, starch, iodine, fructose, glucose, rhamnose, maltose and sucrose (Interlab, Adana, Turkey)
- Tartaric acid, malic acid, citric acid, succinic acid and lactic acid (Interlab, Adana, Turkey)
- Tintometer (Colorflex, Hunterlab, Reston, U.S.A)
- Ultrasound (Branson 2200, Shelton, U.S.A)
- Ultra Pure Water System (Millipore Q, Darmstadt, Germany)
- Analytical Balance (0.01 g scaled, Sartorius, Goettingen)
- pH-meter (Hanna Instruments, pH 211, Darmstadt, Germany)
- Refractometer (Index Instruments, PTR 46 X, Florida, U.S)
- Electronic ebulliometer (Bulteh 2000, Stara Zagora, Bulgaria)
- Centrifuge (5810 R, Eppendorf, Hamburg, Germany)
- 10 L glass and 19 L pet carboys
- Glass materials are pipette, Erlenmeyer flask, balloon joje, beaker, petri dish, spreading rod, burette, steam distillation apparatus, green bottles of 75 cc, air lock and pyconometer (PC001, 25 ml).

3.1.3 Preparation of Solutions and Reagents

3.1.3.1 Preparation of 0.1 N NaOH

NaOH (4 g) was weighed and dissolved in 1 L of distilled water. Normality was checked by standardization and adjusted to desired concentration by following formula:

$$V_1 \times N_1 = V_2 \times N_2$$

where N₁, N₂ and V₁, V₂ represent normality (N) and volume (L) of stock solution respectively.

Standardization of NaOH solution: About 0.7 g potassium hydrogen phthalate, (KHC₈H₄O₄: 204,229 g/mol) was dissolved in 100 ml of distilled water. A few drops

of phenolphthalein was added as indicator. The NaOH solution was prepared as approximately 0.1 N and filled to the zero line of the burette. Titration with NaOH was continued until the color of the indicator changes from colorless to pink. The amount of base spent was read from the burette.

$$\text{Normality (N)} = \frac{\text{g KHC}_8\text{H}_4\text{O}_4 \times 1000}{(\text{ml of NaOH} \times 204.229)}$$

3.1.3.2 Preparation of Phenolphthalein

One g of phenolphthalein was dissolved in 50 ml of 95% ethyl alcohol and transferred to a 100 ml flask. Completed with 95% ethyl alcohol up to volume line.

3.1.3.3 Preparation of 4% NaOH

Twenty g NaOH was weighed into 500 ml conical flask. Completed with distilled water up to volume line. NaOH and distilled water were thoroughly dissolved.

3.1.3.4 Preparation of 25% H₂SO₄

The amount to be taken from 95% H₂SO₄ to prepare 250 ml of solution was calculated according to the following formula:

$$M_1 \times V_1 = M_2 \times V_2$$

where M₁, M₂ and V₁, V₂ represent molarity (M) and volume (L) of 25% solution respectively, M₂ and V₂ represent molarity and volume of 95% solution, respectively.

3.2 Preparation of Culture and Media

3.2.1 Yeast Culture

Dry activated *Saccharomyces cerevisiae* D47 culture was used in the production of red wine and it was used by 20 g/hL.

3.2.2 Preparation of Medium

Potato dextrose agar (PDA) medium was used for microbiological analysis. 39 g of agar media was dissolved in 1000 ml distilled water by boiling. After, it was sterilized in autoclave at 121°C for 15 min. The medium was cooled to 50°C and poured about 20 ml into sterile petri plates in thick layer under the aseptic conditions.

After allowing the agar on the table top (within 10 min) to solidify, plates were turned down and incubated at 25°C for 48 hours.

3.2.3 Sterilization

The medium used in the microbiological analysis was sterilized in autoclave at 121°C for 15 min. All glass wares were sterilized in dry air oven at 180°C for 2 hours.

3.3 Method

3.3.1 Must Preparation

The grapes were separated from the stems, garbage and rotten, and crushed by hand under sanitary conditions without damaging the seeds (Figure A.1 and Figure A.2). Three musts from crushed grapes (14.25 kg for each lot) were prepared together with juice, skin and seeds in duplicate; wine-1: 100% Hönüsü grape must, wine-2: 85% Hönüsü + 15% Horozkarası grape musts and wine-3: 70% Hönüsü + 30% Horozkarası grape musts. Brix values for juice were ranged from 18 to 21.

3.3.1.1 Adjusting of Brix of Must Mixtures

The brix value of lots was adjusted by adding sucrose into musts. The conversion of sugar to alcohol is accepted as 0.55% for 1 brix must. One degree brix means 1 g of sucrose per 100 g of aqueous solution. The required sucrose amount for each must was calculated using this ratio and added into must. Before adding into must, sucrose was dissolved in own must juice. Thus, the final brix values for musts of wines-1, 2 and 3 after addition of sucrose were 22.78, 22.57 and 22.50 % (w/v) respectively.

3.3.2 Production of Red Wines

Grapes were processed into wine according to the process procedure as given in Figure 3.3. The vinifications were carried out in a small-scale winery in the Department of Food Engineering, Faculty of Engineering, Gaziantep University (Gaziantep, Turkey). The grapes were first separated from the garbage and crushed by hand under sanitary conditions. Three wine types of crushed grapes were used in the production; wine-1: 100% Hönüsü grape must, wine-2: 85% Hönüsü grape must + 15% Horoz Karası grape must and wine-3: 70% Hönüsü grape must + 30% Horoz

Karasi grape must. Each must (juice, skin and seeds) was filled up to 75% of clean 19 L plastic demijohns under aseptic condition. The yeast nutrient (0.2 g/kg) is melted in the water and added to the crushed grape. The yeast nutrient (0.2 g/kg) and potassium metabisulfide (PMB, 25 mg/kg) are dissolved in must juice separately and added into each of must. *S. cerevisia* (0.22 g/kg, dry yeast) is rehydrated in must juice, allowed for 10 min at room temperature and then added into each of musts. In each of these preparation, own must juice was used. Airlock was fitted on the each of demijohns. They allowed to fermentation in dark room at 25°C for 5 days under climatic conditions. Fermentation stage in plastic demijohn was shown in Figure A.3. All plastic demijohns were mixed every day twice in order to prevent the cap formation and temperature increase, and improve extraction of phenolics from skin. By the onset of active fermentation, the skins and seeds rise to the top of the fermenting liquid and form a cap. Thus the skins and juice in a fermenter are somewhat separated. A portion of the heat released leads to a temperature increase in the cap as compared to the fermenting liquid below. In order to maximize the extraction of color and flavor from skin it is important to keep skins in close contact with the juice during fermentation. To achieve this, the cap is periodically punched and skins and juice are mixed. For smaller lots, punching the cap twice daily is sufficient to facilitate extraction and control of temperature with releasing heat from cap. Small volumes of raw wine were sampled periodically at the same time every day until the fermentation ended and three parallels were analyzed by using various methods. After fermentation, mixtures were filtered through disinfected cheesecloth to remove skin, seeds, mare and coagulant. Subsequently, each musts were filled into 90% of the 10 L glass carboy with air lock, and the oak chip 2.5 g/L was added to them and they were sulfurized with 15 mg/L. They were placed in a dark room of 20°C to complete the resting. Oak chips were blooded into juices every day twice to release flavor. The rested wines for 7 days were filtered to remove oak chips and coagulants through disinfected cheesecloth. Each of wines was placed into 10 L disinfected glass carboy containing PMB (15 mg/L) which was dissolved in 20 ml own wine and carboy were completely filled to reduce air. Airlock was fitted on the each of carboys. The rested wines were matured (aged) in glass carboy for 45 days at 20°C in dark room. Then the SO₂ (20 mg/L) was added. Airlock was fitted on the each of carboys. After this process, The maturated wines were separated from the sediments by filtration through disinfected cheesecloth. Wines were filled into dark

green colored bottles (75 cc) and the bottles were capped with cork using cork stopper closing machine (Atlantis Cam Ambalaj Ltd.Şti., İzmir, Turkey) (Figure B.1). The bottles were stored in the horizontal position for 3 months in the dark room at 20°C (Figure B.3). The bottles, wine storage and cork closure at the semi automatic sealing machine are shown in Appendix B.



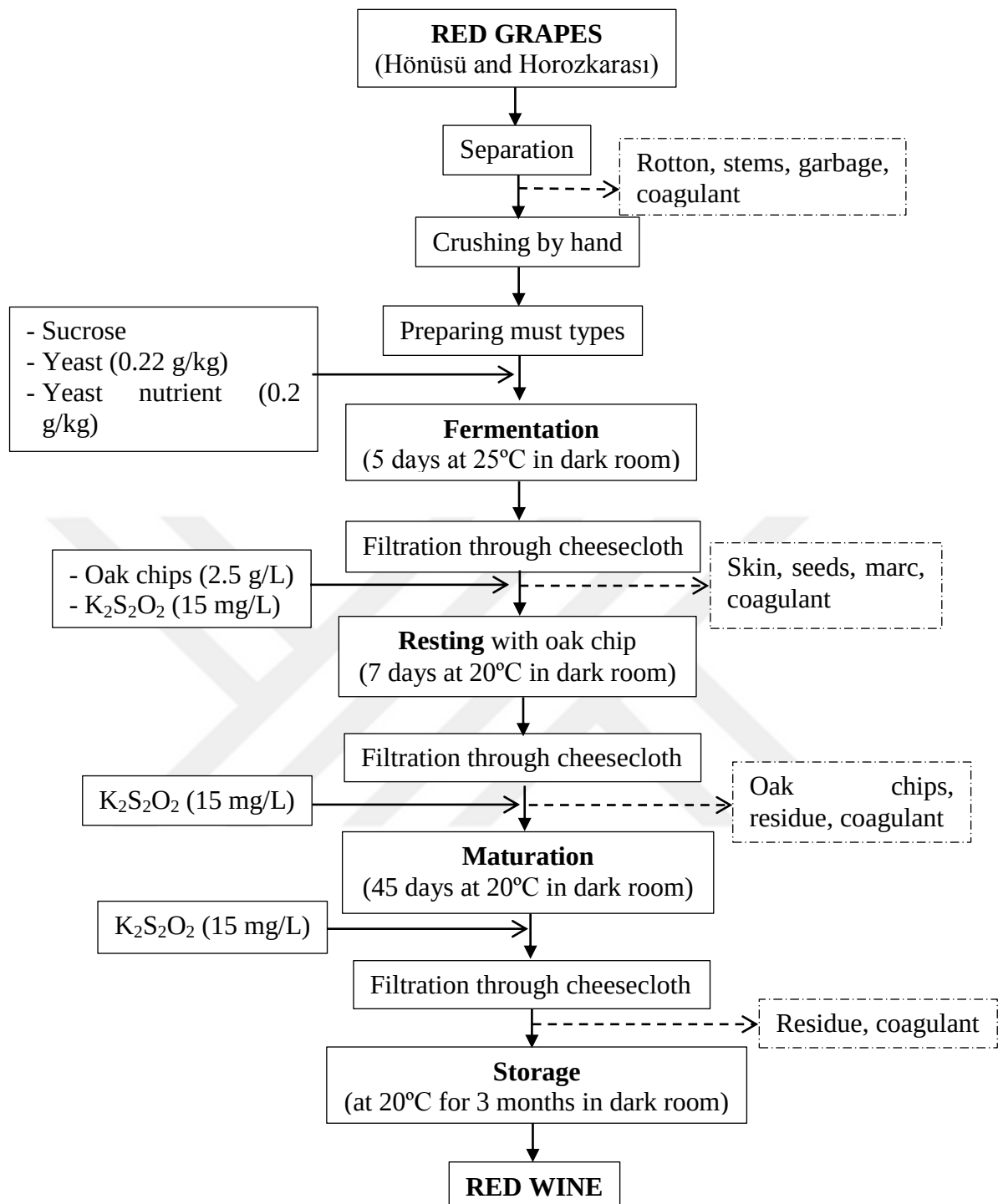


Figure 3.3 Red wine production scheme

3.4 Analysis

3.4.1 Sampling

About 150 ml of the samples were removed in triplicate during the fermentation (1, 2, 3, 4 and 5 days), after resting with oak chips (7 days), after maturation (45 days) and after storage (90 days). The chemical and microbiological analysis were made from these samples.

3.4.2 Analysis on Must and Wines

Total acidity, pH, dry matter, sugar, density, phenol compounds, color intensity, color tone and microbiological analyzes were conducted in must. In addition to these analyzes, alcohol, volatile acidity, free and total SO₂ analyzes were carried out for wines.

3.4.2.1 pH

The pH of the must and wine were measured directly by using pH-meter with glass electrodes (Ough and Amerine, 1988). Twenty milliliter sample was used in pH analysis. The pH meter is shown in Figure A.4.

3.4.2.2 Total Acidity

Five milliliters were removed from each sample and 20 mL of water was added. Two or three drops of phenolphthalein reagent was added to the homogenized mixture and titrated with 0.1 N NaOH. The NaOH spent in titration was recorded and the results were calculated in terms of tartaric acid (Ough and Amerine, 1988). The total acidity apparatus was shown in Figure A.5. The results were determined in terms of tartaric acid. Total acidity (TA) was calculated using following formula:

$$\text{Total acidity (g tartaric acid/L)} = [(V \times F \times E) \times 1000] / m$$

V= volume of NaOH (mL), F=Normality of NaOH, m= amount of sample (g)

E=0.075=milliequivalent weight of tartaric acid

Tartaric acid: 1mL 0.1 N NaOH= 0.0075g tartaric acid

3.4.2.3 Water Soluble Dry Matter

The amount of water-soluble dry matter of must and wine was determined by the refractometer and expressed as Brix (Aktan and Kalkan, 2000). The refractometer

was firstly calibrated with water. Then, one drop of homogenized sample was added to the refractometer (20°C). The reading from screen was expressed as in terms of Brix. The refractometer device is shown in Figure A.6.

3.4.2.4 Alcohol Analysis (Alcoholometric Method)

Electronic ebulliometer was used as an alcoholometer in the experiment (OIV, 2005). Alcohol content of the wine samples was determined by using the difference of boiling points of water and alcohol by using electronic ebulliometer. Fifty ml of distilled water was into the boiling chamber of the ebulliometer for cleaning. Distilled water in the boiling chamber of the ebulliometer was removed. Distilled water was filled upto the line of chamber and allowed to heating. When the distilled water started to boil and boiling point of the distilled water was fixed. The water was removed. The 50 ml sample was placed into the glass chamber of the ebulliometer and allowed to heat. The boiling point of sample was fixed with the thermometer of the alcoholometer, immediately at the same time alcoholic volume from screen was read. The reading was recorded as % alcohol. The electronic ebulliometer device is shown in Figure A.7.

3.4.2.5 Density

The dry and clean 25 mL pycnometer (ISOLAB) was weighed. The sample was filled to the marked level on the pycnometer at 20-25°C. The sample-filled pycnometer was weighed (Ough and Amerine, 1988). The density was calculated according to the following formula:

$$\rho = (m_2 - m_1) / v$$

ρ : density of liquid (g/mL), m_2 : filled weight of pycnometer (g), m_1 : empty weight of pycnometer (g), v : volume of pycnometer (mL)

3.4.2.6 Color Measurement

Color measurement was performed using a Hunter Lab Color Flex colormeter (USA). Prior to each measurement, the instrument was standardized against a white and black plate (Bakker et al., 1999). After that, about 25 ml sample was poured into glass rounded hopper and closed by cap. The results for the color measurements of the samples taken before and after the process are given in the CSI system as L^* (0:

darkness, 100: clearness), a^* (-: green, +: redness) and b^* (-: blueness, +: yellow) values. The used Hunter Lab Color device was shown in Figure A.8.

Calculation of Chroma (C^*) value: $\text{Chroma } (C^*) = [a^2 + b^2]^{1/2}$

Calculation of Hue (H^*) value: $\text{Hue } (H^*) = \arctan [b/a]$

The calculated Hue values were negatively marked, they were evaluated by summing with 360 degrees. Chroma value is a color parameter that measures the saturation of color. The Hue value is a parameter that expresses colors such as red, yellow, green, and blue.

3.4.2.7 Total SO₂ Determination

Twenty five ml of 4% NaOH was pipetted into 250 ml flask. Fifty ml of wine was added. The mouth of the flask was closed and kept in a dark place for 15 min (Aktan and Kalkan 2000). At the end of the period, 10 ml 25% H₂SO₄ and 2-3 ml 1% starch were added. The solution was mixed rapidly with the 0.02 N iodine solution and titrated until the blue color was reached and this color remained constant even after stirring 4-5 times (TS, 522). Calculation of total SO₂:

Amount of total SO₂ (mg/L) = $a \times 10$

a = used 0.02 N iodine solution (mL)

3.4.2.8 Free SO₂ Determination

Fifty ml of wine was pipetted to a 250 ml flask. Five ml of 25% H₂SO₄ and 2-3 ml of 1% starch solution were added (Aktan and Kalkan 2000). Finally, it was titrated rapidly with 0.02 N iodine solution until the blue color remained constant for 30 sec (TS, 522). Calculation of free SO₂:

Amount of free SO₂ mg/L = $a \times 10$

a = used 0.02 N iodine solution (mL)

3.4.2.9 Volatile Acidity

Volatile acidity was made according to the steam distillation method and the results were given in g/L in acetic acid (Ough and Amerine, 1988). About 300-400 mL of pure water was filled into the 500 ml flask which provides steam. Five mL of wine was placed in the flask in this balloon. A 500 ml flask was provided to evaporate and

several porous boiling stones were disposed into water. As the boiling was started, the vapor from sample was recovered by collecting in the collection container. About 60 ml of distillation was collected. 1-3 drops of phenolphthalein was added into distillate and distillate was titrated with N/10 of NaOH until violet color (TS, 522). Volatile acidity was obtained using “Steam Distillation Method” as given in Figure A.9. Results were calculated in terms of acetic acid. Calculation of volatile acid:

$$\text{Volatile acid amount g/L (in terms of acetic acid)} = [(60 \times N \times a) / V]$$

60 = molecular weight of acetic acid,

N = Normality of NaOH

a = amount of sodium hydroxide used in titration (mL),

V = volume of sample (mL)

3.4.2.10 Yeast Count

In the preparation of potato dextrose agar (PDA; Difco, Detroit), 39 g of PDA was mixed in 1000 ml distilled water and boiled to dissolve the agar completely. Agar medium in flask were sterilized in autoclave at 121°C for 15 min. Agar allowed to cool up to 50°C. About 20-25 ml of cooled (at 50°C) PDA agar was poured into sterile PDA plates from flask under aseptic conditions and allow to solidify the agar in plates. Petri plates are placed into an incubator at 35°C for overnight to detect possible contamination.

The samples were homogenized and diluted with 0.1% peptone water from 10^{-2} to 10^{-7} dilutions. Diluted (or nondiluted) sample (1 mL) was spread plated on the potato dextrose agar and the plates were incubated at 25°C for 3-5 days for yeast counts (Erkmen, 2015). The plates containing 30 to 300 colonies were counted. The results were given in colony forming unit per milliliter (cfu/mL).

3.4.3 HPLC Analysis of Phenolic Compounds

3.4.3.1 Preparation of Standards

The gallic acid, (+)-catechin hydrate, routine hydrate, procyanidin B₂, *p*-coumaric acid, chlorogenic acid, resveratrol, tyrosol, myricetin and quercetin standards were determined by HPLC and the results were given in mg/L. The water used in the analysis was obtained from a Milli-Q water purification system manufactured by

Millipore. For all standards, stock solutions were prepared by dissolving the phenolic compounds at different concentrations with the 50% solution (methyl alcohol/water, 50:50 v/v) mixture. The samples were centrifuged for 5 min at 5000 rpm. All solvents used as the mobile phase were previously filtered through 0.45- μ m membranes (Millipore) and degassed prior to use. Samples were added into 1.5 mL colored vials and analysed as soon as possible using HPLC. The HPLC equipment was shown in the Figure A.10.

3.4.3.2 HPLC Conditions and Standard Calibration Curves

In the quantitative analysis of phenolic acids, the HPLC method applied by Gomez-Alonso et al. (2007) was modified. The characteristics of the HPLC instrument and chromatographic condition used for phenolic components analysis are given in Table 3.1. Chromatographic analysis were performed using a Shimadzu LC-20AB (Shimadzu Corporation, Kyoto, Japan) high performance liquid chromatography (HPLC) equipped with a vacuum degasser (DGU-20A5), quaternary-pump LC-10AT, UV detector (SPD-20A), SIL- autosampler (20A HT) and VP column furnace (CTO-10AS). The LCsolution (v.1.25; 2002-2009 Shimadzu Corporation) was used to control the gradient settings, UV and data acquisition. Chromatography was carried out at 280 and 320 nm wavelengths and the separation was performed using a C18 analytical column (GL Sciences, Kyoto, Japan) of 4.6 mm x 250 mm 5 μ m. A C18 guard column of 4.6 mm x 12.5 mm, 5 μ m particle size (GL Sciences, Kyoto, Japan) was used to prevent contamination of analytic column from any non-soluble residues coming from the samples. Approximately 2 mL of the resulting solution was removed with 0.45 μ m PTFE syringe filter (Millipore) and added into 1.5 mL colored vial. The injection volume was adjusted to 25 μ L and the flow rate to 1 mL/min and the temperature to 30 $\pm$ 1 $^{\circ}$ C. The flow is given as a gradient. Gradient program solvent flow concentration used in the analysis is given in Table 3.2. Two solvent gradient elution was used in the study. Solvent A is acetic acid-water mixture (2:98 v/v) and solvent B is methanol-water mixture (50:50 v/v). As the flow program; the system B is arranged in a concentration of 5% at the end of 3 min and then 20% B solvent at the end of 20 min, 25% B at the end of 30 min, 30% B at the end of 40 min, 40% B at the end of 50 min, 50% B at the end of 55, and it is set to 100% B at the end of 65. 100% B was passed through the system for 10 minutes. Finally, 0.01 min A solvent was passed and the system was brought to the initial

conditions. External standard method was used to determine 10 different phenol compound concentrations in the samples. For this purpose, calibration solutions were prepared at 3 different concentrations of phenolic component standards. The phenolic standards used, retention times and maximum absorbance values were shown in the Table C.1. The example to a standard curve for a phenolic compound is shown in the Figure C.1. The examples to HPLC phenolic compound chromatograms for standard and a wine sample were shown in Figures C.2 and C.3 respectively.

Table 3.1 Characteristics and conditions of HPLC for phenolic compounds

Equipment: Shimadzu
Degasser: DGU-20 A5 Prominence (gradient valve)
Pump: LC-20AB Prominence
Control Unit: CBM-20Alite Prominence
Detector: SPD-20A Prominence (UV/VIS)
Automatic Sample Injection Unit: SIL-20A
Column Furnace: CTO-10ASvp
Oven temperature: 30 °C
Total flow: 1 mL/min
Injection volume: 25 µL
Column: C18 GL Sciences analytic coloum (4.6 mm x 250 mm, 5 µm)
Computer: ZENON
Solvent A: 2% Acetic acid-water and solvent B: 50% Methanol-water

Table 3.2 Gradient program solvent flow concentration of phenolic compounds

Time (min)	Action	Value (Solvent A%)
0.01	Pump A B concentration	0
3.00	Pump A B concentration	5
20.00	Pump A B concentration	20
30.00	Pump A B concentration	25
40.00	Pump A B concentration	30
50.00	Pump A B concentration	40
55.00	Pump A B concentration	50
65.00	Pump A B concentration	100
75.00	Stop	0

3.4.4 HPLC Analysis of Sugar

3.4.4.1 Preparation of Standards

The glucose, fructose, sucrose, maltose and rhamnose as sugar contents of the samples were determined by HPLC and the results were given in g/L. The samples

were centrifuged for 5 min at 5000 rpm. For all standards, stock solutions were prepared by dissolving at different concentrations with the pure water-ethanol (99.9%) mixture. Approximately 2 mL of the resulting solution was taken by 0.45 μm PTFE syringe filter and added into 1.5 mL vials. The samples were analysed as soon as possible, using HPLC.

3.4.4.2 HPLC Conditions

The analytical procedure was performed by applying modified chromatographic conditions for wine and grape juice samples previously described by Ball (2011). The characteristics and chromatography of the HPLC instrument used for sugar analysis were given in Table 3.3. In the determination of sugar in wine; The Shimadzu LC-20AB Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) was used and the system components were RID-10A detector, SIL-20A HT autosampler, CTO-10AS VP column furnace and DGU-20A5 degasser units. Chromatography was carried out at 210 nm wavelengths and the separation was performed using a Shodex SUGAR SH1011 (8 mm $\times$ 33 mm, 7 μm) column. The injection volume was adjusted to 25 μL and the flow rate to 0.6 mL/min and the temperature to 50°C. External standard method was used to determine sugar concentrations in the samples. For this purpose, calibration solutions were prepared 5 different concentrations of sugar standards using pure water-ethanol mixture (99.9%). The sugar standards used, retention times were shown in the Table C.2. The standard curve example for sugar example is shown in the Figure C.4. The HPLC chromatogram of sugar standards is given in the Figure C.5.

Table 3.3 Characteristics and onditions of HPLC for sugar analysis

Equipment: Shimadzu
Degasser: DGU-20 A5 Prominence
Pump: LC-20AB Prominence
Control Unit: CBM-20Alite Prominence
Detector for sugar analysis: RID-10A Detector
Automatic Sample Injection Unit: SIL-20A
Column Furnace: CTO-10ASvp
Oven temperature: 50°C
Total flow: 0.6 mL/min
Maximum time: 25 min
Injection volume: 25 µl
Wavelength: 210 nm
Column: Shodex SUGAR SH1011, 8 mm × 33 mm, 7 µm
Computer: ZENON
Solvent: Pure water-ethanol (99.9%)

3.4.5 HPLC Analysis of Organic Acids

3.4.5.1 Preparation of Standards

The lactic acid, malic acid, citric acid, succinic acid and tartaric acid as organic acid contents of the samples were determined by HPLC and the results were given in g/L. The samples were centrifuged for 5 min at 5000 rpm. For all standards, stock solutions were prepared by dissolving at different concentrations with the pure water-ethanol (99.9%) mixture. Approximately 2 mL of the resulting solution was taken by 0.45 µm PTFE syringe filter and added into 1.5 mL vials. The samples were analysed as soon as possible, using HPLC method.

3.4.5.2 HPLC Conditions

The analytical procedure was performed by applying modified chromatographic conditions for wine and grape juice samples previously described by Ball (2011). The characteristics of the HPLC instrument chromatography conditions used for organic acid analysis are given in Table 3.4. In the determination of organic acid in wine; The Shimadzu LC-20AB Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) was used and the system components were SPD-20A UV/VIS detector, SIL-20A HT autosampler, CTO-10AS VP column furnace and DGU-20A5 degasser units. Chromatography was carried out at 210 nm wavelengths and the separation was performed using a Shodex SH1011 (8 mm × 33 mm, 7 µm). The injection volume was adjusted to 25 µL and the flow rate to 0.6 mL/min and the

temperature to 50°C. External standard method was used to determine organic acid in the samples. For this purpose, calibration solutions were prepared 4 different concentrations of organic acids. The organic acid standards used, retention times were shown in the Table C.3. A standard curve as an example for organic acid was shown in the Figure C.6. The HPLC chromatogram of organic acid as an example is given in the Figure C.7.

Table 3.4 Characteristics and conditions of HPLC for organic acid analysis

Equipment: Shimadzu
Degasser: DGU-20 A5 Prominence
Pump: LC-20AB Prominence
Control Unit: CBM-20Alite Prominence
Detector for organic acid analysis: SPD-20A detector (UV/VIS)
Automatic Sample Injection Unit: SIL-20A
Column Furnace: CTO-10ASvp
Oven temperature: 50°C
Total flow: 0.6 mL/min
Maximum time: 25 min
Injection volume: 25 µl
Wavelength: 210 nm
Column: Shodex SUGAR SH1011, 8 mm × 33 mm, 7 µm
Computer: ZENON
Solvent: Pure water-ethanol (99.9%)

3.4.6 Sensory Analysis

The sensory properties of red wines were evaluated by flavor profile analysis tests (Lawless and Heymann, 2010). The method used in sensory analysis of red wine samples were randomly assigned to the wine glasses with three-digit numbers and letters, and presented to the panelists with a tray of glass containing some water to eliminate the odor remaining at nose and taste remaining at mouth between the two sample tastes. The panelists were asked to complete a sensory evaluation form consisting of visual examination, sniffing and tasting examination. In this form, the visual examination section includes color, clarity and turbidity parameters; sniffing section of the alcohol and yeast odor parameter; tasting examination consists of sweetness, presence of bitter taste, presence of acid taste, sourness, astringency whether or not the taste remains in the mouth for a long time and the feeling or taste parameters left in the mouth. Sensory analyzes were tested by 11 panelists, aged between 22-55, consisting of students and teachers from the Food Engineering and

Gastronomy departments of Gaziantep University. For the accuracy of the results, the panelists tasted 3 replications at 1 hour intervals. The panelists evaluated the wine for each criterion out of 10 points. The form, color chart, sensory analysis room and a view from evaluation of panelists shown in Appendix D was used for sensory analysis.

3.4.7 Statistical Analysis

The results obtained from the analysis of red wine (wine-1, 2 and 3) were evaluated. The wine production was repeated three times. Three samples were taken in parallel from each step of wine production and the results of the analyzes were given as the mean $\pm$ standard deviation values of the three experiments unless otherwise stated in the tables. The wines were compared depending on process time and with each other by analysis of variance with ANOVA test using SPSS v.22 (IBM SPSS Corporation, Chicago, IL, USA) with a 95% confidence level (a confidence interval of $\alpha = 0.95$).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Physical and Chemical Analysis of Must and Wines

In this study, three types of wines were produced from two grapes in the following percent: wine-1: 100% Hönüsü grapes, wine-2: 85% Hönüsü grape + 15% Horoz Karası grape, wine-3: 70% Hönüsü grape + 30% Horoz Karası grape. The fermentations were carried out at 25°C for 5 days. The fermentation was performed in plastic demijohns. Wines were rested with oak chip (7 days) and matured in glass carboy (45 days). The storage were carried out at 20-25°C for 90 days in colored glass bottle (75 cc). Physical and chemical analysis of must and wines were determined every day during fermentation, the end of resting with oak chip, at the end of maturation in glass carboy and at the end of storage in the bottle. The total acidity, pH, brix, density, color analysis, organic acid, yeast count, sugar content and phenolic compounds content were determined for the musts obtained from Hönüsü and Horoz Karası grapes. In addition to these analysis for three types of wine, volatile acidity, alcohol and sensory analysis were performed.

4.1.1 Changing of pH and Total Acidity in Musts and Red Wines

The pH changes of the red wine for wine-1, wine-2 and wine-3 are given in Figure 4.1. The pH values of Hönüsü and Horoz Karası grape must mixtures for wines-1, 2 and 3 were found 3.52, 3.47 and 3.42 respectively. Since yeast reduced pH below 2.8, both growth and fermentation are inhibited. Yeast growth rate is higher in the range of 3.5 and 4.0 pH values. In addition, fermentation starts earlier at higher pH values (Reed and Peppler, 1973). Fermentation of wine was completed in 5 days. According to the “ANOVA” the pH results during processing steps of red wines are given in Table F.1. The pH values of wines were founded as 3.47, 3.57 and 3.56 for wines-1, 2 and 3, respectively at the end of fermentation. The pH values for wines-1, 2 and 3 were statistically different between must and at the end of fermentation

($p < 0.05$). After the three of wines were kept with oak chip, the pH were found to be 3.55, 3.47 and 3.40, respectively. After 45 days maturation in glass carboys, the pH were found as 3.67, 3.62 and 3.63 for three types of wine respectively. The pH values of red wines were increased from resting with oak chips to maturation step. It was found that there was significant increase in the pH values of red wines ($p < 0.05$) from resting to maturation. Small amount of increase in pH value can be attributed to precipitation of tartaric acid as potassium bitartrate and breakdown of malic acid by lactic acid bacteria. Finally, after the storage in the bottle for 3 months, the pH values for wines in order were 3.64, 3.61 and 3.58. The pH values of red wines were decreased from maturation to storage in bottle. The pH values for wine-1 and 3 were statistically different between maturation and storage ($p < 0.05$) but for wine-2 was not significantly different ($p > 0.05$). The pH of the wine should not be higher than 3.5. This is because the higher the pH of the wine, the more susceptible it is to oxidative reactions, unwanted color changes, protein instability and bacterial fermentation. At the same time, the efficacy of SO_2 decreases in wines (Esteman et al., 1999). During fermentation, organic acids, together with their salts, remain stable in the wine and maintain the pH of the wine in the range of 2.9–4.0, ensuring that the fermentation is carried out in a healthy manner (Torija et al., 2003). The pH of the wine varies according to the type of acids contains, the amount of potassium ions and other wine components present in the environment (Boulton, 1996). The total acid content of the wine depends on the ratio of tartaric acid to malic acid and the amount of potassium ions in the medium. If the tartaric acid content of the wine is high and the amount of potassium ions is low, the pH of the wine will also be low (Boulton, 1996). For color, microbiological, chemical and oxidative stabilities pH of wines should be between 3.0-3.5 (Boulton et al., 1996). In maturation process; wine-1, 2 and 3, a small amount of increase in pH value and decrease in total acid was attributed to precipitation of tartaric acid as potassium bitartrate and breakdown of malic acid by lactic acid bacteria. It is stated that pH values may vary between 2.8-4.0 in wine (Clarke and Bakker, 2004).

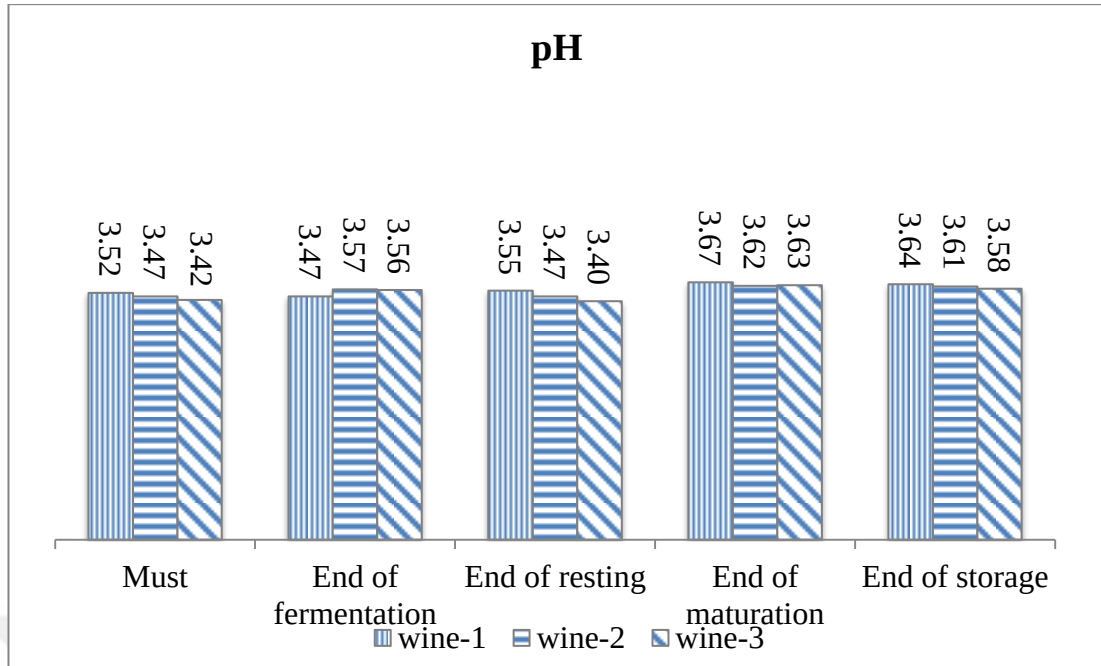


Figure 4.1 pH changes during red wine production

Kelebek (2009) indicated that the pH value of Bogazkere wine obtained from the grapes grown in Denizli and Elazig regions were 3.10-3.40; Kalecik Karası wine obtained from the grapes grown in Ankara and Nevşehir regions were 3.23 and 3.40, and Öküzgözü wine obtained from the grapes grown in Denizli and Elazig region between 3.04 and 3.20. The pH values of the wine above 4.0 is not desirable for wine production. This is because; The higher the pH of the wine results with the more susceptibility to oxidative reactions, unwanted color changes, protein instability and bacterial fermentation. The pH of the wine varies according to the type of acids, the amount of potassium ions and other wine components (Boulton et al., 1996). The pH value in wines plays an important role in the physiological evaluation of wine. The pH value is too low causes the taste of sourness in wines. In this study, the lowest pH value at the end of process was found 3.58 for wine-3 and the highest pH value was found for wine-1 with 3.64. The pH values of the wines are in different groups, the difference between the wines is statistically significant ($p < 0.05$). The pH values obtained from this study are within the normal range and are similar to previous studies.

The total acidity changes of the red wine for wine-1, wine-2 and wine-3 is given in Figure 4.2. According to the “ANOVA” the total acidity results during processing

steps of red wines are given in Table F.1. The acidity values (in tartaric acid) of musts-1, 2 and 3 were found 3.80, 4.84 and 4.95 g/L respectively. According to the “ANOVA” analyzes, there are significant differences ($p<0.05$) between musts-1, 2 and 3 according to total acidity values. The total acidity values of wines were founded as 6.45, 6.55 and 6.61 g/L for wines-1, 2 and 3 respectively at the end of fermentation. It was found that there was significant ($p<0.05$) increase in the total acidity values for wines-1, 2 and 3 during fermentation. After the three types of wine were kept with oak chip (resting), the total acidity were found to be 6.61, 7.08 and 7.34 g/L respectively. After 45 days maturation in glass carboys, total acidity were found as 6.01, 6.30 and 6.21 g/L for three types of wine respectively. The total acidity values of red wines were decreased from resting to maturation step. It was found that there was significant decrease in the total acidity values of red wine types ($p<0.05$). Amount of decrease in total acidity value can be attributed to precipitation of tartaric acid as potassium bitartrate and breakdown of malic acid by lactic acid bacteria. Finally, after the storage in the bottle for 3 months, the total acidity values for wines in order were 6.11, 6.40 and 6.51 g/L. The total acidity values of red wines were increased from maturation to storage in bottle. While this increase was not statistically significant between wine-1 and wine-2 ($p>0.05$), the total acidity value for wine-3 was significantly different ($p<0.05$).

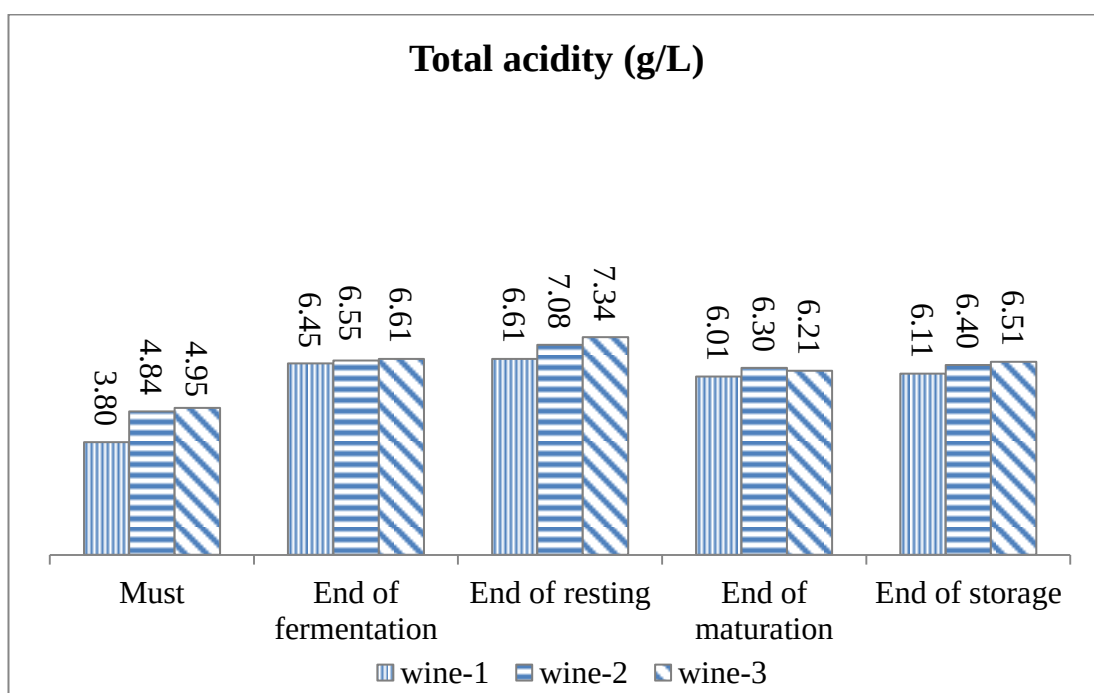


Figure 4.2 Total acidity changes during red wine production

The pH of the must is an important factor in the fermentation process. Yeasts usually grow in a weak acid medium. They are negatively affected at very low (below pH = 2.8) and high pH. In this research, the pH values of 3.52, 3.47 and 3.42 in the initial musts were found suitable for the initiation of fermentation. It has been reported that the pH value of quality black wine grapes should be between 3.20 and 3.40 (Boulton et al., 1996). In this study, results were close to this range. It has been reported that total acidity value in tartaric acid should be between 6.50 and 7.50 for a quality black wine grapes (Boulton et al., 1996). Acidity is one of the elements that makes the taste balance of wine as well as it protects the must against the deterioration caused by microorganisms. Akgün et al. (2007) found that the average total acid content of Hönüsü and Horoz Karası grapes grown in the Gaziantep region was 2.93 g/L and 5.33 g/L, respectively. It has been reported that the acidity value of early harvested grape varieties is high and the acidity value of late harvested grape varieties is low. Kelebek (2009) reported that the total acid content (in tartaric acid) and pH value of Öküzgözü and Boğazkere grapes grown in Denizli region varied between 6.63-7.08 g/L and 3.12-3.32 respectively. The total acidity and pH values were 5.60-8.70 and 3.20 to 3.60 for Öküzgözü grapes (Canbaş et al., 2001). The pH value of Hönüsü and Horoz Karası grapes was slightly higher than the results of Kelebek (2009) but was in agreement with the results of Canbaş et al. (2001). The acidity value of Hönüsü and Horoz Karası grapes was less than the results of Kelebek (2009) and Canbaş et al. (2001). When the grapes are harvested late, the amount of sugar increases, whereas the acid decreases. It is seen that the Hönüsü and Horoz Karası grapes were harvested late. The substances in the composition of must, especially sugar and acid, are closely related to the degree of maturity of grapes. As the grapes get close to maturity, sugar increases and acidity decreases. Most of the other substances reach their maximum values at full maturity (Altındışli et al., 2005). One of the biggest problems encountered in wine grapes in Turkey climatic conditions fall below the desired value the amount of acid for the quality wine production, while sufficient amount of sugar in the grapes.

The acidity affects the taste and durability of the wine. Organic acids facilitates the dissolution of color substances and makes the color more vivid during fermentation (Canbas, 2003). While the amount of acid in wines varies between 4.5–9.0 g/L in tartaric acid, the most suitable acid amount in good quality wines is 6–7 g/L (Ough

and Amerine, 1988). The total acid content of the wine depends on the ratio of tartaric acid to malic acid and the amount of potassium ions in the medium. After tartaric acid, the highest amount of acid in wine is malic acid (Delcourt et al., 1995). If the tartaric acid content of the wine is high and the amount of potassium ions is low; the pH of the wine is low (Boulton et al., 1996). But sometimes, the pH value may or may not be related to the tartaric acid content (Güven, 2008). The amount of acid in the wine varies depending on the grape grown climate and variety. The overall acid content has a great effect on the taste and longevity of the wine. Total acidity, also expressed as potential acidity, plays an important role in wine process. Acidic condition prevents disease of microorganisms and provides resistance to wine. In addition, sensory analysis gives freshness to the wine, increasing the grain's sense of sourness as well as contributing to the color tone (Canbas, 1992). According to the Turkish Food Codex (TFC) for wine notification (2008/67), the total acid amount of the wine should be at least 3.5 g/L in terms of tartaric acid (TGK, 2009). pH (from 3.58 to 3.65) and total acidity (from 6.1 to 6.5) of the red wines obtained in our study corresponds to the standard values. Anlı et al. (2006) reported that the pH and total acidity values of wines obtained from Öküzgözü grape in the range 3.45-3.50 and 4.30 g/L-4.50 g/L respectively. Arpa (2015) reported that the total acid contents of red wine obtained from Kösetevik grapes were 5.9 g/L and pH 3.6. Ünal et al. (2007) indicated that the total acid content of the wines obtained from black grapes grown in Pozanti region was found to be between 6.15 and 8.17 g/L in tartaric acid. The pH values of the wines obtained from Hönüsü and Horoz Karası grapes were higher than the results of Anlı et al. (2006) and compatible with Arpa (2015), whereas low than the results of Ünal et al. (2007). There is no direct correlation between total acidity and pH. Sometimes the total acidity is within the desired range, but the pH may be high. The presence higher total acidity can indicate growth of deteriorating microorganisms to produce organic acid, as well as the grape being too mature or the soil contains a large amount of usable potassium (Güven, 2008).

4.1.2 Changing of Brix and Volatile Acidity in Grape Musts and Red Wines

The Brix changes in wines are given in Figure 4.3. In order to monitor the changes in the amount of water soluble dry matter in wines, the initial must Brix value was determined. The sugar concentration in the medium affects the fermentation activity of yeast in alcohol production. One of the main changes observed during

fermentation is the reduction of sugar concentration in the medium. The decrease in sugar concentration during wine production was monitored by Brix measurements. Because; Brix refers to the amount of dry matter soluble in water and constitutes 95% of the total soluble matter in sugar (Boulton et al., 1996). In terms of the criterion of grape variety in wine grapes content of white varieties 19.0-23.0 Brix, red varieties 20.5-23.5 Brix (Rieger, 2006). According to the “ANOVA” the Brix results during processing steps of red wines are given in Table F.2. The Brix of the musts obtained from Hönüsü and Horoz Karası grapes were 22.78, 22.73 and 22.57 respectively. The Brix values of wines were founded as 7.58, 6.77 and 7.42 for wines-1, 2 and 3, respectively at the end of fermentation. Wine-2 had the lowest Brix value and wine-1 had highest value at the end of fermentation. The Brix values of red wines were decreased from beginning to end of fermentation. It was found that there was significant decrease in the Brix values for wines during fermentation ($p<0.05$). Fermentation activity of yeast is influenced by factors such as sugar concentration, temperature, pH, alcohol concentration, oxygen content, etc. One of the main changes observed during fermentation is the reduction of sugar concentration in the medium. After the three types of wine were kept with oak chip, the Brix was found to be 7.71, 6.44 and 6.64 for three types of wine in the order. After 45 days maturation in glass carboys, Brix values were found as 7.58, 6.52 and 6.48 for wines-1, 2 and 3, respectively. Finally, after the storage in the bottle for 3 months, the Brix values for wines in the order were 6.94, 6.23 and 6.10. The Brix values of red wines were decreased from maturation to storage in bottle. While this increase was statistically significant ($p<0.05$) between wines-1 and 3, the brix value for wine-2 was not statistically significant ($p>0.05$) with other wines. According to the “ANOVA”, values of Brix for wines-2 and 3 are significantly different ($p<0.05$) from wine-1. While wine-1 had the highest Brix value, wine-3 had the lowest value after storage. Different amount of Brix may be due to microbiological reasons. It may be thought that yeast may result from the yield. The main sugars found in grapes are glucose and fructose, which are expressed as the amount of water-soluble dry matter (Brix). The amount of dry matter depends on the condition of the grapes to grow, the type of wine, age of the wine (oxidation and precipitation of color matter, separation of potassium bitartrate from the environment affected by the cold and a small amount of alcohol and water loss by evaporation) (Canbas, 1992). The total amount of dry matter depends on the type of grape cultivation. And also, Brix refers

to the total water soluble solids in the wine and it was determined by refractometer. It is seen that the grape must having the highest degree of Brix has the highest possible alcohol degree. Oxidation of color substances and precipitation of potassium bitartrate are affected by the cold temperature and evaporation of alcohol and water as a result of a small amount of dry matter in wine (Canbaş, 2003). The amount of sugar in must is important for yeast growth and metabolism as well as for the amount and taste of alcohol in the wine. Demiray (2006) reported that Brix values between 19.25 and 22.83 in the must from black grape resulted with high quality wine. The study also reported that the dry matter content of wine is between 6.7 and 7.66. Borazan (2008) reported that Brix values of Öküzgözü in grape must was 15.50 and Brix values changed between 5 and 9 after fermentation and brix values of wines before bottling were reported that between 5 and 5.5.

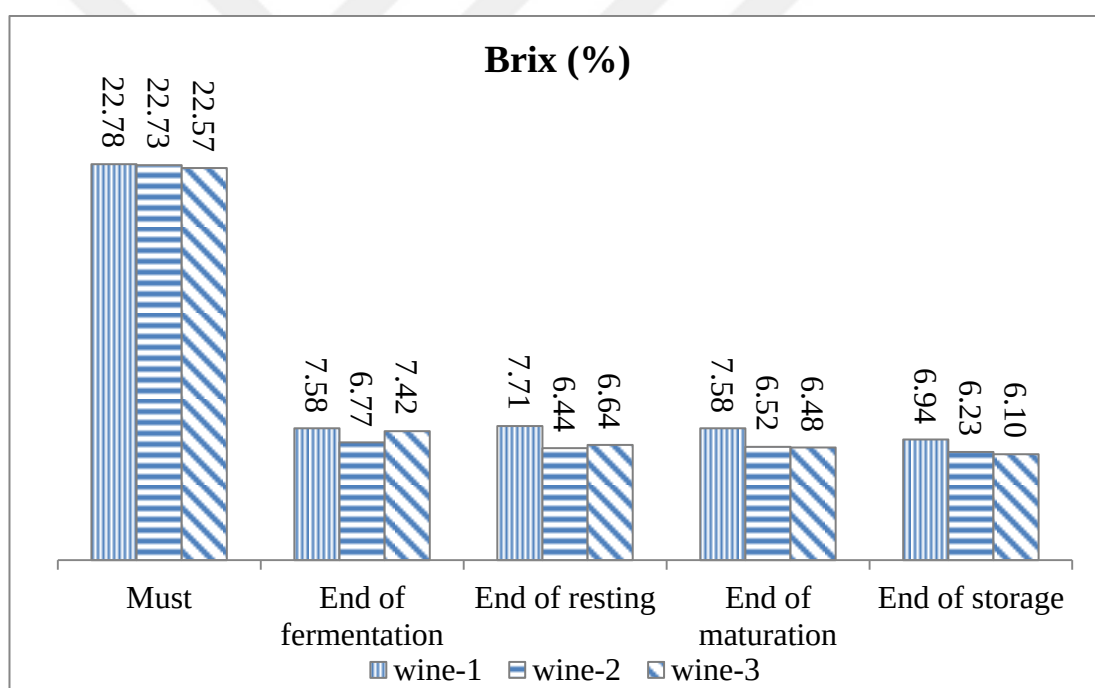


Figure 4.3 Brix changes during red wine production

Volatile acids are formed during the production of alcohol and a significant portion of them are acetic acid. The amount of volatile acid formed depends on the composition of the must (acid, sugar and nitrogenous substance), yeast strain and fermentation conditions (Ough and Amerine, 1988). The volatile acidity changes in wines are given in Figure 4.4. According to the “ANOVA” the volatile acidity results during processing steps of red wines are given in Table F.2. At the end of

fermentation the volatile acidity values of wines were founded as 0.50, 0.47 and 0.43 g/L for wines-1, 2 and 3 respectively. After the three types of wine were kept with oak chip, the volatile acidity were found to be 0.54, 0.48 and 0.44 g/L respectively. After 45 days maturation in glass carboys, volatile acidity were found as 0.56, 0.52 and 0.49 g/L for three types of wine in the order. After the storage in the bottle for 3 months, the volatile acidity values for wines in the order were 0.57, 0.54 and 0.50 g/L. The volatile acidity values of red wines were increased during wine processing. It was found that there was significant ($p<0.05$) increase in the volatile acidity of red wines from at the end of fermentation to storage. The volatile acidity values of a for three type of wines are significantly different ($p<0.05$) from each other. While wine-1 had the highest volatile acidity value, wine-3 had the lowest value at the storage in bottle. Acetic acid (CH_3COOH) is formed in very small amounts during fermentation, while acetic acid bacteria grow in the presence of air and oxidize ethyl alcohol in much higher amounts (Güven, 2008).

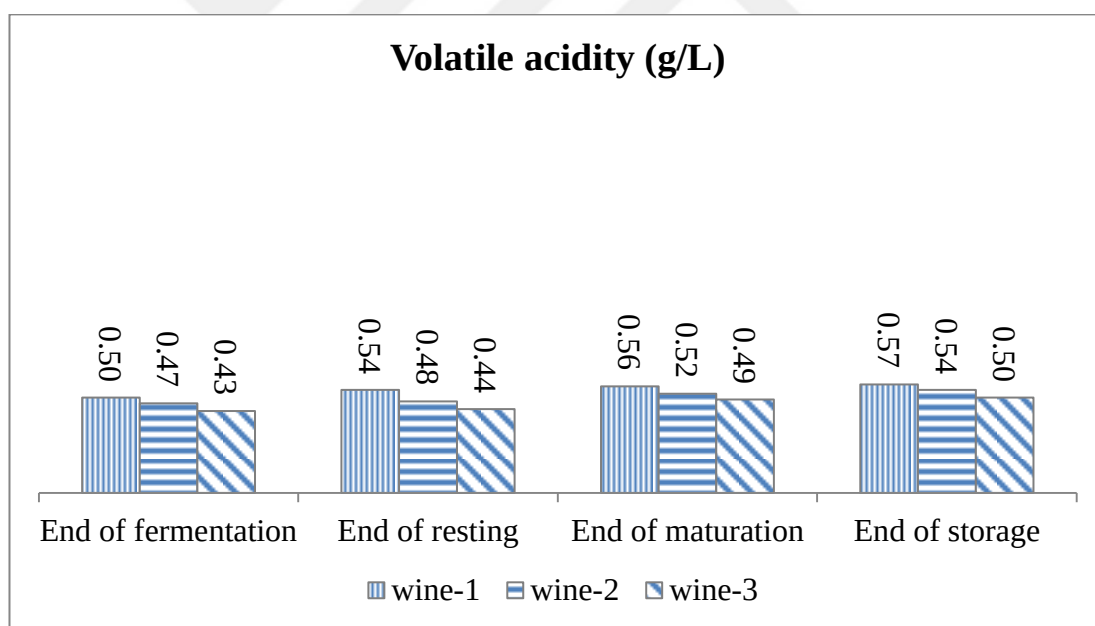


Figure 4.4 Volatile acidity changes during red wine production

According to the legislation in Turkey; in wines with an alcoholic volume of less than 14%, the amount of volatile acid should be less than 1.8 g/L in acetic acid. According to EU regulation, this amount is maximum 1.2 g/L in terms of acetic acid (Yavuzeser, 1989). The amount of volatile acid in the wine is an important factor for the quality of the wine. Because the amount of volatile acid above the values stated in the regulations and codexes indicates that the wine is exposed to microbial

activity. High volatility of volatile acid negatively affects the sensory character of the wine and gives the wine a sharp aroma. The amount of volatile acid in wine is very important and an indication of vinegar. The volatile acids are produced by acetic acid and lactic acid bacteria during the fermentation of ethyl alcohol by yeast, and acetic acid forms an important part of them. Acetic acid occurs in wine together with a small amount of formic, propionic and succinic acids (Ribereau-Gayon et al., 2000b). Rotation disease in wine is caused by microbial reduction of tartaric acid. Reduction in the amount of tartaric acid in wine reduces total acidity and raises the volatile acid. Reduction of some or all of the tartaric acid is known to cause a reduction in wine quality; little information is available about tartaric acid metabolism of lactic acid bacteria (Moreno-Arribas and Lonvaud-Funel, 2000). The amount of acetic acid may be slightly increased, especially during the maturation of wines due to the growth of unwanted microorganisms that cause disease and oxidation of ethyl alcohol. Because of the high amount of volatile acid has a negative effect on wine quality, the amount of volatile acid is used as a parameter in determining the health status of wine (Canbaşı, 2003). In general, high pH, high amount of sugar, low and high temperatures increase the amount of acetic acid depending on the type of yeast (Etiévant, 1991). The level of acetic acid produced by *S. cerevisiae* is normally 0.3-0.8 g/L in wine (Orlic et al., 2005; Nikolaou et al., 2006). Aktan and Kalkan (2000) indicated that the amount of volatile acid in wine in terms of acetic acid ranged from 0.1 and 1.6 g/L. Gutierrez et al. (2005) indicated that the amount of volatile acidity of Cabernet Sauvignon and Shiraz wines were 0.38 and 0.43 g/L respectively. Kelebek et al. (2006) determined the volatile acidity values of Bogazkere and Öküzgözü wines as 0.30-0.31 and 0.36-0.42 g/L, respectively. It is stated that if the amount of volatile acid in the wine is more than 1 g/L, the wine becomes unmarketable (Swiegers et al., 2005) and the amount of the volatile acid that we determined in this study was found to be below 1 g/L. So, the amount of volatile acid did not have a negative effect on wine quality in our study. The amount of volatile acid should be present in red wines should not be more than 1.2 g/L in terms of acetic acid according to the (TGK, 2009). Since the majority of the volatile acid in the wine is acetic acid. Acetic acid (CH_3COOH) is formed during fermentation by acetic acid bacteria with oxidizing ethyl alcohol in the presence of oxygen (Güven, 2008). According to these values, the volatile acid contents of the wines obtained from Hönüsü and Horoz Karası grapes were found appropriate. Therefore, red wines are

drinkable and there is no state to turn into vinegar. The results also indicated that wine production conditions were aseptic and growth of acetic acid bacteria, lactic acid bacteria and wild yeasts were restricted.

4.1.3 Changing of Alcohol and Density in Red Wines

The maturity and variety of grapes have effect on the amount of the alcohol in the wine (Canbaş, 2001). The alcohol, which is characteristic of wine, gives power and warmth and sweetness to wine. Alcohol degree also plays an important role on the durability of wines. Wines with low alcohol levels are more sensitive to the effects of wild yeasts and bacteria. Therefore, the amount of alcohol is important in wine. Alcohol is also important for phenolic compounds of wine. Because phenolic compounds gain more and better soluble properties with the help of alcohol that occurs during fermentation (Canbaş, 1992). Alcohol analysis was carried out by ebulliometric method. The density of the samples was determined by picnometric method. Alcohol changes in wines were given in Figure 4.5. According to the “ANOVA” the alcohol results during processing steps of red wines are given in Table F.3. The alcohol values of wines were found as 13.56, 13.46 and 13.26 for wines-1, 2 and 3, respectively, at the end of fermentation. It was found that there was significant increase in the alcohol values of red wines during fermentation ($p < 0.05$). After the three types of wine were kept with oak chip, the alcohol were found to be in the order 13.20, 13.40 and 13.09, respectively. After 45 days maturation in glass carboys, alcohol were found as in the order 12.91, 13.10 and 12.85 for three types of wine respectively. After the storage in the bottle for 3 months, the alcohol values for wines in order were 12.66, 12.59 and 12.58. The alcohol values of red wines were decreased from end of fermentation to storage in bottle. It was found that this decreases were significant for red wines ($p < 0.05$). While wine-1 had the highest alcohol value, wine-3 had the lowest value at the end of storage in bottle. The alcohol values of a for three types of wines are not significantly different ($p > 0.05$) from each other. The reason for the slight reduction of alcohol may be caused by the breakdown of sugar to alcohol, and CO_2 and cell structures, but also substances such as glycerin, acetic acid and acetaldehyde can also be produced (Albut, 1989). On the other hand, it is due to the use of a small portion of the sugar by the yeast and the use of some sugar by the other microorganisms during the start of fermentation and the evaporation of some alcohol with the CO_2 released during fermentation. The alcohol

content in wine depends on the amount of sugar and grape varieties. In various studies, it was reported that alcohol content in Öküzgözü wines ranged between 10.65 and 13.92% (Akman et al., 1971; Topaloğlu, 1984; Canbaş et al., 2001a). It is seen that the grape must having the highest degree of brix has the highest alcohol degree. It is stated in the Tebliğ (2008) that the actual amount of alcohol by volume should be at least 9% and the highest total amount of alcohol in wine should be 15%. The alcohol contents of red wines obtained from Hönüsü and Horoz Karası grapes are between these value. Kondrashov et al. (2009) examined 10 red wines, alcohol contents ranged between 12.5 and 13.5% for Cabernet Sauvignon and 11.5 and 12.5% for Merlot.

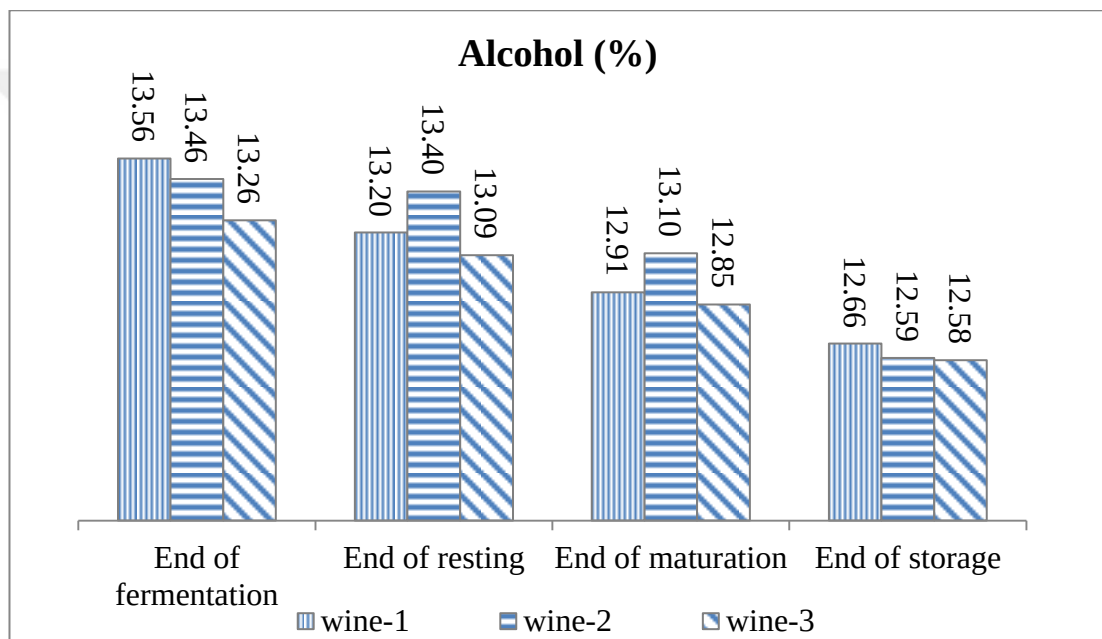


Figure 4.5 Alcohol changes during red wine production

The density of the wine is the ratio of the weight of a certain volume of wine at the same temperature and the same volume of pure water at 20 °C. The density of the wine is generally less than 1.00. The density is related to alcohol and dry matter. The weight of one liter of wine is equal to the total of water, alcohol and dry matter it contains. As the amount of dry matter increases, the density increases. Density is low in alcohol-rich wines. The weight of one liter of wine is equal to the total amount of water (alcohol + dry matter). As the amount of alcohol increases the density decreases (Canbaş, 1992). Because of yeast uses sugar in grape musts during fermentation. Density changes in wines were given in Figure 4.6. According to the “ANOVA” the density results during processing steps of red wines are given in

Table F.3. The density values of the grapes obtained from Hönüsü and Horoz Karası grape musts were 1.0812, 1.0785 and 1.0733 g/mL respectively. The density values of wines were obtained as 0.9975, 0.9969 and 0.9970 g/mL for wines-1, 2 and 3 respectively at the end of fermentation. It was found that there was significant decrease in the density values of red wines during fermentation ($p < 0.05$). After the three types of wine were kept with oak chip, the density were found to be 0.9957, 0.9945 and 0.9962 g/mL respectively. After 45 days maturation in glass carboys, density were found as 0.9952, 0.9935 and 0.9960 g/mL for three types of wine respectively. After the storage in the bottle for 3 months, the density values for wines in order were 0.9932, 0.9927 and 0.9948 g/mL. The density values of red wines were decreased from at the end of fermentation to storage in bottle. There are no significant ($p > 0.05$) differences between wines-1, 2 and 3 during wine processing. While wine-3 had the highest density value and wine-2 had the lowest value at the end storage in bottle. Density decreased during process due to the amount of alcohol increases. Kelebek (2009) indicated that the density values of wines obtained from Kalecik Karası, Öküzgözü and Boğazkere grapes were 0.9881-0.9939, 0.9907-0.9918 and 0.9913-0.9918 respectively. Doyuran (2005) founded that the densities of Karasakız and Karalahna wines were 0.9903-0.9946 and 0.9925-0.9953, respectively. The differences in the density values of wines are mostly based on grape variety, grape maturity, fermentation process, growth of yeasts and level of alcohol.

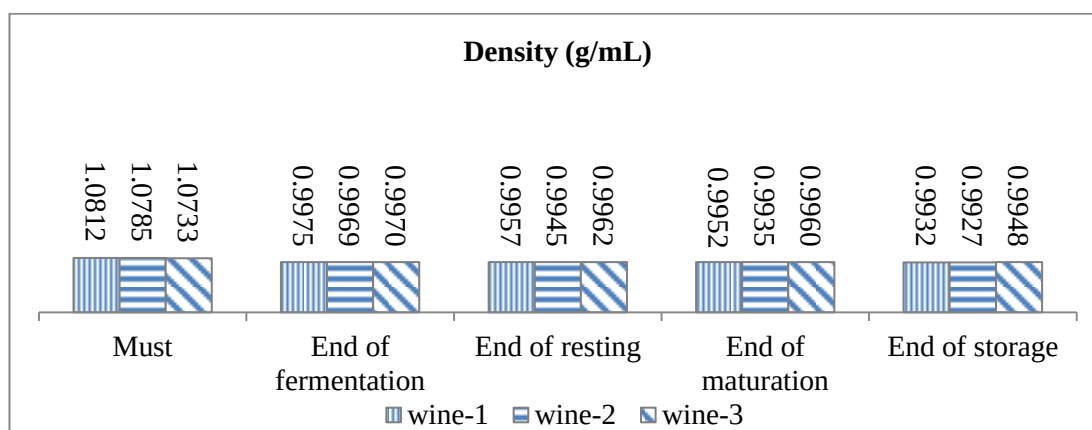


Figure 4.6 Density changes during red wine production

4.1.4 Total SO₂ and Free SO₂ in Musts and Red Wines

In wine production potassium metabisulfite (K₂S₂O₅) was used because of the risk of wild yeast spoilages that pure sulphur would prevent the wild yeasts growth during

wine production. Potassium metabisulfite helps to form wine stone and SO_2 in the presence of tartaric acid. SO_2 protects wine against enzymatic browning and unwanted microbial growth including wild yeasts. SO_2 was also used as an active agent against unwanted yeast, mold and bacterial activity. The slow mixing of air during resting mostly prevents partially non-enzymatic browning (Aktan and Kalkan, 2000; Güven, 2003; Şahin, 1995). SO_2 inhibits bacterial growth and wild yeasts in wine, and generally the amount of volatile acid is lower in sulfurized fermentations. SO_2 is also added to wines to prevent second fermentation before bottling (Reed and Peppler, 1973). The total amount of SO_2 in wines should not exceed 300 mg/L and the amount of free SO_2 should not exceed 30 mg/L (Aktan and Kalkan, 2000). The added SO_2 protects the wine. However, the amount of SO_2 present in the wine is under certain limits is essential for health. SO_2 added to the wine is important for preventing spoilage of wine. SO_2 has an important role in wine production and maturation in the prevention of wine diseases and defects (Cabaroğlu and Canbaş, 1993). SO_2 makes an antiseptic effect on microorganisms and prevents oxidation by binding oxygen (Akman, 1985). During production, crushed grapes and must can be sulfurized. Sulfurized crushed grapes must can protect wines against unwanted microorganisms and oxidation. It also has a positive effect on the maturation and sensory properties of the wine. Because of SO_2 facilitates the dissolution of phenol compounds by affecting cells in tissues (Ribéreau Gayon and Glories, 1986; Canbaş, 1992; Glories, 1999). The amount of SO_2 varies according to the raw material, the condition of the products, and the application method. SO_2 is present in wine in two forms, bound and free. The combination of bound and free SO_2 forms the total SO_2 (Aksoy, 2009).

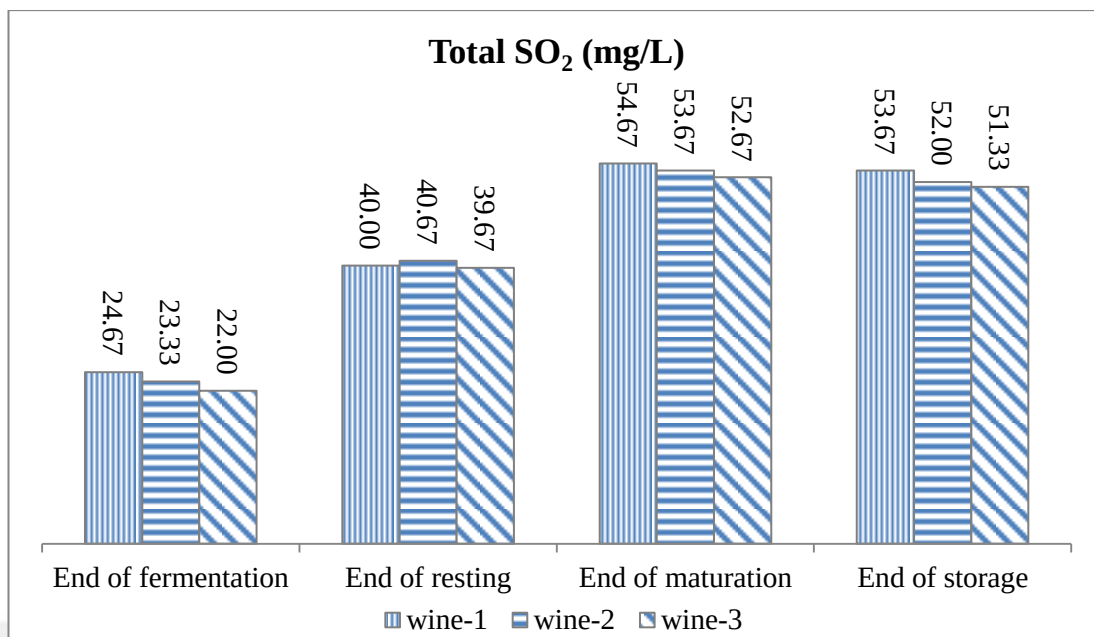


Figure 4.7 Total SO₂ (mg/L) changes during red wine production

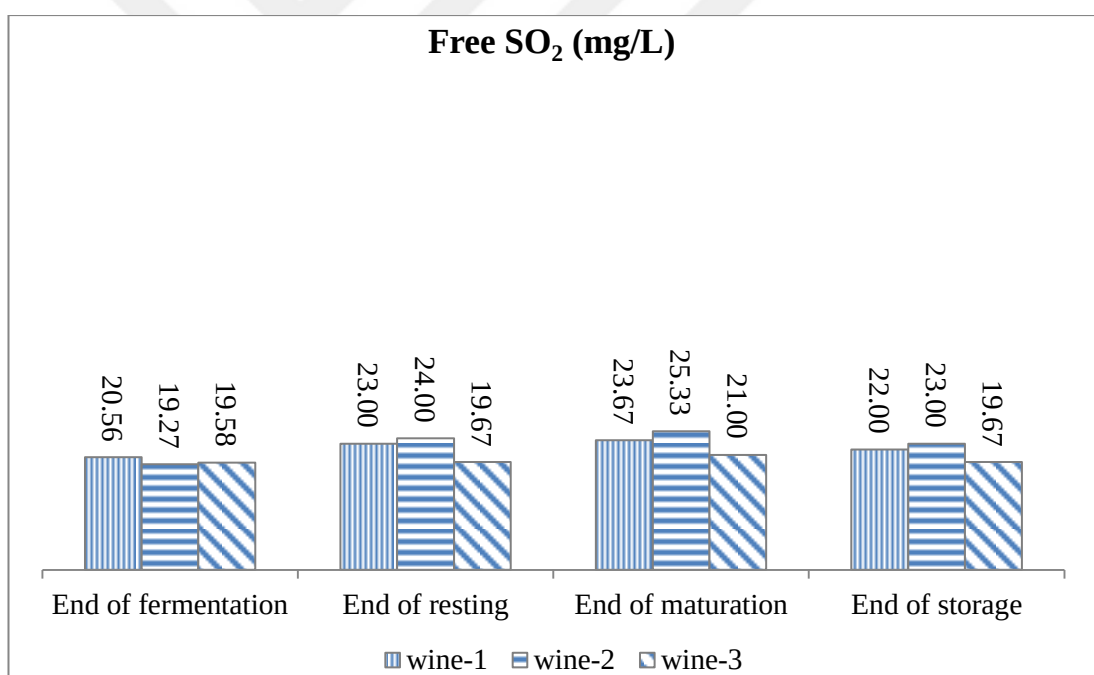


Figure 4.8 Free SO₂ (mg/L) changes during red wine production

Total and free SO₂ changes in wines were given in Figures 4.7 and 4.8. According to the “ANOVA” the total SO₂ and free SO₂ results during processing steps of red wines are given in Table F.4. The total SO₂ content of wines were obtained as 24.67, 23.33 and 22.00 mg/L for wines-1, 2 and 3, respectively, at the end of fermentation. After the three types of wine kept with oak chip, the total SO₂ content were found to be 40.00, 40.67 and 39.67 mg/L respectively. After 45 days maturation in glass

carboys, the total SO₂ content were found as 54.67, 53.67 and 52.67 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the total SO₂ content for wines in the order were 53.67, 52.00 and 51.33 mg/L. The free SO₂ content of wines were obtained as 20.56, 19.27 and 19.58 mg/L for wines-1, 2 and 3, respectively, at the end of fermentation. After the three types of wine were kept with oak chip, the free SO₂ content were found to be 23.00, 24.00 and 19.67 mg/L respectively. After 45 days maturation in glass carboys, the free SO₂ content were found as 23.67, 25.33 and 21.00 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the the free SO₂ content for wines in order were 22.00, 23.00 and 19.67 mg/L. According to the “ANOVA”; total SO₂ results of wines-2 and 3 are significantly different ($p<0.05$) from wine-1, but values of free SO₂ for wines-1 and 2 are significantly different ($p<0.05$) from wine-3 at the end of storage. Between at the end of maturation and storage, the reason for total and free SO₂ decreasing is that SO₂ added to must or wine does not remain as it is. While some are in free form, some are bound to some substances such as aldehyde, sugar and protein in must or wine (Ribereau-Gayon et al., 2000). These values are positive in terms of health, but not sufficient in terms of long preservation of wine and protection from diseases. Anlı (2004) investigated the total SO₂ content of the Kalecik Karası wine was determined to be between 78-80 mg/L. Amendment to TFC regulation on additives indicated that the maximum limit for total SO₂ is 150 mg/L for red wines with a sugar content of less than 5 g/L and 200 mg/L for sugar content of more than 5 g/L (TGK, 2018). Anli (2004) reported that can be in the red wine contains free SO₂ at levels of 20-30 mg/L. In this study, the values of total and free SO₂ contents of the wines are in agreement with the TFC.

4.1.5 Results of Color Analysis of Wines

The CIE (Commission Internationale de l’Eclairage) L* (luminance) value is defined as the reflection or absorption of light from a surface, regardless of wavelength. When light is completely reflected from a surface, the object appears as white ($L=100$), when it is fully absorbed, the object is black ($L=0$) and partially reflected or absorbed, it appears as gray ($0<L<100$) (ETS, 2003). L* color changes in wines were given in Figure 4.9. According to the “ANOVA”, the L* values during processing steps of red wines are given in Table F.5. The L* values of the musts-1, 2 and 3 were 15.25, 14.99 and 13.82 respectively. The L* values of wines were 7.35, 5.62 and

5.31 for wines-1, 2 and 3, respectively, at the end of fermentation. It was found that there was significant decrease in the L^* values of red wines during fermentation ($p<0.05$). After the three types of wine were kept with oak chip, the L^* were found to be 2.74, 3.17 and 2.92 respectively. After 45 days maturation in glass carboys, L^* were found as 2.31, 2.10 and 2.80 for three types of wine respectively. After the storage in the bottle for 3 months, the L^* values for wines in order were 1.26, 0.96 and 0.94. The L^* values of red wines were decreased from end of fermentation to storage in bottle. This decrease is statistically significant ($p<0.05$). While wine-1 had the highest L^* value, wine-3 had the lowest value at the end of storage in bottle. According to the “ANOVA”; total SO_2 results of wines-2 and 3 are significantly different ($p<0.05$) from wine-1 among three types of wines after storage.

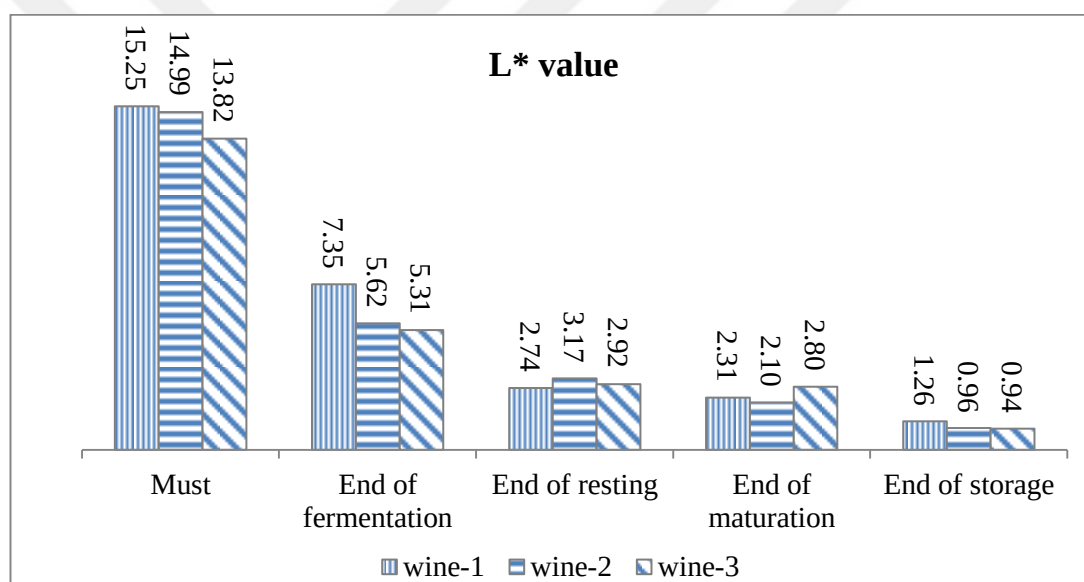


Figure 4.9 L^* color analysis results during red wine production

L^* value (luminance) defines how light or dark color is occurred on wines. The color of anthocyanins, which is the main compound that gives color to red wine, varies according to the pH and SO_2 content of the environment (Canbaş, 2006; Glories, 1999; Ribéreau-Gayon et al., 2000). Anthocyanin compounds lose color when reacted with free SO_2 . However, this reaction is reversible (Bakker et al., 1998; Ribéreau-Gayon et al., 2000). In general, the L^* values of musts higher than wines, because of they lighter color than wine color. Morris et al. (1980) indicated that the L^* value found to be 11.7 as the average of 5 years in Concord grape (red grape) juice. Wine-2 and wine-3 had lower L^* values than wine-1 at the end of storage, thus

they were observed darker color than wine-1. Darıcı and Cabaroğlu (2017) indicated that L^* values of Kalecik Karası wines in Ankara region ranged between 7.2-20.6 and in Denizli region ranged from 4.1 to 11.2. Şener (2008) reported that CIE L^* values change between 21.00 and 32.50 in wines produced from Cabernet sauvignon grapes. The L^* values of the wines obtained from Hönüsü and Horoz Karası grapes were found to be lower than those determined by Darıcı and Cabaroğlu (2017) and Şener (2008). Therefore, wine colors were darker in this study than other studies. Mazza (1995), indicated that increase the amount of grape's color such as factors light, temperature and weakness of the plant.

The a^* color changes in wines are given in Figure 4.10. According to the “ANOVA”, the a^* and b^* values during processing steps of red wines are given in Table F.6. The a^* value indicates positive (+a) color with red and negative (-a) color is green. The b^* value indicates yellow color when positive sign (+b) and blue color when negative sign (-b) (Gil-Munoz et al., 1998). The a^* values of the musts-1, 2 and 3 were 8.69, 9.22 and 9.73 respectively. Values of “ a^* ” for three types of musts are significantly different ($p < 0.05$) from each other. The a^* values of wines were obtained as 10.96, 11.93 and 2.50 for wines-1, 2 and 3, respectively, at the end of fermentation. After the three types of wine were kept with oak chip, the a^* were found to be 3.46, 5.79 and 0.91 respectively. After 45 days maturation in glass carboys, a^* were found as 0.88, 1.34 and 0.82 for three types of wine respectively. After the storage in the bottle for 3 months, the a^* values for wines in the order were 0.49, 0.70 and 0.64. The a^* values of red wines were decreased from at the end of fermentation to storage in bottle. There were significant decrease ($p < 0.05$) for wine-1, 2 and 3 from end of fermentation to end of storage, but except wine-1 also this decrease not significant between resting and end of storage ($p > 0.05$). The reasons for the significant reduction after from resting are explained by the adsorption of anthocyanins on oak chips or tannins. While wine-2 had the highest a^* value, wine-1 had the lowest value at the end of storage in bottle. The values of “ a^* ” for wines-1 and 2 are significantly different ($p < 0.05$) from wine-3. The a^* values of three types of must are positive marked, so it refers to red color. Morris et al. (1980) indicated that the a^* value found to be 9.8 as the average of 5 years in Concord grape juice. CIE a^* values in all of the wines are positive sign indicating red color. Şener (2008) reported that CIE a^* values change between -0.23 and -0.04 in wines produced from Cabernet sauvignon grapes.

Darıcı and Cabaroğlu (2017) indicated that a^* values of Kalecik Karası wines in Ankara region ranged between 25.41 and 35.55. The a^* values of Kalecik Karası wines in Denizli region ranged from 21.03 to 27.12. The a^* values of the red wines obtained from Hönüsü and Horoz Karası grapes were found to be quite lower than those determined by Darıcı and Cabaroğlu (2017). However, the a^* values of the red wines obtained from Hönüsü and Horoz Karası grapes were found to be higher than determined by Şener (2008). The wines which has higher a^* value in this study, show more a red character than the wines in the study of Şener (2008). But also, the wines which has lower a^* value in this study, show less a red character than the wines in the study of Darıcı and Cabaroğlu (2017).

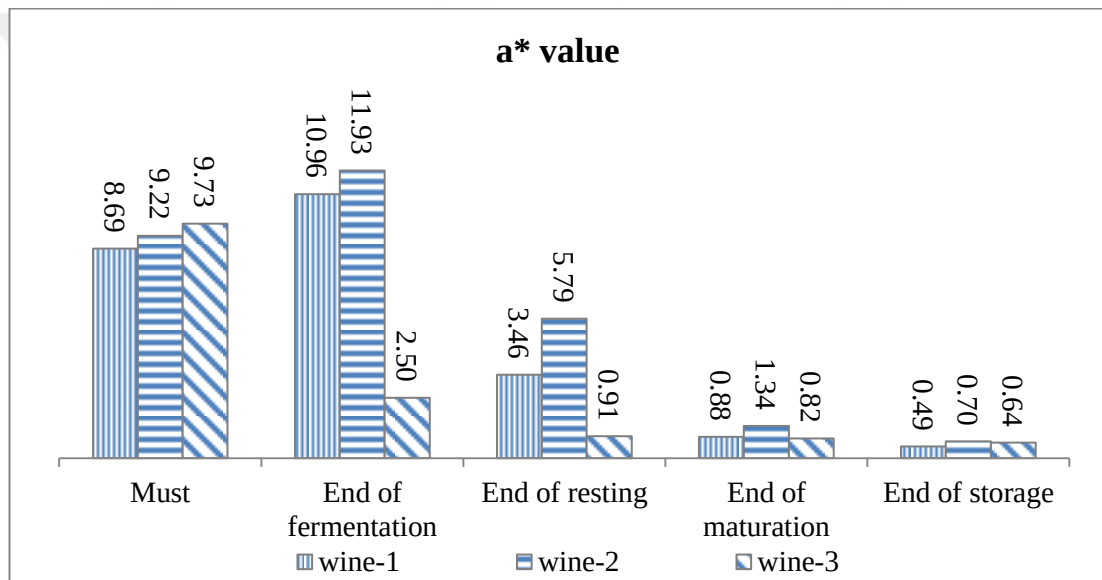


Figure 4.10 a^* color analysis results during red wine production

The b^* color changes in wines were given in Figure 4.11. The b^* values of the musts-1, 2 and 3 were 9.52, 7.93 and 6.39 respectively. The values of “ b^* ” for three types of musts were significantly different ($p < 0.05$) from each other. The b^* values of three types of must are positive marked, so it refers to yellow color. The b^* values of wines were obtained as 2.05, 0.66 and 0.45 for wines-1, 2 and 3 respectively at the end of fermentation. It was found that there was significant decrease in the b^* values of red wines during fermentation ($p < 0.05$). After the three types of wine were kept with oak chip, the b^* values were found to be 1.89, 0.48 and -0.63 respectively. After 45 days maturation in glass carboys, b^* were found as 0.39, -0.32 and -1.24 for three types of wine respectively. After the storage in the bottle for 3 months, the b^* values

for wines in order were -0.18, -0.36 and -0.31. There are significant decreases ($p<0.05$) for wines-1, 2 and 3 according to mean b^* values from end of fermentation to end of storage. The lower the b^* value, shows more the blue pigment in the wine. While wine-1 had the highest b^* value, wine-2 had the lowest value at the end of storage in bottle. The values of “ b^* ” for wines-2 and 3 are significantly different ($p<0.05$) from wine-1 at the end of storage. The b^* values in the study, wines are negative sign indicating blue color. Şener (2008) reported that b^* values change between 0.11 and 0.24 in wines produced from Cabernet sauvignon grapes. Darıcı and Cabaroğlu (2017) indicated that b^* values of Kalecik Karası wines in Ankara region between 9.03 and 13.69. The b^* values of Kalecik Karası wines in Denizli region ranged from 6.23 to 18.20. The b^* values of the red wines obtained from Hönüsü and Horoz Karası grapes were found to be quite lower than those determined by Darıcı and Cabaroğlu (2017) and also by Şener (2008). The wines which has lower b^* value in this study, show more a blue character than the wines in the study of Şener (2008) and Darıcı and Cabaroğlu (2017).

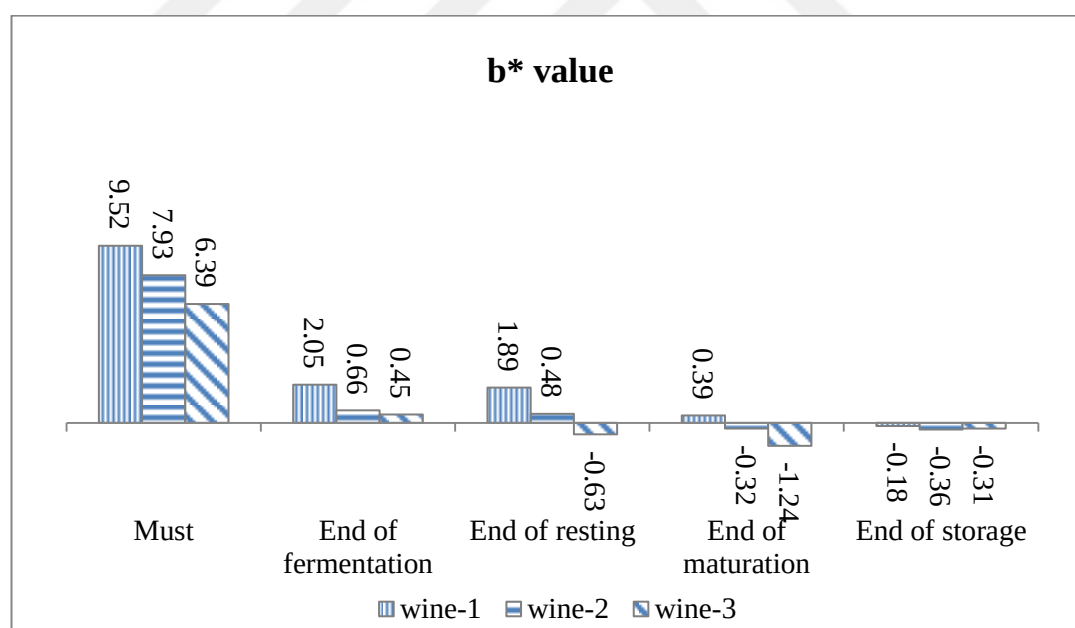


Figure 4.11 b^* color analysis results during red wine production

Anthocyanins can be converted to different forms depending on various factors (pH, temperature, metal ions, enzymes, oxygen, ascorbic acid, sugars and SO_2). There are two important factors that can affect the color of the wine; the pH of the medium and the effect of SO_2 . The primary factor affecting the conversion of anthocyanins to

different forms is pH. When wines $\text{pH} < 2.0$, anthocyanins are in the form of flavyllium cations. $4.5 < \text{pH} < 6.0$ in the form of quinoidal base and $2.0 < \text{pH} < 7.0$ in the form of carbinol base (Cheynier et al., 2006). Since the pH of the wines in this study is around 3.5 at the end of storage, they are in carbinol base form. When SO_2 is added to the wine, it is dissolved as bisulfite and flavyllium cations are easily discolored by the formation of bisulfite (HSO_3^-). Each form of anthocyanins has different color properties. Flavilium cation is red, quinoidal base is blue, carbinol and chalcone are colorless (Harbertson and Spayd, 2006). Flavilium cations of anthocyanins are the main pigments that give color to red wine (Mazza and Miniati, 1993; Gutierrez et al., 2005). Glories (1984) in a study of young red wine ($\text{pH}=3.5$); flavyllium cation was found to be 12.2%, colorless carbinol 45.2%, colorless chalcone 27.6% and blue quinoidal 15.0%. In this case, the predominant form of anthocyanin in young red wines appears to be the carbinol base form (colorless). Red wines in this study belong to young red wine group due to pH and it is understood that anthocyanin form is carbinol base form (colorless). The transformation of anthocyanins into different forms and the joining mechanisms called copigmentation play a role in the formation of red wine color. Substances forming complexes with anthocyanins forming more stable and denser colored compounds are called copigments. Copigments, which are usually colorless on their own, combine with anthocyanins to become colored. Compounds that act as copigments in wine are flavonoids, polyphenols, amino acids, organic acids and anthocyanins themselves (Mazza and Miniati, 1993). The colors of anthocyanin-derived pigments range from orange to blue. The pigments from the association of anthocyanins with flavanols are red while the pigments from the association with tannins are orange. In addition to these compounds, anthocyanins may form colorless or different pigments due to their combination with other compounds found in wine (Cheynier et al., 2006). Tetik and Selli (2018) found that L^* value was 3.49, a^* value was 20.21 and b^* value was 5.93 in Öküzgözü red wines. Larrauri et al. (1999) indicated that L^* value in Spanish red wine (Joven, Crianza, Reserva) between 3.7-9.9. Lago-Vanzela et al. (2013) found that the red wines obtained from hybrid BRS Violeta grape varieties ranged between 1.10-5.20, a^* values between 8.64-30.94, and b^* values between 1.91-9.01. Uysal (2010), indicated that L^* , a^* and b^* values in red wines were 2.02, 13.28 and 2.64 respectively. The L^* , a^* and b^* values of the red wines obtained from Hönüsü and Horoz Karası grapes were found to be lower than those values in the literature.

Chroma (C*) changes in wines are given in Figure 4.12. According to the “ANOVA”, the C* and H* values during processing steps of red wines are given in Table F.7. The C* values of the musts obtained from Hönüsü and Horoz Karası grapes were 12.89, 12.55 and 11.22 respectively. The values of C* for musts-1 and 2 were significantly different ($p<0.05$) from must-3. The C* values of color defines intensity or brightness. The C* values of wines were obtained as 11.08, 11.53 and 12.28 for wines-1, 2 and 3, respectively at the end of fermentation. It was found that there was significant decrease in the C* values of red wines during fermentation ($p<0.05$). It is emphasized that color intensities tend to decrease after reaching the highest level in the first days of the fermentation period (Peynaud, 1981). After the three types of wine were kept with oak chip, the C* values for wines in the order were found to be 3.82, 5.81 and 6.05. After 45 days maturation in glass carboys, C* values were found as 0.89, 1.20 and 1.31 for three types of wine in the order. After the storage in the bottle for 3 months, the C* values for wines in order were 0.42, 0.80 and 0.93. The lower the C* value of stored wine shows the slightly duller to must because of added SO₂ to wine. Organic acids facilitates the dissolution of color substances and makes the color more vivid during fermentation (Canbas, 2003). While wine-3 had the highest C* value, wine-1 had the lowest value at the end of storage in bottle. There is significant differences in C* mean values among three types of wines ($p<0.05$) at the end of storage. In result of wine-3 (0.93) was more brightly colored than wine-1 (0.42) and wine-2 (0.80).

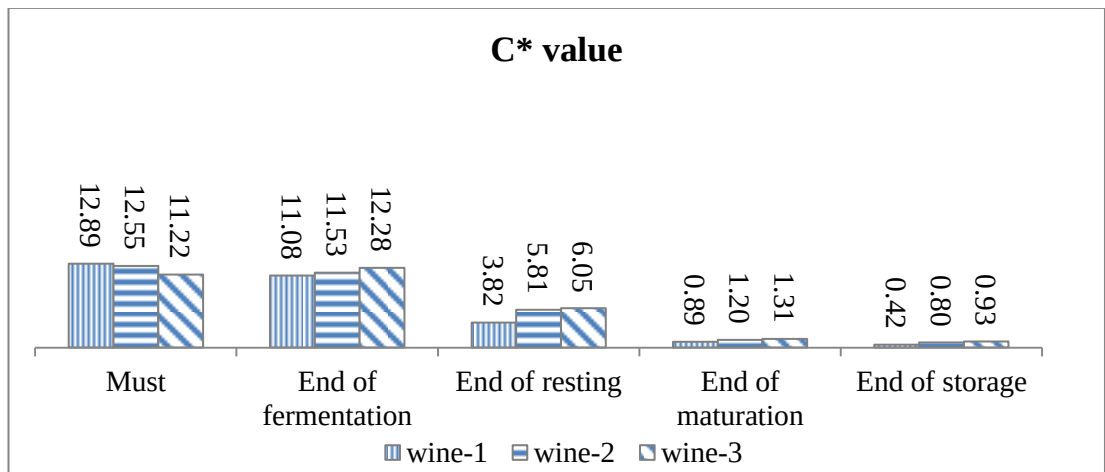


Figure 4.12 C* color analysis results during red wine production

Chroma value is a color parameter that measures the saturation of color. As the chroma value decreases, the saturation (intensity) of the color decreases and the color becomes more dull (pale). High chroma value makes the color look more vivid (ETS, 2003). Color density, as well as the amount of anthocyanin, pH, tannins and tannins are related to reactions between anthocyanins (Deryaoglu et al., 1997). Since anthocyanins are substances that give color to the wine, the decrease in color intensity and the decrease in the amount of anthocyanins are linear. After a certain maceration period, anthocyanins react with proanthocyanins to convert to copolymerization products, or together with the adsorption of anthocyanins by yeasts, together they form sediment and cause discoloration in wine (Vasserot et al., 1997). In addition, a decrease in total anthocyanin content occurs during the aging of wine (Fulcrand et al., 1998; Mateus et al., 2003). Chroma values were ranged from 0.3 to 1.8 in wines (Ribereau-Gayon et al., 2000). Darıcı and Cabaroğlu (2017) indicated that the C* values were found to be 33.48 on Kalecik Karası wines in Ankara region and 26.63 on Denizli region wines. Şener (2008) reported that C* values change between 14.86 and 32.93 in wines produced from Cabernet sauvignon grapes. The C* values of the red wines obtained from Hönüsü and Horoz Karası grapes were found to be lower than those values in the literature. In this study, this difference the C* value of wine shows the slightly duller than values in the those literature. Chroma values are lower than the results of other studies may be due to method difference. While wine is included gelatin or etc. for clarification process in other studies, no additives were added for clarification in our study. Filtration with cheese cloth was applied only to remove wine from the residues in this study.

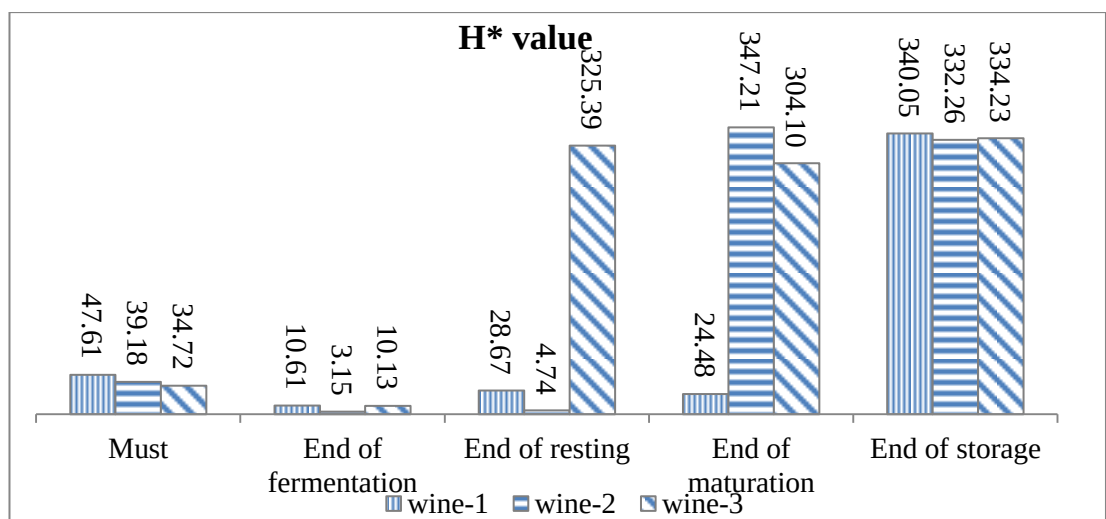


Figure 4.13 H* color analysis results during red wine production

The H^* values of color defines initial color observed e.g. violet-red, red or red-orange. Since the calculated Hue (H^*) values are negatively marked, they were evaluated by summing with 360 degrees (Bakker et al., 1999). The H^* value is a color parameter that measures the dominant wavelength (color). The H^* value is a parameter that expresses colors such as red, yellow, green, and blue. In standard calculations, 0 degrees Hue angle means red color, 90 degrees yellow color, 180 degrees green color and 270 degrees blue color (ETS, 2003). Hue (H^*) changes in wines were given in Figure 4.13. The H^* values of the musts obtained from Hönüsü and Horoz Karası grape were 47.61, 39.18 and 34.72 respectively. The H^* values of wines were obtained as 10.61, 3.15 and 10.13 for wines-1, 2 and 3 respectively at the end of fermentation. It was found that there was significant decrease in the H^* values of red wines during fermentation ($p < 0.05$). This decrease reason was close to more red hue of wine colors. After the three types of wine were kept with oak chip, the H^* values were found to be 28.67, 4.74 and 325.39 respectively. After 45 days maturation in glass carboys, H^* values were found as 24.48, 347.21 and 304.10 for three types of wine respectively. After the storage in the bottle for 3 months, the H^* values for wines in order were 340.05, 332.26 and 334.23. While wine-1 had the highest H^* value, wine-2 had the lowest value closely with wine-3 at the end of storage in bottle. The values of “ H^* ” for three types of wines are significantly different ($p > 0.05$) from each other at the end of storage. In result of wines-1, 2 and 3 had a purple-red hue because of values more than 270° by its hue degree. Darıcı and Cabaroğlu (2017) found H^* values were 21.17 for Kalecik Karası wine in Ankara and 21.91 for Denizli region wine. Şener (2008) reported that H^* values change between 287.74 and 314.38 in wines produced from Cabernet sauvignon grapes. The H^* values of the red wines obtained from Hönüsü and Horoz Karası grapes were found to be higher than those values in the literature. The wines which has higher H^* value in this study, show more a red-purple hue than the wines in the study of Şener (2008) and Darıcı and Cabaroğlu (2017). This difference can be caused by the method of wine production. The yeast and SO_2 are important factors affecting color.

4.1.6 Microbiology Analysis for Musts and Red Wines

Yeasts were determined in the must and wines during fermentation, resting with oak chip, maturation in the glass carboy and storage in bottle. The changes in must and wines are given for yeast count in Figure 4.14. The results were found in log cfu/ml.

According to the “ANOVA”, the yeast amounts during processing steps of red wines are given in Table F.8. The number of yeasts were determined between 5.38 and 6.40 log cfu/ml in musts after adding commercial yeast. According to the “ANOVA”, amount of yeasts results of musts-1, and 3 were significantly different ($p<0.05$) from wine-2. Yeast count of wine-1 was increased from 6.61 to 7.65 log cfu/mL, wine-2 was increased from 6.56 to 7.52 log cfu/mL, and wine-3 was increased from 6.53 to 7.55 log cfu/mL during 3 days of fermentation. It was found that there was significant increase in the yeast amount of red wines during 3 days of fermentation ($p<0.05$). The highest yeast amount for wines-1, 2 and 3 were obtained at the third day of fermentation. However, a significant decrease ($p<0.05$) was observed from the 3rd day of fermentation to the last day of fermentation. After the wines were kept with oak chip, the yeast count was found to be 5.61, 5.81 and 5.77 log cfu/mL for three types of wine respectively. After 45 days maturation in glass carboys, yeast count was 4.78, 4.38 and 4.29 log cfu/mL for them respectively. Finally, after the storage in the bottle 2.38, 2.34 and 2.30 log cfu/mL for three types of wine. There are significant decreases ($p<0.05$) for wines from end of resting to end of storage. After each production step, a decrease in the total yeast amount is observed. Fermentation of wine occurs as a result of the activity of yeast.

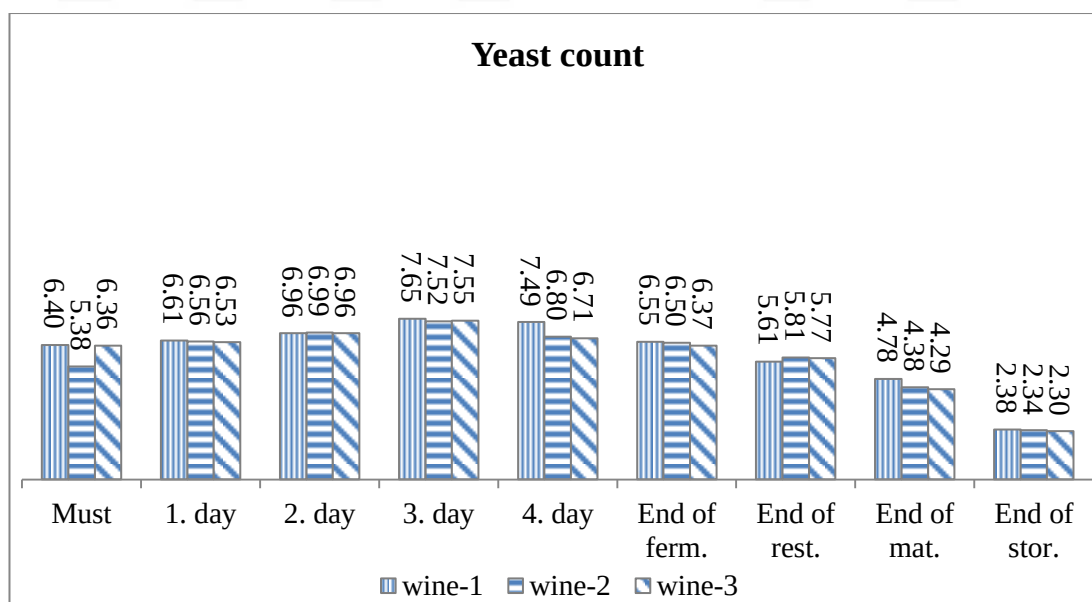


Figure 4.14 Yeast count changes in the must and wines (log cfu/mL)

As shown in Figure 4.14, at the end of the fermentation stage, the highest numbers of yeast were observed in the wine-1. The highest yeast content was also determined in wine-1 at the end of the bottling stage for 90 days. The main function of yeasts is to

realize alcohol fermentation in wine. Wild yeasts are found in many natural sources (such as air, empty bottles, corks, bottling equipments).

The wild yeasts can cause that have a detrimental effect in wine. When these yeasts find suitable conditions in which they can reproduce in wine (such as residual sugar), they cause deteriorating of wine (Gültekin et al., 2006). Wild species that are naturally found in wine but causing deterioration are *Saccharomyces bayanus*, *S.ludwigii*, *Schzosaccharomyces pombe*, *Pichia membranalefuciens*, *Torulopsis bacilleris*, *Rhodotorula rubra*, *Kloeckera apiculata* and *Hansenula anomalies*. As a result of the activities of these yeasts, problems such as scratching, sweet-sour, odor resembling sauerkraut, turbidity and sedimentation occur due to the changes in the ingredients in the wine. Nutrient content of wines containing less than 2 g of glucose and fructose per liter are usually sufficient for the growth of wild yeasts. Wild yeasts sometimes grow up to 16.5% ethanol. The number of yeasts were increased during 3 days fermentation and decreased during later processing steps. Yeast is necessary for the formation of alcohol. The results showed that the higher the yeast growth, the higher the alcohol production. When the number of these yeasts exceeds 5 log cfu/mL in wine, a noticeable turbidity occurs in the wine (Ünlütürk and Turantaş, 1999). The number of yeasts at the end of bottling stage of wines remained below this limit. Therefore, the number of yeast at the end of the bottle did not affect the color, smell, taste and appearance of the wine. *S. cerevisiae*, due to its ability to grow in grape juice, which is characterized by high sugar and low nitrogen content, it is accepted as the main yeast species responsible for alcohol fermentation. *S. cerevisiae* produce high amounts of ethyl alcohol, inhibit the growth of other yeasts and becomes dominant during must fermentation (Cocolin et al., 2004). The growth of yeast species other than *Saccharomyces* during wine fermentation is limited to the first three days of fermentation and then their number decreases rapidly (Bağder and Özçelik, 2008). Ripe and intact grapes are generally considered to have a microbial population of 10^3 - 10^5 cfu/g and this population is composed of various lactic and acetic acid bacteria, especially yeasts. In alcohol fermentation, the growth of other microorganisms is suppressed by the growth of yeast; Because yeast rapidly grow at low pH values (3.0-3.5) of must and producing ethyl alcohol, and molds and bacteria are inhibit (Fleet, 1999). Freshly squeezed grape musts contain yeast at the level of 10^3 - 10^4 cells/mL. These yeasts consist of a mixture of species the grapes and from

the surface of the winery equipment (presses, pumps, tanks, etc.) that come into contact with the grape juice. When alcohol fermentation is completed, the yeast number increases by approximately 100,000-1,000,000 times, reaching 10^8 - 10^9 cells/mL (Fleet, 1990). On the 3rd day of fermentation, the number of yeasts reached the highest value (about 10^8 cfu/mL) in this study. *Saccharomyces* yeasts are resistant to low pH values and can easily develop in musts in the pH range of 2.8-4.2 (Bisson, 1999). Film yeasts can develop on must and wine surfaces in contact with air. They oxidize alcohol and organic acids to form a thick membrane called wine flower on the surface. Film yeast must be restricted by mixing periodically and wine is not in contact with air to prevent wild yeast growth and oxidation when wild yeasts problems (Ünlütürk and Turantaş, 1999).

At the end of the alcoholic fermentation, growth begins to cause defects on wine and cell numbers increase to about 10^7 cells/mL. Wild yeast numbers at harvest range from 10^4 to 10^6 cfu/mL. *Rhodotorula*, *Cryptococcus*, *Candida*, *Kloeckera* and *Metschnikowia* are the most dominant species of wild yeasts (Fleet, 2003). Some fermentation by-products that occur in the fermentation process affect the wine quality depending on the yeast. These products, which are closely related to the yeast numbers, are known to be glycerol, high alcohol, aldehyde, ester and organic acids. These substances are one of the most important quality factors in wine composition (Özçelik and Denli, 1999; Quilter et al., 2003). Uysal (2010) determined the number of *S.cerevisiae* in red wine about 7.21 log cfu/mL. Azabağaoğlu (2006) indicated that wild yeast numbers of red wine determined between 10^2 and 1.5×10^3 cfu/mL.

4.1.7 Phenolic Compounds in Musts and Wines

Phenolic compounds were determined in three types of must and wine during fermentation, resting with oak chips for 7 days, maturation in glass carboy for 45 days and storage in glass bottles for 90 days. The most important factors affecting the content of phenolic compounds in wines are the concentration of these compounds in grapes, applied winemaking technology, contact time of skin and seed, ethyl alcohol concentration, fermentation temperature and transformations during wine ripening (Uylaşer and İnce, 2008). The region where grapes are grown, soil properties and agricultural activities are also effective on the color substances and phenol

composition in grapes (Ünsal, 2007). In this study, two different grape varieties (Hönüsü and Horoz Karası) obtained from Gaziantep province were used in wine production. The HPLC analysis results were carried out for phenolics at 280 and 320 nm wavelength. The phenolic components found in wine are mainly divided into two groups as non-flavonoids and flavonoids. In this study, the following phenolic compounds were detected: flavonoids such as flavan-3-ol group ((+)-catechin and procyanidin B₂), flavonols (quercetin, myricetin and rutin), and non-flavonoids such as hydroxycinnamic acids (p-coumaric acid and chlorogenic acid), hydroxy-benzoic acid (gallic acid), stilbene (resveratrol), and phenolic alcohol (tyrosol) were analyzed quantitatively in must and red wines.

4.1.7.1 Flavonoid Content of Must and Wine Varieties

According to the “ANOVA”, the flavonoid contents during processing steps of red wines are given in Table F.9. The myricetin changes in must and wines are given in Figure 4.15. The myricetin content of the musts-1, 2 and 3 were 0.54, 0.67 and 1.11 mg/L respectively. Mean values of “myricetin content” for three types of musts are significantly different ($p < 0.05$) from each other. At the end of fermentation, myricetin content of wines were founded as 4.80, 5.43 and 5.62 mg/L for wines-1, 2 and 3 respectively. It was found that there was significant increase in the myricetin content of red wines during fermentation ($p < 0.05$). After the three types of wine were kept with oak chip, the myricetin contents were found to be 4.56, 4.90 and 4.44 mg/L respectively. After 45 days maturation in glass carboys, myricetin contents were found as 1.38, 1.42 and 1.73 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the myricetin contents for wines in order were 1.12, 1.26 and 1.68 mg/L. The myricetin contents of red wines were decreased from end of fermentation to storage in bottle. This decrease are statistically significant ($p < 0.05$). While wine-3 had the highest myricetin content, wine-1 had the lowest content at the storage in bottle. The concentration of phenolic compounds in grapes depends upon the variety of grape and is affected by viticulture and environmental factors (Broussaud et al., 1999; Cheynier et al., 1998; Ojeda et al., 2002). Myricetin and quercetin are essential flavonol components in red wine. Flavonol content depends on the intensity of sunlight, the thickness of the grape skin and the technological process (McDonald et al., 1998). The amounts of myricetin and

quercetin increase during fermentation. Later, these compound continued to decrease. Gambuti et al. (2007) stated that long maceration periods caused an increase in the total phenol components of wine, and decreased amounts of anthocyanin, (+)-catechin, (-)-epicatechin, trans-resveratrol and quercetin at maturation in oak barrels, but an increase in total phenolic compounds. They also found that pectolytic enzyme and SO₂ application increased polyphenolic compounds. Vuorinen et al. (2000) have determined that the amounts of myricetin and quercetin in wines changed between 2.5-22.0 mg/L and 1.6-17.7 mg/L respectively. The amount of flavonol in wines varies depending on the type of grape, the region in which it is grown and the technological processes applied, but it is around 100 mg/L in red wines and 1-3 mg/L in white wines (Ribéreau-Gayon et al., 2000b). Orban et al. (2006) determined that the amount of myricetin in wines obtained from grapes grown in Hungary varies between 1.58-11.36 mg/L and quercetin amount between 1.24-12.93 mg/L. Flavonols (glycerides such as quercetin, myricetin and quercetin-3-rutinoside) that contribute to bitterness are mainly found in the skin and grape seeds. Flavonols are the most abundant phenolic compounds in grape peel, while grape seed is rich in flavan-3-ol (catechin) (Cheynier and Rigaud, 1986; Souquet et al., 2000). Flavonols are found in the shell of grapes. Hönüsü grape is lower with flavonols than Horoz Karası grape, and we can understand that the bark of Hönüsü grape is thinner and weaker than Horoz Karası grape variety. It is known that grape seed is especially rich in flavonols. It is known that the amount of quercetin in grape peel is high.

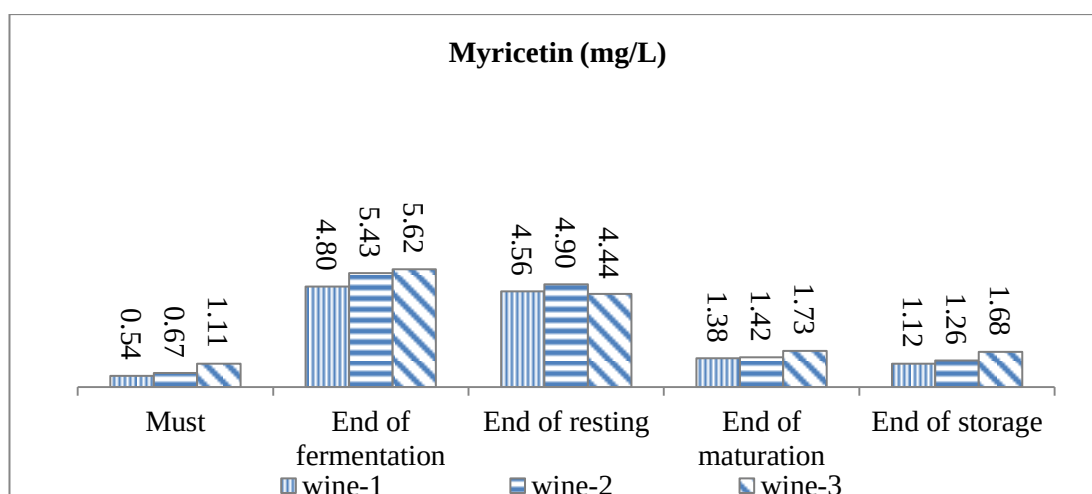


Figure 4.15 Myricetin content of must and red wine samples (mg/L)

The quercetin changes in must and wines are given in Figure 4.16. The quercetin content of the musts-1, 2 and 3 were 0.11, 0.23 and 0.28 mg/L respectively. The values of “quercetin content” for three types of musts are significantly different ($p<0.05$) from each other. At the end of fermentation, quercetin content of wines were found as 3.40, 7.57 and 8.52 mg/L for wines-1, 2 and 3 respectively. It was found that there was significant ($p<0.05$) increase in the quercetin content of red wines during fermentation. After the three types of wine were kept with oak chip, the quercetin contents were found to be 3.63, 4.11 and 6.93 mg/L respectively. After 45 days maturation in glass carboys, quercetin contents were found as 3.26, 3.94 and 6.27 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the quercetin contents for wines in order were 2.86, 3.89 and 4.73 mg/L. The quercetin contents of red wines were decreased from end of fermentation to storage in bottle. This decrease is significant ($p<0.05$). While wine-3 had the highest quercetin content, wine-1 had the lowest content at the end of storage in bottle. The values of “quercetin content” for three types of wines are significantly different ($p<0.05$) from each other at the end of storage. Flavonols are found in the glycoside structure of grapes and are located in the skin portion of the grapes. Since the maceration process is not applied in white wine production, the amount of white wines is less than that of red wines. Flavonols are hydrolyzed during fermentation and are therefore aglycated in wines (Ribéreau-Gayon and Glories, 1986). It is known that grape seed is especially rich in flavonols. However, there are also flavanols in the grape skin. However, the main phenolic compounds in the skin are flavonols. It is known that the amount of quercetin as flavonols in grape skin is high. Quercetin and their glycosides cause bitterness in wine (Jackson, 2000). Vuorinen et al. 2000 determined that quercetin amount in Cabernet Sauvignon and Merlot varieties were found to be <1.2-10.2 mg/L and 5.4-19.4 mg/L respectively. Ritchey and Waterhouse (1999) found that the amount of quercetin in Cabernet Sauvignon wines was 0.92-63.01 mg/L. The quantities of quercetin in Merlot, Cabernet Sauvignon and Pinot Noir wines were found to be between 2.0-5.26 mg/L and quercetin amounts were found to vary between varieties (Soleas et al., 1997a). Lopez et al. (2001) indicated that red wines contain 0-4.66 $\mu\text{g/mL}$ quercetin. Careri et al. (2003) determined that the content of quercetin in red wines was 0.89-8.84 $\mu\text{g/mL}$. Kelebek et al. (2010) found that Öküzgözü's wines contained 0.76-2.01 mg/L quercetin in wines from different vineyard regions. These results are consistent with

our data. The difference between flavonol content may be a marker for different grape species and for wines. Hönüsü wines had very low flavonol levels, while the highest flavonol content in Horoz Karası wines. This change in flavonol content can be explained by the accumulation of flavonols in the skins of red grapes and different grape skin thicknesses. Hönüsü grape is a “thin-skinned” variety and wines contain low amounts of flavonol. Horoz Karası wines are a “thick-skinned” grape variety with higher flavonol concentrations. Quercetin, which belongs to the flavonol group, contributes to bitterness in wines. The sunny and dry climate in Gaziantep regions are good conditions for the synthesis and accumulation of flavonols in grapes.

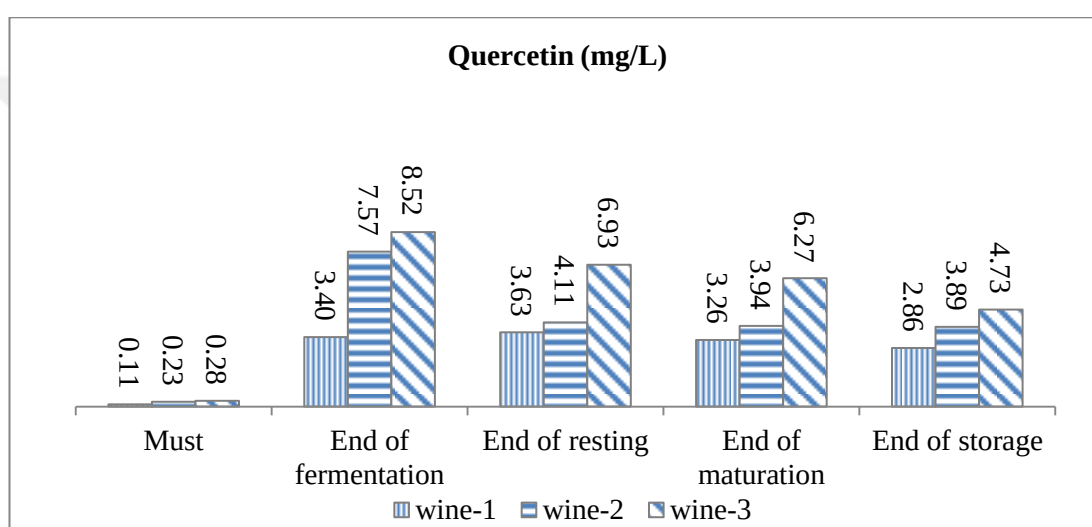


Figure 4.16 Quercetin content of must and red wine samples (mg/L)

The rutin changes in must and wines are given in Figure 4.17. The rutin content of the musts-1, 2 and 3 were 0.12, 0.24 and 0.28 mg/L respectively. The values of “rutin content” for three types of musts are significantly different ($p < 0.05$) from each other. At the end of fermentation, rutin content of wines were founded as 1.60, 2.00 and 2.21 mg/L for wines-1, 2 and 3 respectively. There was significant ($p < 0.05$) increase in the rutin content of red wines during fermentation. After the three types of wine were kept with oak chip, the rutin contents were found to be 1.58, 1.97 and 2.13 mg/L respectively. After 45 days maturation in glass carboys, rutin contents were found as 1.51, 1.83 and 2.11 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the rutin contents for wines in order were 1.38, 1.58 and 1.61 mg/L. The rutin contents of red wines were decreased from end of fermentation to storage in bottle. This decreases, statistically significant ($p < 0.05$).

While wine-3 had the highest rutin content, wine-1 had the lowest content at the storage in bottle. The values of quercetin content for three types of wines are significantly different ($p < 0.05$) from each other at the end of storage.

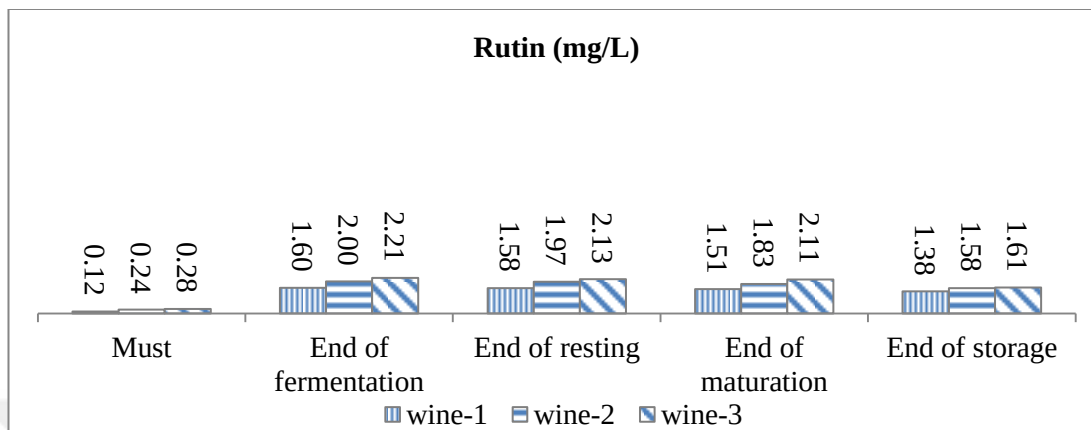


Figure 4.17 Rutin content of must and red wine samples (mg/L)

Flavonols (quercetin, myricetin and their glycosides such as quercetin-3-rutinoside) which contribute to bitterness, are found mainly in phenol compounds and in skin and seeds of grapes. Flavonols are the most abundant phenolic compounds in grape skin, otherwise the grape seed is rich in flavan-3-ol (Cheynier and Rigaud, 1986; Souquet et al., 2000). In flavonoids, rutin are the only flavonol capable of chelating metal ions such as iron, which cause the formation of oxygen radicals with their high antioxidant activity. It is also reported to stabilize vitamin C and increase the antioxidant value of ascorbic acid (Grassmann, 2004). Hallaç (2009) found that the amount of rutin was 0.67 $\mu\text{g/mL}$ in red grape juice. Simonetti et al. (1997) determined that the amount of quercetin and rutin were more than the other flavonols in wines. In the same study, it was determined that the amount of flavonol was very low in red wine grapes. The amount of rutin, like other flavonols, increased depending on fermentation time. Kocabey (2013) reported that the amount of rutin was 1.25 mg/L in red wine produced from Karaoğlan grape at the end of fermentation for 5 days, and 0.69 mg/L for 15 days. Şen (2014) reported that the rutin amount of red wines is between 0.78-6.03 mg/L. Among the wines used in the study, wine-1 flavonol (myricetin, quercetin and rutin) content was lower than other grape varieties. Since flavonols generally cause bitterness, we can infer that wine-2 and wine-3 have more bitter taste than wine-1.

The (+)-catechin hydrate and procyanidin B₂ changes in must and wines are given in Figures 4.18 and 4.19. The (+)-catechin hydrate and procyanidin B₂ contents of the musts obtained from Hönüsü and Horoz Karası grapes were ranged between 60.98-61.49 and 45.42-51.16 mg/L respectively. The values of “(+)-catechin hydrate and procyanidin B₂” for three types of musts are no significantly different ($p>0.05$) from each other. At the end of fermentation, (+)-catechin hydrate and procyanidin B₂ content of wines were ranged between 63.08-66.00 and 247.43-278.53 mg/L, respectively for wines-1, 2 and 3. There was significant ($p<0.05$) increase in the (+)-catechin hydrate and procyanidin B₂ content of red wines during fermentation. After the three types of wine were kept with oak chip, the (+)-catechin hydrate and procyanidin B₂ contents were ranged between 72.00-75.53 and 531.37-732.34 mg/L respectively. After 45 days maturation in glass carboys, (+)-catechin hydrate and procyanidin B₂ contents were founded between 66.62-72.77 and 423.60-643.67 mg/L, respectively, for three types of wine. After the storage in the bottle for 3 months, the (+)-catechin hydrate and procyanidin B₂ contents for wines in order were changed between 62.12-71.71 and 258.30-373.50 mg/L respectively. The (+)-catechin hydrate and procyanidin B₂ contents of red wines were decreased from end of resting to storage in bottle. This decrease was significant ($p<0.05$). While wine-3 had the highest (+)-catechin hydrate and procyanidin B₂ content, wine-1 had the lowest content at the storage in bottle. The values of (+)-catechin hydrate and procyanidin B₂ content for wines-1 and 2 are significantly different ($p<0.05$) from wine-3 at the end of storage.

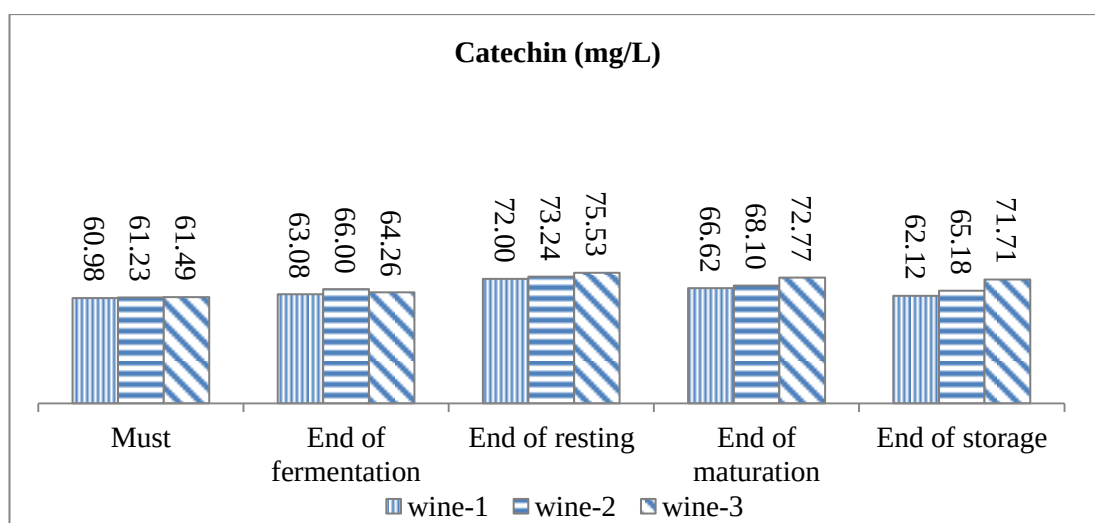


Figure 4.18 Catechin content of must and red wine samples (mg/L)

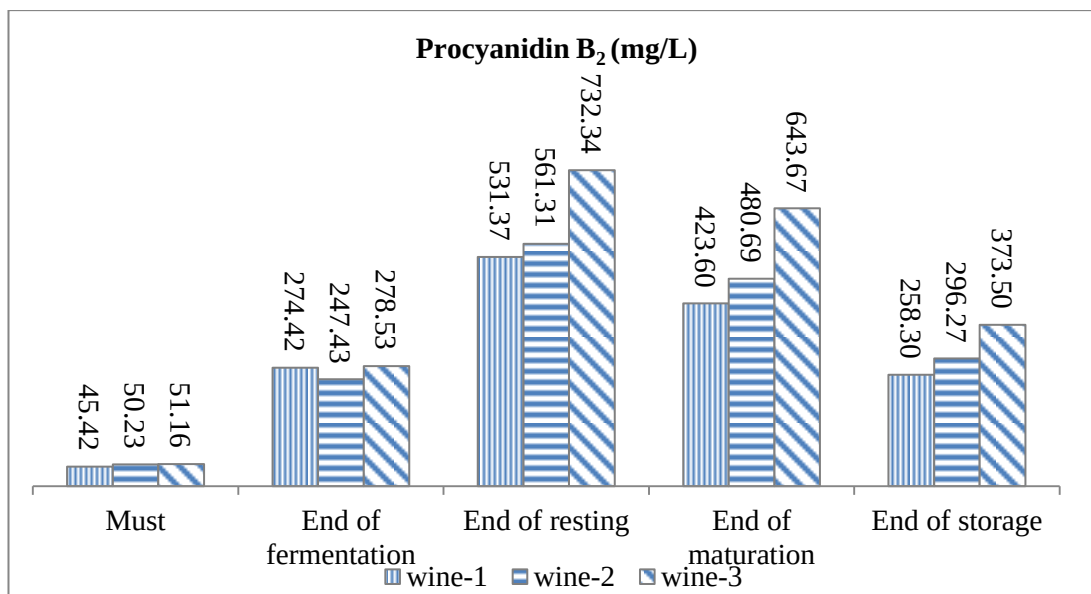


Figure 4.19 Procyanidin B₂ content of must and red wine samples (mg/L)

The amounts of catechin and procyanidin B₂ can vary depending on the grape variety, whether the must is seeded and whether the grape skin is thin or thick or the colored skin. Catechins react easily with oxygen in the air chemically and enzymatically to form condensed tannins (proanthocyanidins). The astringency or bitterness of these compounds is determined by their molecular weight. Sixty-eight monomeric proanthocyanidins produce acid taste, while thirty-five monomeric ones produce bitter taste (Akalin, 2011). Tannins are chemically divided into two groups as hydrolysable tannins and condensed tannins (Haslam, 1998). The condensed tannins ((+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin and (-)-epicatechin 3-gallate) are the most common tannin structures in grapes. The basis of these tannins are (+)-catechin and (-)-epicatechin. When heated in an acid medium, these compounds are converted to highly unstable carbocations, which turn into brown clusters, often forming red cyanidines. Condensed tannins, especially procyanidins and catechins, are present in all parts of the grape (skin, seed, stem). The most known characteristics of tannins are that they combine with glycoproteins in the saliva, leaving a acrid feeling in the mouth and binding to the protein parts of the enzymes (Saucier et al., 2001, Ribéreau-Gayon et al., 2006). The tannin content of grape skin significantly affects the phenolic composition of the wine (Harbertson et al., 2011, Ribéreau-Gayon et al., 2006a). Procyanidin B₂ amounts of wines were increased in parallel with fermentation time and decreased during storage. However, the amount of procyanidin B₂ of wine-3 was highest than others. This may be

because the color of wine-3 is darker than the others. It was found that catechin was found to be less in the shell portion of the grapes, and was found to be more in the core and stalk portion and this monomeric structure was responsible for browning in white wines and astringency in red wines (Hornsey, 2007). Catechins are colorless flavan-3-ol. Procyanidin is effective on color and taste of fruits and vegetables. The chain length of procyanidins is short and colorless. As the chain length increases, they change from yellow to brown. The astringent taste in fruits is provided by tannin. The bitter and astringent taste in fruits and wines varies with the molecular weight of the procyanidins. Anthocyanins responsible for the color of wine are known to be polymerized with tannins, procyanidins and catechins. The polymerization rate increases by almost 40% after 1 year of storage. The color of matured red wine reflects the proportion of anthocyanin tannin polymers. Because of most of the polymers are brown, the aged wine color is usually in brick tones. Color density also decreases over time with the disappearance of free anthocyanins (Jackson, 2000). Hydrolysis of procyanidins is associated with the separation of the central flavan unit from the oligomer by carbocation and subsequent oxidation to the colored anthocyanin (Ribéreau-Gayon and Glories, 2006). During the resting of the wine, the amount of the tannins in the wine increases with time. During the aging in oak barrels, hydrolysable tannins had important effects on the taste and bouquets of the wine pass from the barrel to the wine (Ünsal, 2007). Tannins are more soluble in alcohol than water. During maceration, they pass from the solid parts of the grape to wine. At the same time, if maturation is carried out in the barrel, it may pass from the barrel to wine. During the production of wine, only a few of the tannins present in the grapes are soluble (approximately 0-50). The solubility of these substances varies according to the grape variety, the maturity of the grapes and also the maceration conditions. Gómez-Plaza et al. (2002) investigated the effect of different fermentation periods (4, 5 and 10 days) on phenol compounds, reported that the amount of catechin was 8.4-9.8 mg/L and the amount of procyanidin B₂ ranged from 2.5 to 3.6 mg/L. Orban et al. (2006) reported that the amount of catechin in Pinot noir wine as 97.35 mg/L, Merlot wines 33.16 mg/L and Shiraz wines were 54.97 mg/L. Makris et al. (2006) indicated that the amount of catechin as 40.5 mg/L and procyanidin B₂ was 14.1 mg/L on average in the wine obtained from Cabernet sauvignon grapes. In addition, they was found the average amounts of catechin and procyanidin B₂ dimers in Syrah grapes as 41.7 and 12.3 mg/L respectively. The same

researchers indicated that the amount of catechin between 26.4 and 80.7 mg/L, and procyanidin B₂ ranged between 2.9 and 19.1 mg/L in the wine obtained from Merlot grapes. Gómez-Plaza et al. (2001) explained that catechin, procyanidin, anthocyanin compounds and color density were increased and polymer content compound content was high in wines with long fermentation period. Laudrault et al. (2001) found the amount of catechin in Cabernet Sauvignon wines grown in Southern France between 34.5 and 48.8 mg/L. Vivas et al. (2003) indicated that the maximum amount of catechin in wine 300 mg/L minimum 20 mg/L. Kelebek (2009) found that the amount of (+)-catechin between 22.53 and 36.8 mg/L in Boğazkere wines obtained from grapes of Denizli and Elazığ regions, the catechin content of the wines was determined between 25.24 and 37.40 mg/L. Kocabey (2013) reported that the amount of procyanidin B₂ between 492.75 and 713.48 mg/L in red wine produced from Karaoğlan grape. Gómez-Plaza et al. (2001) reported that the amount of catechin, procyanidine, anthocyanin compounds and color density of the compound is increased and the polymer content is high in wine with long fermentation. The findings obtained in this study are in agreement with the results obtained from the above studies. The reason for the difference between the varieties can be considered as the thickness and color of the shell. It is reported that changes in the synthesis of tannins are influenced by both environmental and periodical differences, and this has the effects of soil mineralogy, pH, water and sunlight (Gaffney et al., 2004).

4.1.7.2 Non-Flavonoid Content of Must and Wine Varieties

According to the “ANOVA”, the flavonoid contents during processing steps of red wines are given in Table F.10. The gallic acid changes in must and wines are given in Figure 4.20. The gallic acid content of the musts-1, 2 and 3 were 2.80, 4.59 and 6.53 mg/L respectively. The gallic acid for three types of musts are significantly different ($p < 0.05$) from each other. At the end of fermentation, gallic acid content of wines were founded as 32.77, 23.94 and 40.54 mg/L for wines-1, 2 and 3 respectively. There was significant ($p < 0.05$) increase in the gallic acid content of red wines during fermentation. After the three types of wine were kept with oak chip, the gallic acid contents were found to be 40.39, 39.59 and 46.67 mg/L respectively. After 45 days maturation in glass carboys, gallic acid contents were found as 21.60, 17.30 and 36.47 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the gallic acid contents for wines in order were 11.49, 15.51 and

24.78 mg/L. The gallic acid contents of red wines were decreased ($p<0.05$) from the end of resting to the end of storage in bottle. While wine-3 had the highest gallic acid content, wine-1 had the lowest content at the storage in bottle. The contents of gallic acid for wines-1 and 2 are significantly different ($p<0.05$) from wine-3 at the end of storage. Hydroxybenzoic acids (gallic acid) are mainly localized in grape peel. Therefore, they are found in higher amounts until the removal of the grape skin. Decreases in hydroxybenzoic acids occur due to the concentration of alcohol formed during fermentation (Gil-Munoz et al. 1999). Kılınç and Kalkan (2003) reported that gallic acid is the most common phenol acids in wines and this is followed by hydroxycinnamic acid, syringic acid and ferulic acid. The amount of gallic acid of Hönüsü and Horoz Karası wines increased depending on the fermentation time. Phenolic compounds in grapes pass to wine during fermentation (maceration) (Macheix et al., 1991). In addition, a significant decrease was observed in the amount of gallic acid waiting after with oak chips. Gallic acid and ellagic acid play an important role in the structure of the tannin (procyanidin B₂) and taste of wine as extracted from oak barrels during the wine production stages (Margalit, 2004). Oak-aged wines contain high levels of hydroxybenzoic acid derivatives (gallic acid), in particular ellagic acid (dilactone formed by the coupling of two molecules of gallic acid). Ellagic acid is formed by the hydrolytic degradation of esterified ellagitannins to glucose. Gallotannins are a hydrolyzable tannin group containing gallic acid polymers. Approximately 5% of the oak contains hydrolysable tannin (Anlı and İnce, 2016). Gallic acid gives astringency aroma to wines due to found in the structure of the tannin. Kelebek (2009) found that the amount of gallic acid in Öküzgözü wines obtained from grapes of Denizli region was 9.03-19.4 mg/L. In the Elazığ region it was determined as 8.66-18.9 mg/L. The amount of gallic acid was found to be 3.46-7.4 mg/L in Boğazkere wines obtained from grapes from Denizli. It was determined as 3.99-7.7 mg/L in the Elazığ region. The amount of gallic acid was found to be 5.03-8.0 mg/L in Kalecik Karası wines obtained from grapes from Ankara region. In the Nevşehir region it was determined to be 6.30-7.4 mg/L. In a study on Cabernet Sauvignon wines of different yields grown in southern France, gallic acid amounts were found to be between 33.47-40.1 mg/L (Landrault et al., 2001). Vivas et al. (2003) determined that the amount of gallic acid in wine minimum 5 mg/L maximum 140 mg/L. Gallic acid 70.2 g/L for Cabernet Sauvignon wine in Hoşk y region, while 52.6 g/L for Gamay grape wine ( nsal, 2007). The findings obtained in this

study are in agreement with the results obtained from the above studies. Phenol acids are chemically divided into two groups: hydroxycinnamic (*p*-coumaric and chlorogenic) and hydroxybenzoic (gallic acid) acids. Budic-Leto and Lovric (2002) reported that the amount of hydroxybenzoic acid in grape juice is more than the amount of hydroxycinnamic acid and protocatechic acid and gallic acid are reported to be the most common phenol acids in grape juice.

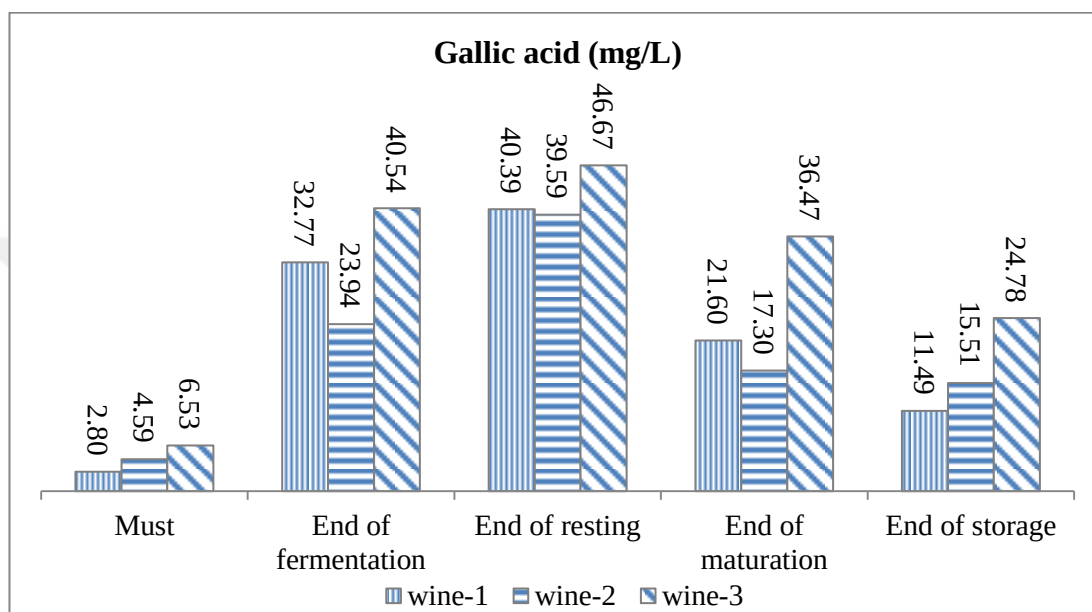


Figure 4.20 Gallic acid content of must and red wine samples (mg/L)

The *p*-coumaric acid changes in must and wines are given in Figure 4.21. The *p*-coumaric acid content of the musts-1, 2 and 3 were 0.05, 0.06 and 0.10 mg/L respectively. The values of “*p*-coumaric acid” for musts-1 and 2 are significantly different ($p < 0.05$) from must-3. At the end of fermentation, *p*-coumaric acid content of wines were founded as 11.27, 11.37 and 12.16 mg/L for wines-1, 2 and 3 respectively. There was significant ($p < 0.05$) increase in the *p*-coumaric acid content of red wines during fermentation. After the three types of wine were kept with oak chip, the *p*-coumaric acid contents were found to be 11.21, 11.31 and 11.92 mg/L respectively. After 45 days maturation in glass carboys, *p*-coumaric acid contents were found as 7.38, 7.21 and 7.30 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the *p*-coumaric acid contents for wines in order were 7.35, 7.15 and 7.33 mg/L. The *p*-coumaric acid contents of red wines were decreased from end of resting to the end of storage in bottle. This decreases are significantly ($p < 0.05$) different during process. But, decrease is not significant

($p > 0.05$) for each wines between maturation and at end of storage. While wine-1 had the highest *p*-coumaric acid content, wine-2 had the lowest content at the storage in bottle. The *p*-coumaric acid content values for wines-1 and 3 are significantly different ($p < 0.05$) from wine-2 at the end of storage. The cinnamic acids having C₆-C₃ carbon structure and the most common cinnamic acids in plants are *p*-coumaric acid, 5-hydroxiferulic acid, caffeic acid, ferulic acid and cinapic acid. The cinnamic acids are not free in plants, but are generally present as esters or sugar esters of tartaric acid, quinic acid and shikimic acid. Phenolic acids have little effect on the sensory properties of wine. However, some microorganisms (*Brettanomyces* and some bacteria) synthesize volatile phenol acids, and some of these adversely affect the aroma. For example, ethyl phenol gives an animal odor. In addition, *p*-coumaric acid and ferulic acid decompose vinyl phenol and this substance similar to oil paint odor (Ribéreau-Gayon et al., 2000b). Volatile phenol compounds are released by the activity of the decarboxylase enzymes of yeasts from phenol acids (*p*-coumaric, ferulic and caffeic acids) during alcohol fermentation. These compounds are biochemically formed in two basic ways. The first stage is decarboxylation of hydroxycinnamic acids with cinnamate decarboxylase enzyme and the second stage is the formation of 4-vinyl phenol and 4-vinyl guaiacol sequentially with the help of vinylphenol reductase enzyme (Chatonnet et al., 1992; Saez et al., 2010). In sensory analysis, according to the panelists commented that wine-2 smells chemical odor. It can be interpreted that *p*-coumaric acid is broken down by the effect of oak chips. *p*-coumaric acid was found between 1-15 mg/L in crushed grapes and wine (Ribereau-Gayon, 2006). Lante et al. (2004) obtained that amount of *p*-coumaric acid ranged from 0.23 to 7.07 mg/L in the analysis of 23 commercial Italian wines. Kelebek (2009) stated that *p*-coumaric acid increased due to fermentation time. In the same study, the amount of *p*-coumaric acid was found to be 0.97 mg/L in 2005 and 0.86 in 2006 at the end of fermentation. In the Elazığ region it was determined as 0.69 and 0.61 mg/L, respectively. The findings obtained in this study are in agreement with the results obtained from the above studies.

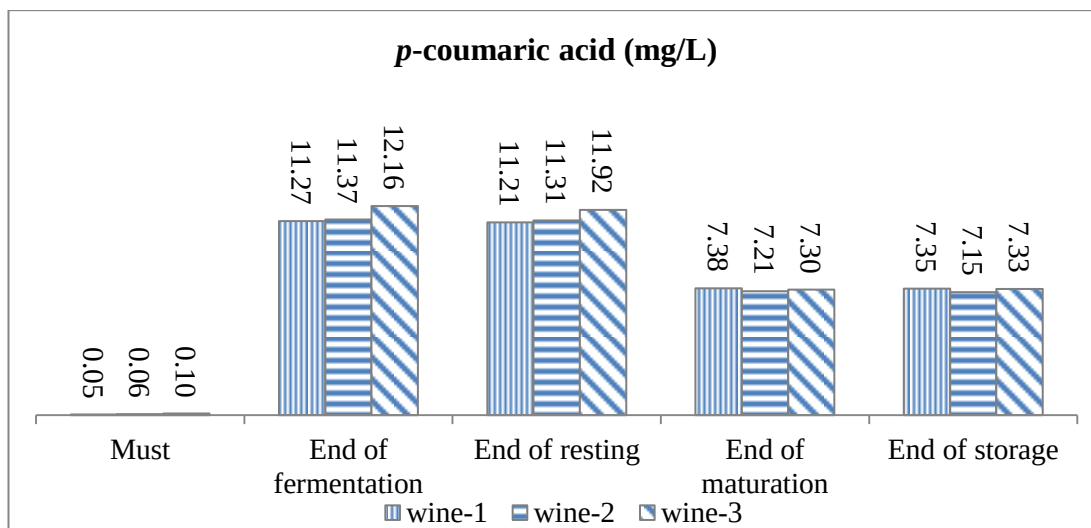


Figure 4.21 *p*-coumaric acid content of must and red wine samples (mg/L)

The chlorogenic acid changes in must and wines are given in Figure 4.22. The chlorogenic acid content of the musts-1, 2 and 3 were 8.42, 8.86 and 9.20 mg/L respectively. The contents of “chlorogenic acid” for three types of musts are not significantly different ($p>0.05$) from each other. At the end of fermentation, chlorogenic acid content of wines were founded as 9.71, 10.90 and 11.20 mg/L for wines-1, 2 and 3 respectively. There was significant ($p<0.05$) increase in the chlorogenic acid content of red wines during fermentation. After the three types of wine were kept with oak chip, the chlorogenic acid contents were found to be 10.50, 11.71 and 12.59 mg/L respectively. After 45 days maturation in glass carboys, chlorogenic acid contents were found as 7.23, 7.27 and 7.33 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the chlorogenic acid contents for wines in order were 7.22, 7.24 and 7.32 mg/L. The chlorogenic acid contents of red wines were decreased from end of resting to the end of storage in bottle and this decrease is significant ($p<0.05$). While wine-3 had the highest chlorogenic acid content, wine-1 had the lowest content at the storage in bottle. There is no significant ($p>0.05$) differences in chlorogenic acid content values among three types of wines at the end of storage. Phenolic acids; in fruits cinnamic acid, caffeic, coumaric acids and ferulic acid. Cinnamic acids are not found in the free state in plants and are generally present as esters or sugar esters of tartaric acid, quinic acid and shikimic acid. The most common cinnamic acid derivative is ester chlorogenic acid ester, the ester of caffeic acid with quinic acid. Chlorogenic acid is responsible for the sour taste in fruit and fruit products and easily oxidizes in the

presence of polyphenol oxidase and can also be converted to brown colored compounds. Amakura et al. (2000) stated that the amount of chlorogenic acid in grape juice is high. In this study, the chlorogenic acid content of red wines increases during fermentation and resting with oak chips. Hydroxycinnamic acids (chlorogenic acid) are mainly stored in grape pulp and their derivatives are found in oak structure and are easily passed to wine during crushing (Jackson, 2008). Kocabey (2013) indicated that the amount of chlorogenic acid determined between 1.46 and 1.67 mg/L as in red wine obtained from Karaoğlan grapes. The chlorogenic acid contents obtained in this study are higher than Karaoğlan wines.

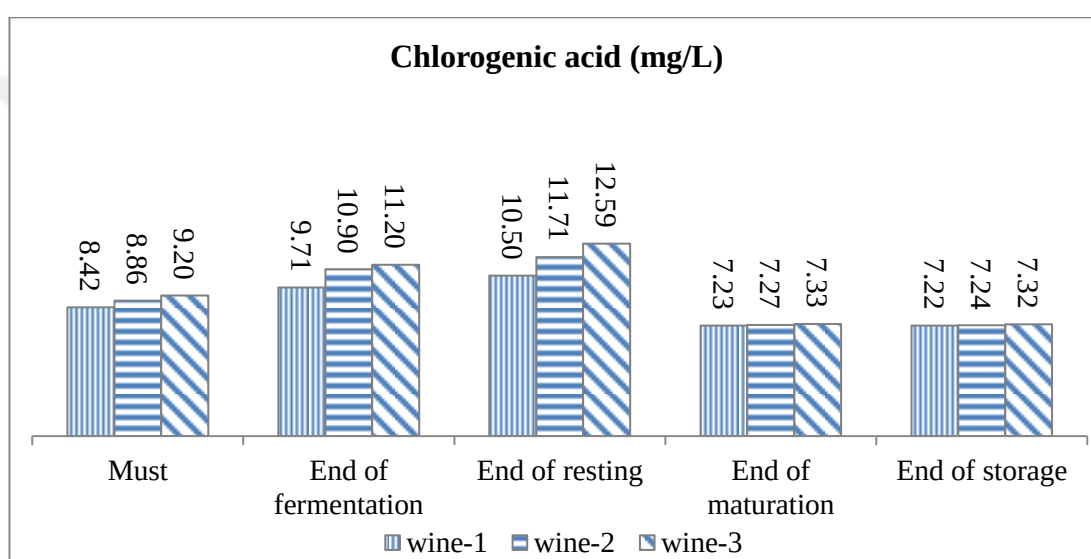


Figure 4.22 Chlorogenic acid content of must and red wine samples (mg/L)

The tyrosol changes in must and wines are given in Figure 4.23. The tyrosol content of the musts-1, 2 and 3 were 1.05, 2.57 and 3.54 mg/L respectively. The values of “tyrosol” of musts are significantly different ($p < 0.05$) from each other. At the end of fermentation, tyrosol content of wines were founded as 13.75, 19.74 and 23.83 mg/L for wines-1, 2 and 3 respectively. There was significant ($p < 0.05$) increase in the tyrosol content of red wines during fermentation. After the three types of wine were kept with oak chip, the tyrosol contents were found to be 12.86, 19.02 and 22.15 mg/L respectively. After 45 days maturation in glass carboys, tyrosol contents were found as 10.56, 11.96 and 18.35 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the tyrosol contents for wines in order were 6.57, 11.05 and 11.49 mg/L. The tyrosol contents of red wines were decreased ($p < 0.05$) from end of fermentation to the end of storage in bottle. While wine-3 had the

highest tyrosol content, wine-1 had the lowest content at the end of storage in bottle. There is significant differences ($p < 0.05$) in tyrosol content for among three types of wines. Tyrosol is produced by *S. cerevisiae* by oxidative decarboxylation from tyrosine amino acid during alcohol fermentation (Ribereau-Gayon et al., 2000c). The synthesis of tyrosine is associated with the amount of glucose. During fermentation, tyrosol, which is composed of tyrosine amino acid by yeasts, is also included in the non-flavonoid phenolic compounds group (Singleton and Esau., 1969). Tyrosol formation content increases in the presence of tyrosine amino acid (Tuck et al., 2001). Pour Nikfardjam and Pickering (2008) found that tyrosol levels in Hungarian and Canadian red wines varied between 38.8 and 82.8 mg/L. Gris et al. (2011) indicated that the amount of tyrosol determined in red wines varied between 23 and 47 mg/L. Pineiro et al. (2011) indicated that the tyrosol contents were 20.38-44.46 mg/L in red Spanish wines. Tyrosol synthesis is correlated with the amount of glucose. It has been reported that the formation of tyrosol increases with the growth of *S. Cerevisiae* (Sentheshanmuganathan and Elsdén, 1957). Since phenolic compounds are highly reactive and sensitive to various reactions, some researchers have stated that the tyrosol content may change during the aging of the wine (Gris et al., 2011). The composition and amount of phenolic compounds in wine have been reported to depend on the grape variety used for wine production, the climatic conditions, the procedures used in wine production and the chemical reactions that occur during the aging of wine (Macheix et al., 1990, Pena et al., 1998). Garde-Cerdan and Ancin-Azpilicueta (2008) indicated that the synthesis of tyrosol is directly proportional to the total amount of amino acids present in the wine. The presence of tyrosol in wines depends on the type of yeast, used in alcohol fermentation, sugar concentration and the amount of tyrosine in grape must (Barcenilla, 1992). As shown in Table F.10, the amount of phenolic compounds increased as the fermentation time increased. The fermentation did not show a selective effect on the compounds and the amount of all compounds increased with increasing time.

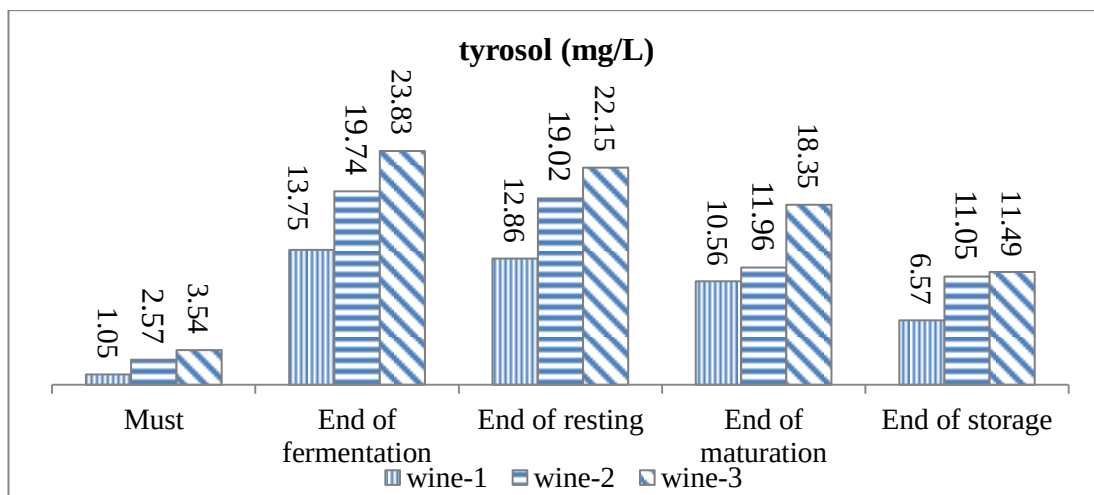


Figure 4.23 Tyrosol content of must and red wine samples (mg/L)

The resveratrol changes in must and wines are given in Figure 4.24. The resveratrol content of the musts-1, 2 and 3 were 0.05, 0.07 and 0.08 mg/L respectively. The values of “resveratrol” for wines-2 and 3 are significantly different ($p < 0.05$) from wine-1. At the end of fermentation, resveratrol content of wines were founded as 2.03, 2.14 and 2.26 mg/L for wines-1, 2 and 3 respectively. There was significant ($p < 0.05$) increase in the resveratrol content of red wines during fermentation. After the three types of wine were kept with oak chip, the resveratrol contents were found to be 2.63, 2.74 and 2.91 mg/L respectively. After 45 days maturation in glass carboys, resveratrol contents were found as 2.55, 2.73 and 2.89 mg/L for three types of wine respectively. After the storage in the bottle for 3 months, the resveratrol contents for wines in order were 2.49, 2.69 and 2.87 mg/L. The resveratrol contents of red wines were decreased from end of resting to the end of storage in bottle. This decrease is no significant ($p > 0.05$) from end of resting to the end of storage. While wine-3 had the highest resveratrol content, wine-1 had the lowest content at the storage in bottle. The resveratrol content values for wines-1 and 2 are significantly different ($p < 0.05$) from wine-3 at the end of storage. Another important group of compounds in phenol acids is stilbenes. Of these compounds, trans isomeric resveratrol (3,5,4-trihydroxystyrene) has been suggested to have a positive effect on health. Resveratrol is located in the shell of the grape. This compound grape protects against various external influences (against pathogens) (Kocaturk and Kavas, 2007). In grape, resveratrol synthesis is generally in shell cell of seeds. Resveratrol is either absent or very small in fruit flesh (Romero-Pérez et al., 1996). Grape juice is

considered a good source of resveratrol; however, the concentration of resveratrol in grapes and consequently in wines depends on climatic conditions, grape variety and grape growing conditions, and the method of wine processing used. Stilbenes consisting of trans-resveratrol, cis-resveratrol and resveratrol glucoside are also included in the non-flavonoid group (Chiou et al., 2007). Resveratrol amounts of three wines increased during fermentation. The end of resting with oak chip has increased again. During maturation and storage in bottle, it began to decrease. The most basic component of stilbene is resveratrol in grape skin. This compound has many health benefits. Resveratrol is located in the skin of the grapes and dissolves during the wine making process. Since red wines have maceration, their amount of resveratrol is higher than white wine. The amount of resveratrol in wines varies between 1-3 mg/L depending on the type of grapes used and the wine-making technique (Ribéreau-Gayon et al., 2000b). Gürbüz et al. (2007) determined that the red grape of Öküzgözü grape contains the highest resveratrol concentration in must and wine (4.403 mg/L). The amount of resveratrol is shown diversity in the wines of the grapes grown in different regions in Turkey. The reasons for this are thought to be related to grape variety, cultural and plant protection practices and ecological conditions of the regions. Cassidy et al. (2000) reported that resveratrol in wines depends on grape variety, climatic conditions of the region, yeast strains and maturation in oak. Adıgüzel (2007) determined the resveratrol levels in Öküzgözü wine obtained from the vineyards of the Eastern Anatolia Region with 6.469 mg/L. The wine produced in the Aegean region (Cabernet Sauvignon) was found to be 2.257 mg/L. Some other resveratrol values obtained in wines are Syrah 1,790 mg/L, Misket 0.278 mg/L and Sauvignon Blanc 0.494 mg/L.

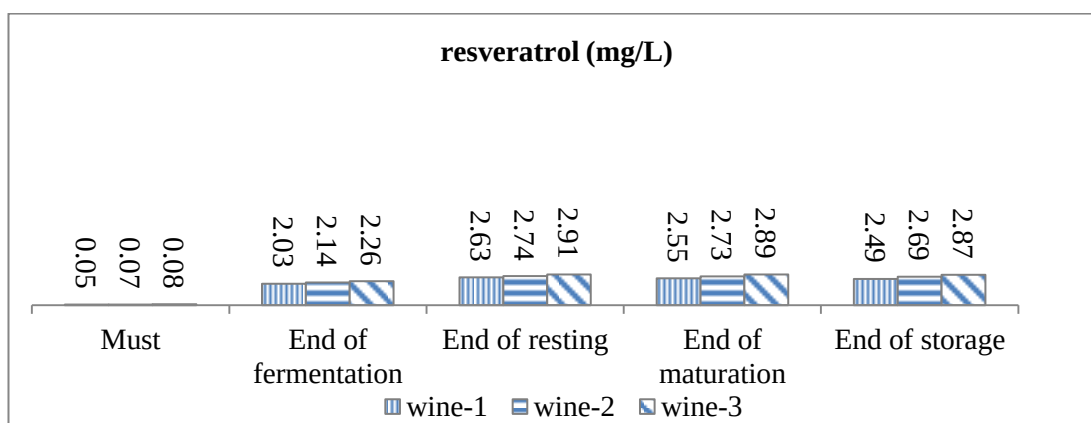


Figure 4.24 Resveratrol content of must and red wine samples (mg/L)

In grape musts-2 and 3, catechin and procyanidin B₂ amounts are high. Procyanidin B₂ and (+)-catechin are thought to be effective in forming the astringency and bitterness of wines (wines-2 and 3). These are more effective than others, since the amount of tannins (procyanidin B₂) increases with astringency and bitterness. The amount of phenol compounds subject to numerous investigations is 1610-10850 mg/kg in grapes (Galet, 1993) and 748-1200 mg/L in wines (Mazza et al., 1999). Hallaç et al. (2009) determined the amount of catechin in red grape juice as 2.94 µg/mL, chlorogenic acid was 2.76 µg/mL, gallic acid was 0.75 µg/mL, catechin was 2.94 µg/mL, p-coumaric acid was 0.05 µg/mL, and rutin was 0.65 µg/mL. Ateş (2015) determined the gallic acid between 0.84 and 18.84 mg/kg, catechin between 5.25 and 149.68 mg/kg, chlorogenic between 9.07 and 1961.6 mg/kg, p-coumaric acid between 3.37 and 7.42 mg/kg, rutin between 1.98 and 125.6 mg/kg, myricetin between 0.51 and 11.89 mg/kg, and procyanidin B₂ between 2.1 and 19.56 mg/kg dry matter on Tahannebi and the Kureyş grape varieties. The findings obtained in this study are in agreement with the results obtained from the above studies.

When the differentiation of the wines in terms of phenolic compounds is statistically evaluated (Tables F.9 and F.10), it can be concluded that the amount of each phenolic component in the wine depends on the type of grapes. The average of phenolic components and Duncan Test results showed that wine-3 phenolic compounds were higher compared to other wine varieties, whereas p-coumaric acid content was second. In this case, it can be said that wine-3 is richer in terms of phenolic compounds than wine-1 and 2. This situation can be explained by the differences of grape varieties. When the research results are evaluated in general; it is seen that the differences among the quality wine varieties grown under similar conditions are primarily related to the variety in the Gaziantep region. In this study, fermentation conditions were kept equivalent for all wines. When the results of the research are evaluated on the basis of varieties of wine; wine-3 is generally rich in phenolic compounds and has shown that more powerful body wines. In addition, wine-3 is thought to be more astringent and has higher antioxidant properties and may be more beneficial for cardiovascular health if consumed in appropriate amounts.

4.1.8 Determination of Sugar Contents in Must and Wine Samples

The sugar contents were determined in the musts and during fermentation, resting with oak chip, maturation in the glass carboy and storage in bottle processes. The changes in sugar contents statistically were given in Table F.11. The fructose and glucose changes in must and wines are given in Figures 4.25 and 4.26. The fructose and glucose content of the musts-1, 2 and 3 were ranged from 93.95 to 111.08 and from 70.97 to 78.13 g/L respectively. The values of “fructose” for musts-1 and 2 are significantly different ($p<0.05$) from must-3. The values of “glucose” for musts-2 and 3 are significantly different ($p<0.05$) from must-1. At the second day of fermentation, fructose and glucose content of wines were significantly ($p>0.05$) reduced to 16.24-56.08 and 8.33-14.48 g/L for wines-1, 2 and 3, respectively. At the end of fermentation, fructose and glucose content of wines were significantly ($p<0.05$) reduced from 1.53 to 1.97 and from 0.93 to 1.07 g/L for wines-1, 2 and 3, respectively. There was significant decrease in the fructose and glucose values of red wines during fermentation ($p<0.05$). After the three types of wine were kept with oak chip, the fructose and glucose contents were ranged from 1.49 to 2.02 and from 0.79 to 0.92 g/L respectively. After 45 days maturation in glass carboys, fructose and glucose contents were found as 1.47-2.93 and 0.72-0.79 g/L for three types of wine respectively. After the storage in the bottle for 3 months, the fructose and glucose contents for wines in order were 1.45-1.84 and 0.70-0.77 g/L. The fructose and glucose contents of red wines were decreased from end of fermentation to the end of storage in bottle. These decrease are not significant ($p>0.05$). While wine-3 had the highest fructose, wine-1 had the lowest content at the end of storage in bottle. However, wine-1 had the highest glucose content, wine-3 had the lowest content at the end of storage in bottle. The values of fructose contents for three types of wine are significantly different ($p<0.05$) from each other, while the glucose content values for wines-2 and 3 are significantly different ($p<0.05$) from wine-1 at the end of storage.

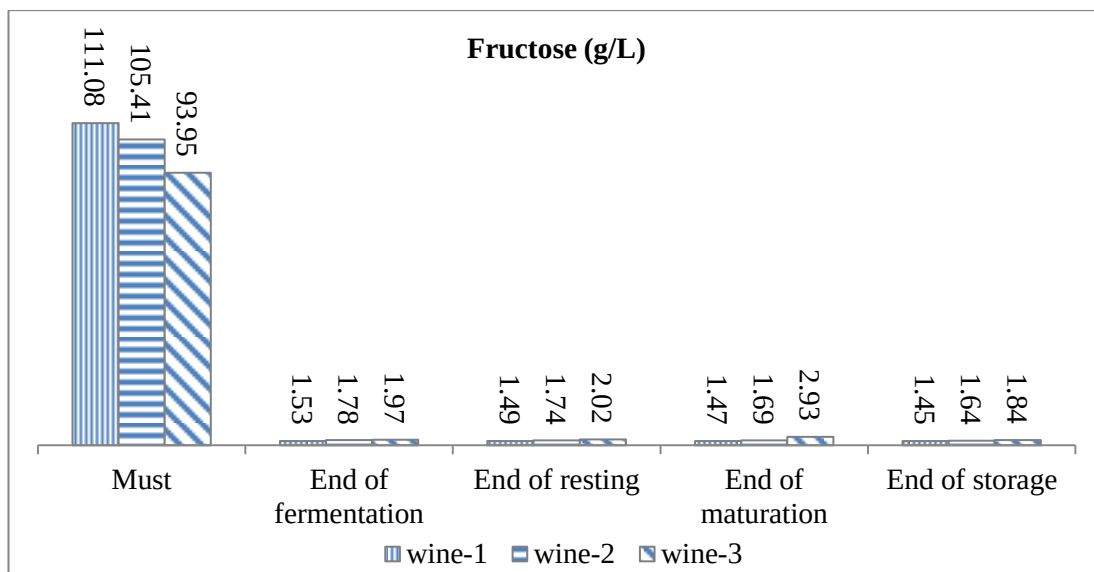


Figure 4.25 Fructose contents of must and wine samples (g/L)

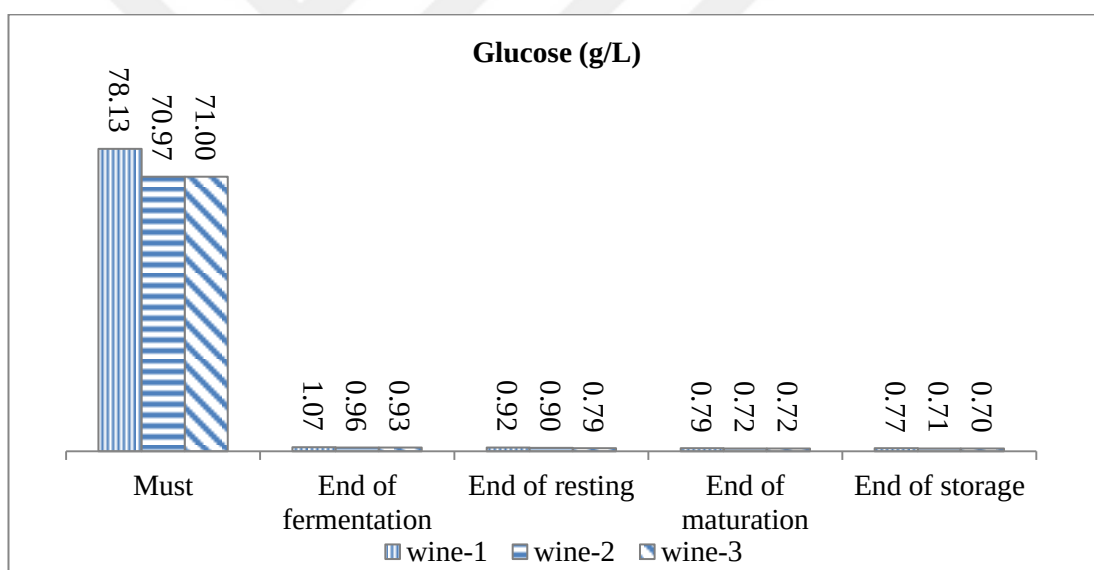


Figure 4.26 Glucose contents of must and wine samples (g/L)

The sucrose changes in must and wines are given in Figure 4.27. The sucrose content of the musts-1, 2 and 3 were 5.89, 3.92 and 2.73 g/L respectively. The values of “sucrose” for three types of musts are significantly different ($p < 0.05$) from each other. At the second day of fermentation, sucrose content of wines were reduced to 1.02, 0.93 and 0.32 g/L for wines-1, 2 and 3 respectively. There was statistically significant decrease in the sucrose content of red wines during fermentation ($p < 0.05$), also significantly different ($p < 0.05$) from each other at the end of fermentation. After

the end of fermentation, sucrose was undetectable. Maltose and rhamnose also was not detectable. The concentration of sucrose is generally low and ranged from 2.0 to 5.0 g/L in musts-1, 2 and 3. Sometimes this amount can be even higher (Canbaş, 2006). Sucrose is broken down by fermentation by yeasts, after conversion to glucose and fructose. For this reason, sucrose is not present in the wine if it is not subsequently added to the fermentation medium illegally. Sucrose is either added before fermentation or rarely naturally present in grapes. Sucrose is the main sugars used to increase the potential alcohol degrees of grapes. The reason for this is the low purity and price (Canbaş, 2006). Grapes are of great importance for yeast growth and metabolism. Non-fermented sugars such as arabinose, rhamnose, xylose and ribose are residual sugars (Jackson, 2000).

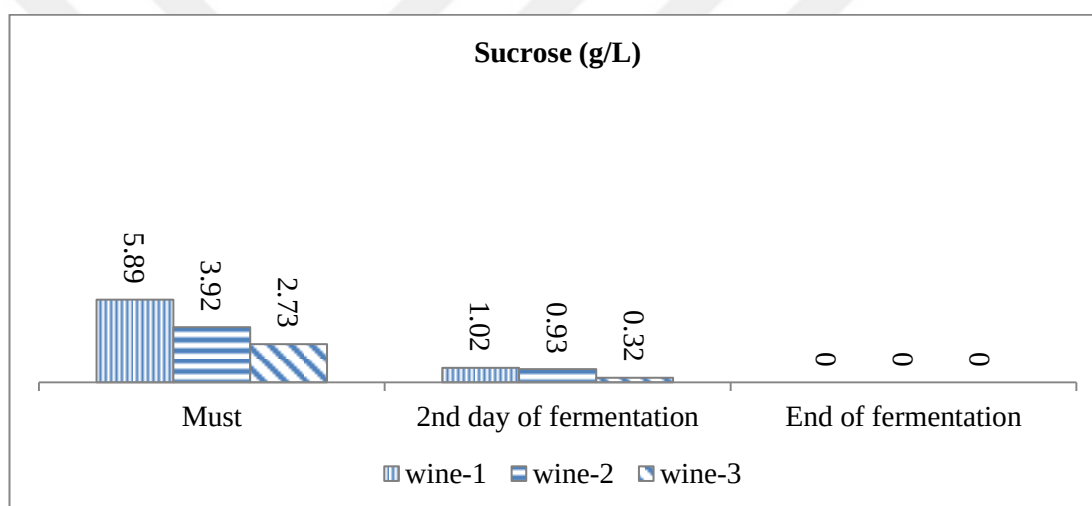


Figure 4.27 Sucrose contents of must and wine samples (g/L)

The main grape sugars (glucose and fructose) are important both in quantity and quality. They are found at equal ratio in mature grapes, but the amount of fructose is higher than glucose of grapes in excessive maturity. The amount of sugars is 150-300 g/L in grapes, sometimes reaching up to 350 g/L. It has reduction features and they may be oxidized. They are converted to gluconic acid as a result of oxidation. Quantities of sugars are determined by using this property (Canbaş, 2006). Fermentable sugars in grapes are broken down with yeast (*S. cerevisiae*) to alcohol. Therefore, there is a close relationship between sugar concentration and wine quality (Canbaş, 2006). The concentration of fructose is higher than glucose in overripe grapes. Intervarietal comparison of glucose/fructose ratio showed that the Hönüsü grape had higher glucose content than the Horoz Karası. Varandas et al. (2004)

observed the glucose content higher than the fructose content in 5 grape varieties at harvest stage. Sabir et al. (2010) indicated that glucose and fructose ranges from 86.4 to 107.0 g/L and from 80.4 to 94.1 g/L in grapes, respectively. Amerine et al. (1972) reported that the ratio of glucose/fructose in normal maturity is around 1, but may vary between 0.71-1.45 g/L depending on the variety. Şen (2014) reported that glucose contents of red wines produced from grape varieties grown in Turkey ranged from 0.267 to 15.687 g/L. In the same study, fructose contents of red wines were determined from 0.263 to 31.955 g/L. Sweetness varied in different sugars, it was higher in fructose than glucose and sucrose (Pangborn,1963; Doty,1976). Alcoholic fermentation is ended when the sugars of grape must are completely utilized (Boulton et al., 1996; Doyle et al., 2001). As a result, it is seen that glucose/fructose ratio is in accordance with literature values in red grape varieties and wines.

After the start of fermentation, sucrose was finished. The grape contains a physically veined structure. This structure consists mainly of xylem and phloem bundles. Xylems carry the substances necessary for the growth of grapes, such as water, minerals, growth factors, nutrient components, from the root to the fruit. Phloem bundles; the sucrose in the leaves is responsible for conveying the fruit of the grape. Sucrose is broken down into fructose and glucose by metabolic pathways (Greenspan et al., 1994). Wine is an alcoholic beverage obtained from grapes and during fermentation, glucose and fructose are converted to ethyl alcohol (Anlı, 2004; Yücel et al., 2001). Rusjan and Korosec-Koruza (2007) found that the of sucrose content ranged from 3.0 to 16.8 g/L, the glucose from 50.9 to 89.9 g/L, and fructose from 54.8 to 83.9 g/L respectively in red grapes. Şen (2014) reported that sucrose contents were not observed in red wines (type of grape wine) produced from grape varieties grown in Turkey.

Rhamnose was not be detected during wine process. Due to the fermentation process, glucose and fructose occur in low concentrations, and there is non-fermentable sugars such as rhamnose, xylose and arabinose in wines. Grape varieties may also include sugars from the hydrolysis of polysaccharides through the action of pectinase enzymes such as rhamnose (Apolinar-Valiente et al., 2015). Some oligosaccharides have physicochemical properties such as the ability to chelate cations in quality wine (Cescutti and Rizzo, 2001). Quijada-Morín et al. (2014) reported that high rhamnose

concentrations will reduce perceived astringency in wines. Coelho et al., (2018) indicated that rhamnose content ranged from 0.05 to 0.24 g/L in red wine.

4.1.9 Determination of Organic Acids in Must and Wine Samples

The organic acids were determined in three types of must and wine during fermentation, resting with oak chips at 7 days, maturation in glass carboy at 45 days and storage in glass bottles at 90 days. The analysis results in organic acids at 210 nm wavelengths are given in Table F.12. The tartaric acid content changes in must and wines are given in Figure 4.28. The tartaric acid content of the musts-1, 2 and 3 were 6.25, 6.27 and 6.17 g/L, respectively. The values of “tartaric acid” for wines-1 and 2 are significantly different ($p < 0.05$) from wine-3. At the second day of fermentation, tartaric acid content of wines were founded as 5.95, 5.65 and 5.41 g/L for wines-1, 2 and 3, respectively. At the end of fermentation, tartaric acid content of wines were founded as 5.81, 5.52 and 5.31 g/L for wines-1, 2 and 3 respectively. There was no significant decrease in the tartaric acid content of red wines during fermentation ($p > 0.05$). After the three types of wine were kept with oak chip, the tartaric acid contents were found to be 5.60, 4.85 and 4.78 g/L respectively. After 45 days maturation in glass carboys, tartaric acid contents were found as 4.85, 4.61 and 4.25 g/L for three types of wine respectively. After the storage in the bottle for 3 months, the tartaric acid contents for wines in order were 4.64, 4.43 and 4.19 g/L. While wine-1 had the highest tartaric acid content, wine-3 had the lowest content at the storage in bottle. The values of “tartaric acid” for wines-1 and 2 are significantly different ($p < 0.05$) from wine-3 at the end of storage.

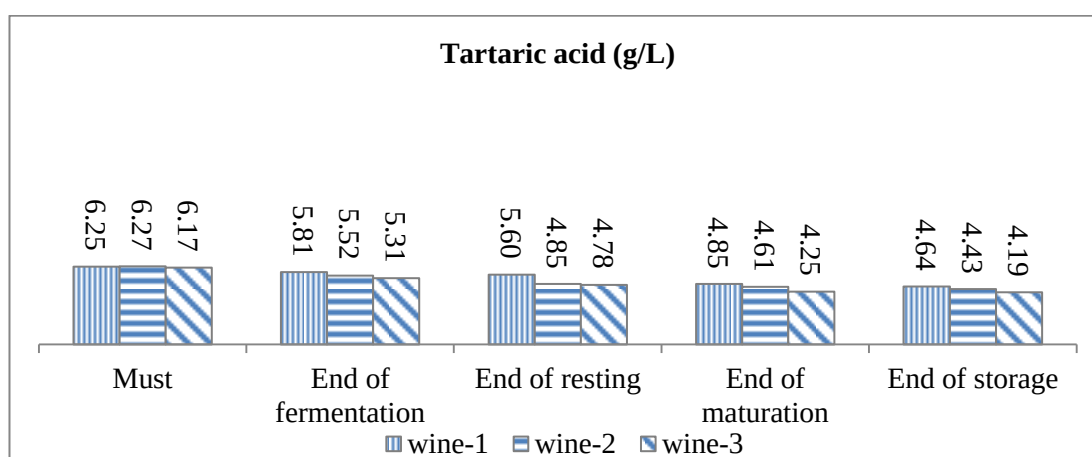


Figure 4.28 Tartaric acid contents of must and wine samples (g/L)

The malic acid content changes in must and wines are given in Figure 4.29. The malic acid content of the musts-1, 2 and 3 were 0.91, 0.86 and 0.81 g/L respectively. The values of “malic acid” for three types of musts are significantly different ($p<0.05$) from each other. At the second day of fermentation, malic acid content of wines were founded as 0.83, 0.80 and 0.79 g/L for wines-1, 2 and 3 respectively. At the end of fermentation, malic acid content of wines were founded as 0.79, 0.80 and 0.78 g/L for wines-1, 2 and 3 respectively. There was no significant decrease in the malic acid content of red wines during fermentation ($p<0.05$) except wine-1. After the three types of wine were kept with oak chip, the malic acid contents were found to be 0.77, 0.77 and 0.72 g/L respectively. After 45 days maturation in glass carboys, malic acid contents were found as 0.76, 0.72 and 0.70 g/L for three types of wine respectively. After the storage in the bottle for 3 months, the malic acid contents for wines in order were 0.75, 0.71 and 0.70 g/L. While wine-1 had the highest malic acid content, wine-3 had the lowest content at the storage in bottle. The values of “malic acid” for wines-2 and 3 are significantly different ($p<0.05$) from wine-1 at the end of storage.

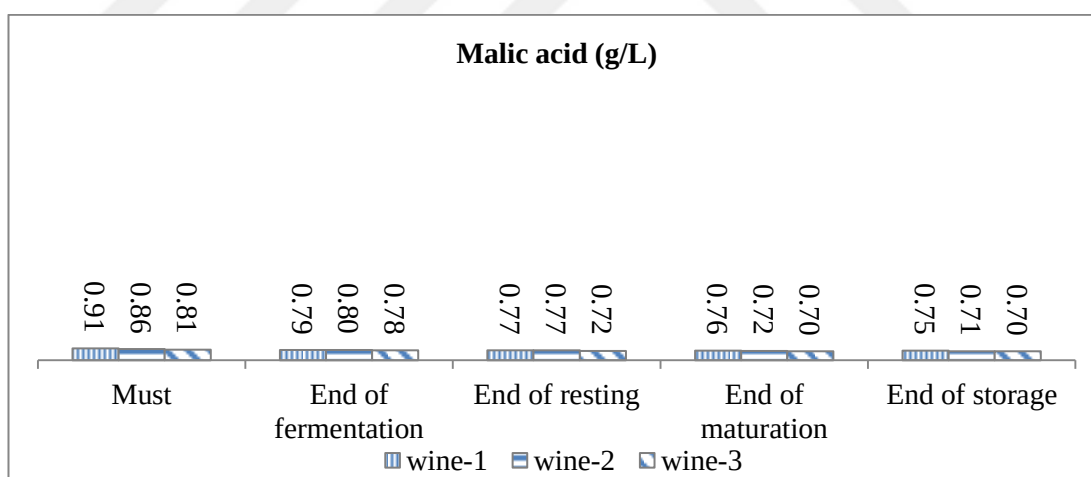


Figure 4.29 Malic acid contents of must and wine samples (g/L)

The citric acid content changes in must and wines are given in Figure 4.30. The citric acid content of the musts-1, 2 and 3 were 0.49, 0.47 and 0.47 g/L respectively. The values of “citric acid” for musts-2 and 3 are significantly different ($p<0.05$) from must-1. At the second day of fermentation, citric acid content of wines were founded as 0.42, 0.42 and 0.40 g/L for wines-1, 2 and 3 respectively. At the end of fermentation, citric acid content of wines were founded as 0.28, 0.27 and 0.24 g/L for

wines-1, 2 and 3 respectively. There was significant decrease in the citric acid content of red wines during fermentation ($p<0.05$). After the three types of wine were kept with oak chip, the citric acid contents were found to be 0.26, 0.24 and 0.21 g/L respectively. After 45 days maturation in glass carboys, citric acid contents were found as 0.25, 0.22 and 0.20 g/L for three types of wine respectively. After the storage in the bottle for 3 months, the citric acid contents for wines in order were 0.24, 0.20 and 0.19 g/L. While wine-1 had the highest citric acid content, wine-3 had the lowest content at the storage in bottle. The values of “citric acid” for wines-2 and 3 are significantly different ($p<0.05$) from wine-1 at the end of storage. The sugars (glucose, fructose, xylose and arabinose) are catabolized to lactic acid, acetic acid, ethyl alcohol and CO_2 , while citric acid is converted to acetic acid and carbonyl compounds, especially diacetyl having butter taste (Lonvaud-Funel, 1993).

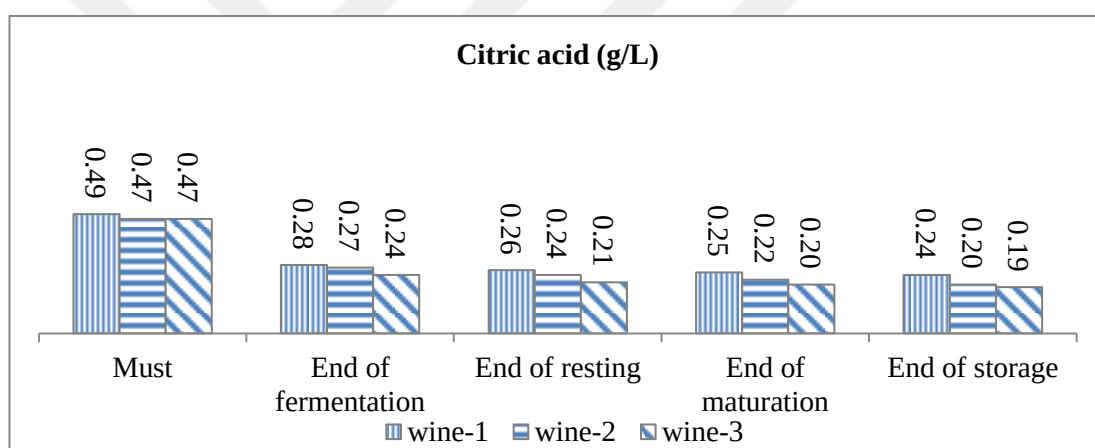


Figure 4.30 Citric acid contents of must and wine samples (g/L)

The succinic acid content changes in must and wines are given in Figure 4.31. The succinic acid content of the musts-1, 2 and 3 were 0.38, 0.39 and 0.37 g/L respectively. The values of “succinic acid” for three types of musts are significantly different ($p<0.05$) from each other. At the second day of fermentation, succinic acid content of wines were founded as 0.45, 0.41 and 0.40 g/L for wines-1, 2 and 3 respectively. At the end of fermentation, succinic acid content of wines were founded as 0.50, 0.48 and 0.45 g/L for wines-1, 2 and 3 respectively. There was significant increase ($p<0.05$) in the succinic acid content of red wines during fermentation. After the three types of wine were kept with oak chip, the succinic acid contents were found to be 0.51, 0.47 and 0.45 g/L respectively. After 45 days maturation in glass

carboys, succinic acid contents were found as 0.57, 0.45 and 0.41 g/L for wines-1, 2 and 3, respectively. After the storage in the bottle for 3 months, the succinic acid contents for wines in order were 0.54, 0.41 and 0.38 g/L. The succinic acid contents of red wines were decreased from end of fermentation to storage in bottle. This decrease is not significantly different ($p>0.05$). While wine-1 had the highest succinic acid content, wine-3 had the lowest content at the storage in bottle. Mean values of succinic acid content for wines are significantly different ($p<0.05$) from each other. Succinic acid occurs after yeast metabolism, so it is present in wine after fermentation (TGK, 2009).

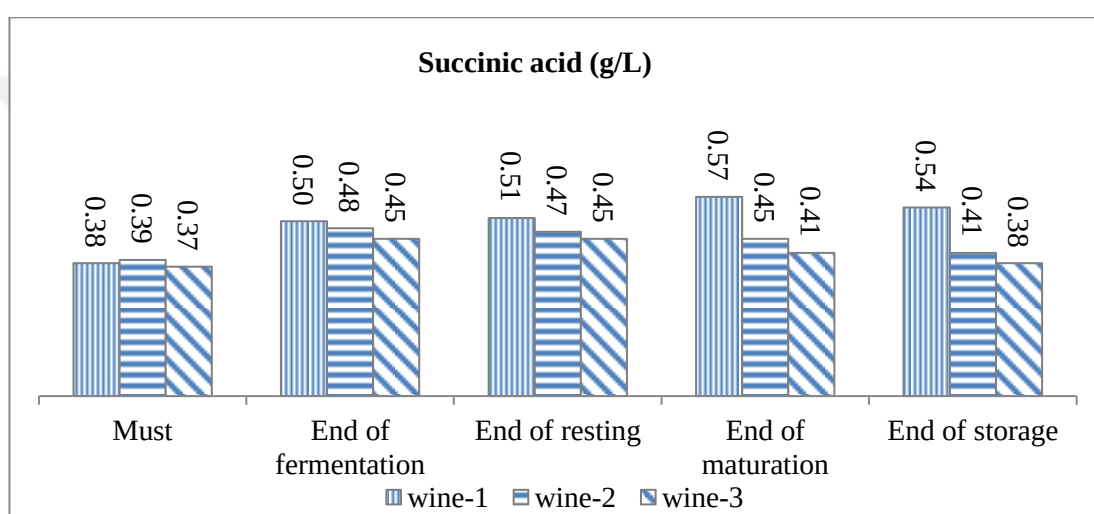


Figure 4.31 Succinic acid contents of must and wine samples (g/L)

Acids give wine fullness and freshness. When they are very small, the taste is perceived as uninspired, and when they are high, the taste is perceived as very harsh. Organic acids also prevent the growth of pathogenic microorganisms. In red wine production, they facilitate the dissolution of color substances during fermentation and make the color more vivid (Canbas, 1992). The presence of sufficient tartaric acid in the wine improves the taste and aroma of the wine. They are also of great importance in the formation of wine acidity and stability of wine (Lamikanra, 1997). Tartaric, malic, citric and succinic acids were found in the must and wines. Tartaric acid was found to be higher than other acids. It is seen that the amount of tartaric acid in wines decrease during process. The wines were not applied malolactic fermentation process. Malolactic fermentation is the breakdown of malic acid by lactic acid bacteria into lactic acid. Tartaric, malic and citric acids result from grape, while

succinic acids are produced during the fermentation (Mato et al., 2005). Tartaric and malic acids are main acids in formation of the acidity of the wine and determine the pH. These two acids serve as indicators for determining the ripening level of grapes in wine science. The amount of tartaric acid in wine is considered a critical control point in determining the stability of the wine (Lamikanra et al., 1995). Tartaric acid increases the natural aroma of fruits. Among the organic acids, it is the most soluble acid in water and has the most sourness (Liebrand, 1992). It is the main acid that determines the acidity of the wine and ensures that the pH of the wine is between 3.0-3.5. The amount of tartaric acid in wine can vary between 1.5 g/L–3.0 g/L (Usseglio-Tomasset, 1995). Malic acid also affects the acidity of the wine. After tartaric acid, the highest amount of acid in wine is malic acid. It is also the substrate of lactic acid bacteria, which leads to deterioration of wine. Therefore, it is not desirable to have more malic acid in the wine. Malic acid in excess; microbial, physical, biochemical stability of wine and negatively affect the quality of wine (Delcourt et al., 1995). Tartaric acid/malic acid ratio is an extremely important factor for wine grape varieties and determines the stability and quality of wine made from grapes (Kanellis and Roubelakis-Angelidakis, 1993). If the ratio of these acids is about 2 or less, the wine will have a sour taste. But, wine have best taste and bouquet with a ratio of tartaric to malic acid equal coefficient 3 and more (Kasai et al., 2004; Spasov et al., 2010). Citric acid is an acid capable of slowing yeast growth and its concentration in wine or wine ranges from 0.5-0.7 g/L (Swiegers et al., 2005). Succinic acid is one of the non-volatile acids formed as a result of fermentation in wine. The concentration in wine is around 1 g/L (Boulton et al., 1996). The total acid content of the wine depends on the ratio of the amount of tartaric acid to the amount of malic acid and the amount of potassium ions in the medium. If the tartaric acid content of the wine is high and the amount of potassium ions is low, the pH of the wine will also be low (Boulton, 1996). Organic acids form potassium, calcium and magnesium salts in wine. Thus, a reduction in the amount of acid can be observed during wine process (Torija et al., 2003). For instance, tartaric acid is neutralized by the formation of tartarate salts and the perception of sour taste is reduced (Mato et al., 2005). This is called “chemical acid reduction” in wine science (Bartowsky et al., 2003). Tartaric acid cannot be used by microorganisms. Therefore, the reason for the decrease in tartaric acid amounts in wines can only be attributed to the chemical acid reduction. The reason why malic acid and citric acid amounts in wines are lower than

that of musts is that they are used by microorganisms and they form salt. Tartaric, malic and citric acids are synthesized in grapes (Romero and Munoz, 1993). The presence of sufficient tartaric acid in the wine improves the taste and aroma such as hard taste, malic acid considered as sour (sourness of green apples) and citric acid yielding a fresh taste. It is also of great importance in the formation of wine acidity and stability of wine (Lamikanra, 1997). Succinic acid adds a bitter and salty aroma to the wine (Boulton et al., 1996). Yeast cells rapidly synthesize succinic acid from the sugar substrate in wine. The formation of succinate depends on the type of yeast or the amount of 2-oxoglutarate in the medium. After the end of alcohol fermentation, the formation of succinic acid stops. Succinic is an acid that cannot be metabolized by bacteria such as tartaric acid. It is a stable acid (Coulter et al., 2004). It can be said that organic acid content differences of grape varieties are due to pH. The potassium content of grapes increases during maturation. The potassium transition to grape, reduces acidity and raises the pH of the fruit juice resulting in the formation of potassium bitartrate. Wine grape varieties form high sugar and tartaric acid. The formation of malic acids in grape depends on the degree of malic enzyme and malic dehydrogenase activity (Hawker, 1969). The low concentrations of citric acid may be in order to that this acid is converted into malic acid during maturation in wine (Mato et al., 2007). The amount of malic acid may vary between 1-10 g/L depending on climate conditions. Malic acid content is higher in grapes grown in cool climatic conditions. Because of cool climatic conditions, the respiration rate of grapes is slower than in warm climatic conditions. This slows down causes the degradation of malic acid in grapes (Redzepovic et al., 2003). Because of the degradation of carbohydrates in places where the temperature is high, acidity decreases due to the use of malic acid in respiration. Since Gaziantep region has warm climatic conditions, the amount of malic acid in grapes is low as indicated in this research from 0.81 to 0.91 g/L.

Demiray (2006) reported that grape must average tartaric, malic, citric and succinic acid amounts respectively; 4.84-5.18 g/L, 1.48-2.64 g/L, 0.25-0.52 g/L and 0.33-0.52 g/L. In that study, the average tartaric acid content of the wines is 4.63-5.34 g/L, the amounts of malic acid are 1.4-2.60 g/L, the amounts of citric acid are 0.20-0.50 g/L and the amounts of succinic acid are 0.30-0.45 g/L in wine obtained from Turkey. Şen (2014) reported that tartaric, malic, citric and succinic acid contents were; 876-

3009 mg/L, 16-1527 mg/L, 0.6-255.0 mg/L and 1050-8478 mg/L respectively in red wines obtained from grapes grown in Turkey. Romero et al. (1993) determined the amount of tartaric acid as 4.07-7.65 g/L, malic acid as 1.99-2.9 g/L, citric acid as 0.25-0.35 g/L in red grape must. It is common for different grape varieties and different climatic conditions to create differences in organic acid distribution in must. Perez-Rui et al. (2004) found that tartaric acid was 3.2-2.2 g/L and citric acid was 0.066-0.054 g/L in red wines. Castineira et al. (2002) found the amount of malic acid in red wine samples between 2.84 and 1.51 g/L. Romero et al. (1993) found that the amounts of succinic acid in red wines mean 0.48-1.22 g/L. Malic acid, citric acid, succinic acid and tartaric acid results were in agreement with the literature. The amount of tartaric acid in wine varies according to the year, grape variety, the alcohol degree of the wine and also the type and character of maturation and resting (Akman, 1951).

4.1.10 Results of Sensory Analysis

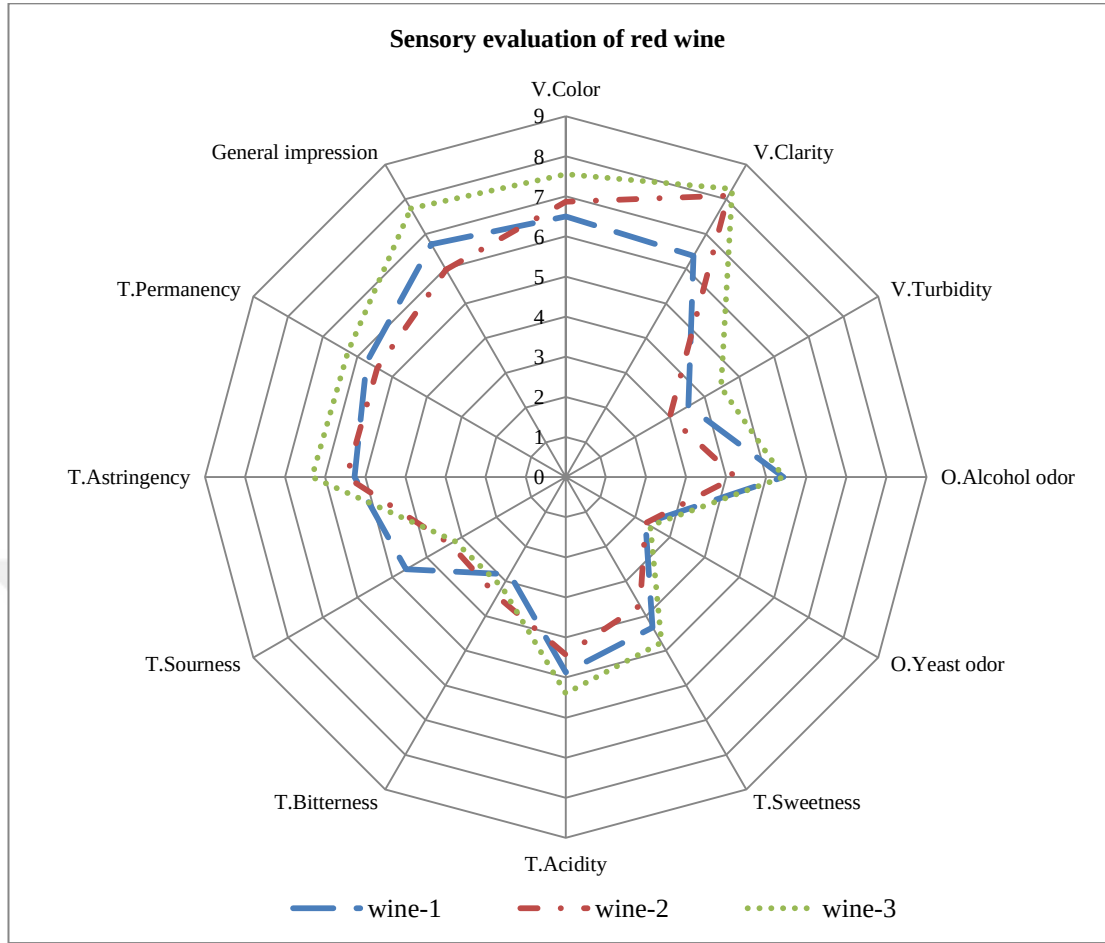
The sensory effect after 90 days storage of red wines made with three different concentrations of Hönüsü and Horoz Karası grapes on visual, odor and taste properties was investigated (Çopanoğlu and Boyacıoğlu, 2006). In the sensory analysis of the wines, the panelists were firstly informed about the terminology; about descriptors (color, aroma, fullness, sweetness and lack of etc.) were clearly determined for each sample. In the analysis performed by a group of 11 panelists consisting of lecturers and students of the Department of Food Engineering and Department of Gastronomy in Gaziantep University, the panelists evaluated the wines according to 12 different criteria over 10 points. ANOVA results of wine during production are given in Tble 4.13 and these results are shown in Figure 4.32 on the spider web diagram for better visualization. In terms of clarity, wine-3 takes the first place with 8.30, wine-2 with 8.10 and wine-1 followed the with 6.37. The clarity evaluation of wine-1, 2 and 3 are significantly ($p < 0.05$) different from each other. In terms of turbidity, wine-3 takes the first place with 4.47, wine-1 with $3.53 \pm$ and wine-2 followed the with 3.00. The highest alcohol odor score with 5.43 was obtained with wines-1 and 3, while the lowest score with 4.23 was obtained for wine-2. The turbidity and alcohol odor evaluation of wine-1, 2 and 3 are significantly ($p < 0.05$) different from each other (except alcohol odor for wines-1 and 3). The

highest yeast odor score was 2.43 for wine-3, while wine-1 and 2 received 2.30 points. The yeast odor evaluation of wine-1, 2 and 3 are not significantly ($p>0.05$) different from each other. In terms of sweetness, wine-3 had the highest score with 4.77, followed by 4.33 and 3.70 to wines-1 and 2 respectively. Wine-1 had the highest tartaric acid which is neutralized by the formation of tartarate salts and the perception of sour taste is reduced (Mato et al., 2005). Wine-3 had the highest score with 5.40 in terms of acidity, which is among the parameters of taste evaluation section, wine-1 and 2 were followed 4.87 and 4.43 value respectively. The sweetness and acidity evaluation of wine-1, 2 and 3 are significantly ($p<0.05$) different from each other. The unwanted bitterness of the wines remained below 3.44 points in all three types of wine. The bitterness evaluation of wine-1, 2 and 3 are not significantly ($p>0.05$) different from each other. Wine-2 and 3 were perceived high bitterness than wine-1, because they had a higher flavonols. Bitterness is also able to be elicited by some flavonols, hydroxycinnamic and hydroxybenzoic acid derivatives. Succinic acid also adds a bitter and salty aroma to wine (Boulton et al., 1996). In terms of sourness, wine-1 was scored the highest value with 4.60. Wine-1 had the highest tartaric, malic and malic acid content, which the presence of sufficient tartaric acid in the wine improves the taste and aroma such as hard taste, malic acid considered as sour (sourness of green apples) and citric acid yielding a fresh taste (Lamikanra, 1997). Red wine is important in terms of quality in terms of astringency, wine-3 scored the highest with 6.33. The astringency is an mouth sensation involving dryness and puckering. Wine-1 and 2 were perceived less astringent than wine-3, because the former they had a lower procyanidin and catechin. In terms of overall impression and permanency, wine-3 received the highest score, followed by wine-1 and wine-2. These difference of wine-1, wine-2 and wine-3 are significantly different from each other ($p<0.05$). As seen in Table F.13 red wine color evaluation as a result of the wine-3 wine color score was found to be higher. The color evaluation of wine-1, wine-2 and wine-3 are significantly different from each other ($p<0.05$). The difference in grape varieties affected the color scores of wine in sensory sense. Horoz Karası grape has caused more phenolic compound and/or color transition to wine. This was also the case in the analysis of color tone (H^*), color intensity (C^*) and phenolic compounds by HPLC. As a result of sensory analysis, high turbidity, perception of less alcohol and high bitterness caused wine to score lower. It was thought that the wine-3 wine scored slightly higher than the other two red wines

because of the relationship between the color, clarity and astringency of the wine. Color is one of the most basic parameters of wine quality and one of the most important factors affecting the total acceptability of consumers (Peynaud, 1987). Anthocyanins, the main compound that gives color to red wine, occur during the ripening of grapes (Kennedy et al., 2006). These coloring compounds in wine are phenolic compounds contribute to the sensory properties of wine as well as color. Some of these compounds give wine astringency or bitterness, while others give odor and aroma (Cheynier et al., 2006). The sources of compounds that provide sensory properties in wine are known as grape-derived compounds, formed during fermentation and aging (Ribereau-Gayon et al., 1974). The bitterness in wine is caused by monomeric flavanols (catechins) in the bitterness nucleus, and the astringency is due to polymeric flavanols (tannins) in the shell (Adams, 2006). It is known that the sensory properties of phenolic compounds change with the interaction of ethyl alcohol, glycerol, salts, acids and other macromolecular components in wine (Cheynier et al., 2006). Phenol acids have little effect on the sensory properties of wine. However, some microorganisms (*Brettanomyces* and some bacteria) synthesize volatile phenol acids, and some of these adversely affect the aroma. For example, ethyl phenol gives an animal odor. In addition, the breakdown of p-coumaric acid and ferulic acid produces vinyl phenol, which gives the wine an odor similar to oil paint odor (Ribereau-Gayon et al., 2000b). Phenolic compounds are a source of astringent taste in foods (6-8 monomers of procyanidins) and bitter (3-5 monomers of procyanidins). These compounds complex with proteins to form sediment and negatively affect clarity (Ekşi and Karadeniz, 2002). They also play an important role in color changes (enzymatic browning and black refraction in wines) (Kılıç, 1990; Ekşi and Karadeniz, 2002). *S. cerevisiae* has been used in the production of alcoholic beverages for at least eight thousand years. This yeast ferments sugars, together with ethanol and CO₂ formation together with an amount of many low molecular weight fragrance compounds. These alcohols, aldehydes, organic acids, esters, organic sulfides and carbonyl compounds have a strong influence on product quality. In fact, the delicate aroma balance of these compounds in fermented foods and beverages is often used as organoleptic fingerprints for certain products and brands (Hazelwood et al., 2008).

The sensory values are between 1 and 10 points. The values increase from the center to the outside of diagram. When the sensory analysis results were examined, it was observed that according to the spider web diagram (Figure 4.1). Wine-1 had the lowest score in terms of color and clarity, and its image was lower than other wines. Wines-1 and 2 had the medium red color, but wine-3 had the deep red color (Figure D.4). At the same time, wine-1 had the highest sourness and low bitterness points in terms of taste. It was observed that wine-2 had the lowest score as turbidity in the determined visual parameters and its appearance was more vivid than other wines. Wine-2 was closed to center of diagram interms of alcohol and yeast odor. Alcohol odor was dominant in wines-1 and 3. Yeast odor was closed to center for all wines, so they were not perceived yeast odor. In terms of taste, wine-3 had the highest scores in sweetness, acidity and astringency. Because, tannins give astringency taste to wines. Wine-3 was richest interms of tannins. Wine-3 had the highest score in terms of color, clarity and turbidity among the determined visual parameters, its overall impression and permanence is higher than other varieties. Sourness of wine-1 was very close to outside of diagram, since it had tartaric acid more than the other wines. Undesirable bitter taste in red wines is the highest in wine-2, because of its succinic acid content is higher than other wines. Because succinic acid gave the wines a bitter and salty taste. As a result of wine-2 had the lowest persistence in the mouth and the most unpopular wine in general impression.

Figure 4.32 Sensory evaluation on the spider web diagram for red wines



Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası

It is almost impossible to determine the unique properties and varieties of wine based on the chemical analysis of the quality of the wine. In order to define the character, type, development, age and other differences of wine, it is necessary to make use of sensory analyzes as well as chemical analyzes. Wines having the same chemical properties of each other may be different in terms of taste and aroma and this difference is determined by sensory analysis (Güven, 2008). In this study, sensory analysis was performed in addition to chemical analysis. In the panel which was attended by eleven people, wines were presented at 18°C. According to Troost (1988), the best sensory analysis temperature for red wines is 16-18°C. In addition, thin-walled goblets with the same characteristics were used. Water was used to neutralize flavors between tastings. In order to ensure the accuracy of the results, three replications were performed at regular intervals. In addition, during the panel, some of the participants gave some opinions about the sensory properties of the

wines. The wine-2 had a chemical odor and is interpreted as not good. The clarity of wine-1 was found to be insufficient. Cliff et al. (2006) stated that wines with high phenolic and tannin content had the highest astringency and contraction in the mouth. In our study, wine-3 had the highest phenolic compounds, it was found the highest astringency compared to the others. It is known that phenolic compounds in wine contribute to sensory properties such as color, bitterness and astringency (Bimpilas et al., 2015). Kelebek et al. (2010) indicated that general impression parameters of the wines of Denizli region is preferred in terms of color, taste, body and general impression. According to the results of sensory analysis, the wine to be more of concentration of Horoz Karası grape is dark red which plays more violet than the others in our study. At the same time, it is wine that is clearer and the smell of alcohol is perceived more intensely. It has been determined that the most sweet taste which is among the parameters of taste evaluation section is found in wine-3. Wine-1 and wine-2 were found to be more sour than the other. The important astringency in red wines was found to be balanced in all wines. Permanence and overall impression left on the palate were the most admired in wine-3.

CHAPTER V

CONCLUSION

In this study, it is aimed to examine the physical, chemical, microbiology, color, phenolic compound, sugar, organic acid and sensory properties of Hönüsü and Horoz Karası grape must mixtures and red wines obtained from these grapes which are one of the domestic wine types of Gaziantep in Turkey. The identification and evaluation of phenolic compounds, sugar and organic acid content of Hönüsü and Horoz Karası must mixtures and red wines obtained from Gaziantep region were performed by HPLC method. As a result of the chemical analyzes carried out on must, total acidities were 3.80 g/L, 4.84 g/L and 4.95 g/L in must-1, 2 and 3 respectively. During fermentation, titration acidity increased continuously, but at the end of maturation, titration acidity of three types of wine decreased due to the precipitation of potassium bi tartarate salt. The density and brix amounts of the three types of wine changed depending on the amount of alcohol present in the wine. The alcohol content of wine samples ranged from 12.58 to 12.66%. The amount of alcohol present in the wine changes depending on the amount of sugar in the must at inially. The brix values were 22.78, 22.73 and 22.77 in must-1, 2 and 3 respectively. As a result, must-1 has higher sugar content than the must-2. Wine-1 had highest alcohol content due to the it has highest Brix value in must. There was a relationship between the value of alcohol, Brix and density of three types of wine. During fermentation, sugar was used by yeasts and produced alcohol. Thus, as the amount of alcohol increases, the Brix (soluble solid) value of the wine varieties decreases. In addition, the density decreased due to alcohol increase. A total of ten phenolic compounds, gallic acid, tyrosol, (+)-catechin hydrate, procyanidin B₂, p-coumaric acid, routine hydrate, chlorogenic acid, myricetin, quercetin and resveratrol compound were identified in three types of must mixtures and wines. Procyanidin B₂ was more abundant than other phenolic compounds. The most common phenolic compounds after procyanidin B₂ in Hönüsü and Horoz Karası mixtures were (+)-catechin and gallic acid respectively. Thus, it is understood that tannin amount of grapes grown in

Gaziantep region is quite high. These wine types had most bitterness and astringency taste. In this study, the amount of the phenolic compounds in all of the wine types increased during fermentation. When the results of the research are evaluated on the basis of varieties of wine; wine-3 is generally rich in phenolic compounds and has shown that more powerful body wines. In addition, wine-3 is thought to be more astringent and has higher antioxidant properties and may be more beneficial for cardiovascular health if consumed in appropriate amounts. A total of three sugar content, fructose, glucose and sucrose were identified in must mixtures. The fructose was more abundant than other sugars. A total of three organic acid content; malic, tartaric, citric and succinic acid content were identified in must mixtures and wines. The amount of fructose, glucose and sucrose was found to be highest in musts. Tartaric acid was more abundant than other organic acid contents in must and wines. According to the analysis results; the amount of sugar and organic acid in must mixtures and wines are generally in agreement or partially higher with other studies. In storage, the organic acid content was highest in wine-1. Yeast count was performed as microbiological analysis in Hönüsü and Horoz Karası wines. The yeasts were not completely inactivated in the process from fermentation to bottling. This is because the yeast is resistant to SO₂, which we add at certain times and amounts in winemaking. It was found that the color of wine-3 was darker than the other two wines from a sensory perspective. The reason for this is that the skin color of the Horoz Karası grape is darker than the Hönüsü grape. During the fermentation, the color of the grape skin effected to wine. According to the sensory analysis general impresssion value was closely each other. High wine color intensity values were observed for wine-3. Accordingly, wine-3 color was darker (lower L* values) and more brightly colored (higher C* values). Wine-1 and 2 had the lowest wine color density were lighter (higher L* values) and slightly duller (low C* values). C* values in the must mixtures higher than wines at the end of bottling. So, the must samples more vivid than wine samples. In this study, the usability of Hönüsü and Horoz Karası grapes in red wine production was investigated as an alternative to wine grapes having wide vineyard soils in Southeastern Anatolia. In general, it was concluded that wine can be obtained from all three concentrations obtained from the Hönüsü and Horoz Karası grapes grown in Gaziantep. Finally, at the end of 147 days production process of the wines, according to sensory analysis and phenolic compound contents results, wine-3 (70% Hönüsü grape must + 30% Horoz Karası

grape must) gives better quality wine in terms of taste and appearance than other varieties. Moreover, wine-3 has higher antioxidant properties and may be more beneficial for cardiovascular health if consumed in appropriate amounts. These results could be of great interest for nutritionists and dietitians for assessment of dietary phenolic compounds intake in Turkey. Considering the different concentrations, sugar content and processing techniques of Hönüsü and Horoz Karası grape varieties, it is useful to carry out similar researches on this subject. In addition to this, the data obtained from these and similar studies should be shared with the industry and guided for the stronger opening of South-east Anatolian wines to the world market in terms of quality. This research can help the economic development of Gaziantep.



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APPENDICES
APPENDIX A THE MATERIALS OF MUST AND WINES



Figure A.1 Grape crushing (Hönüsü)



Figure A.2 Grape crushing (Horoz Karası)



Figure A.3 Fermentation stage in plastic demijohns with airlock



Figure A.4 pH meter



Figure A.5 Acidity experiment apparatus



Figure A.6 Refractometer



Figure A.7 Ebulliometer



Figure A.8 Hunter Lab apparatus



Figure A.9 Volatile acidity apparatus



Figure A.10 HPLC equipment

APPENDIX B THE STORAGE STAGES



Figure B.1 Cork closure at the semi automatic sealing machine



Figure B.2 Ready-to-store bottles



Figure B.3 Wine storage

APPENDIX C HPLC

Table C.1 Retention time and maximum absorbance of phenolic compounds

Phenolic compound standards	Retention time (minute)	Maximum absorbance (nm)
Procyanidin B ₂	67.9	280
Myricetin	72.1	280
Quercetin anhydrous	68.7	320
(+)-Catechin Hydrate	41.2	280
Rutin Hydrate	70.0	280
p-coumaric acid crystalline	69.4	320
Gallic acid-1-Hydrate extra pure	8.5	280
Chlorogenic acid crystalline	35.7	280
Resveratrol	68.2	320
Tyrosol(4-hydroxyphenylethyl alcohol)	24.0	280

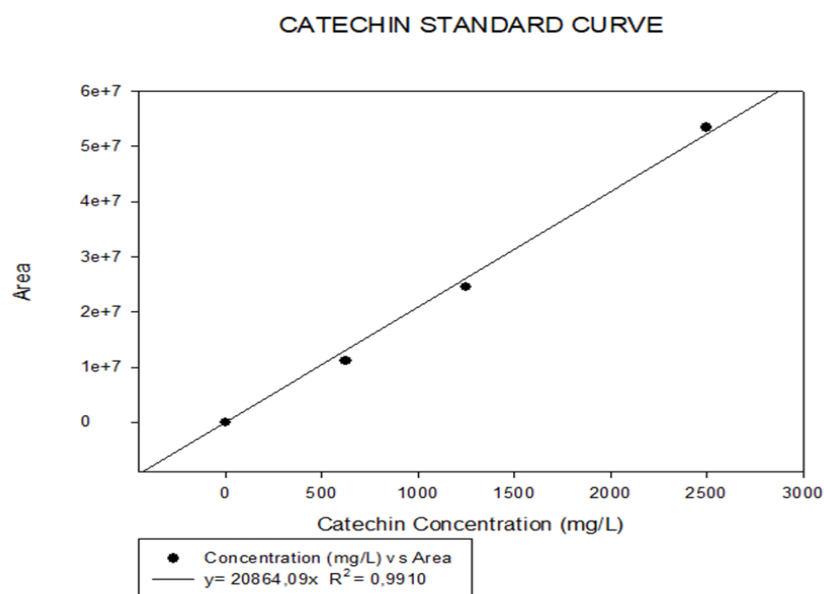


Figure C.1 Standard curve example for phenolics

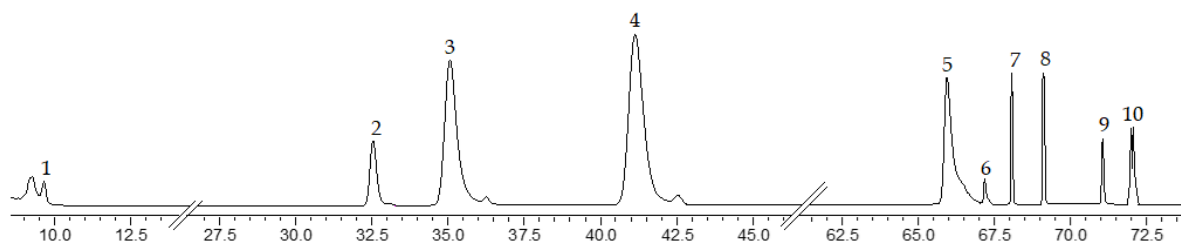


Figure C.2 Chromatogram of 10 phenolic standards. (1) gallic acid, (2) tyrosol, (3) chlorogenic acid, (4) (+)-catechin, (5) procyanidin B₂, (6) resveratrol, (7) quercetin, (8) *p*-coumaric acid, (9) rutin, (10) myricetin.

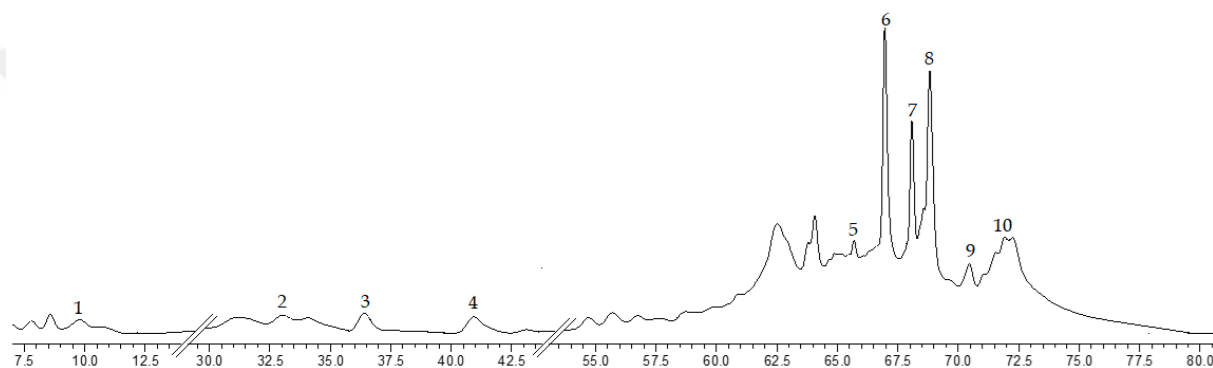


Figure C.3 Phenolic compounds detected in the red wine samples after maturation. (1) gallic acid, (2) tyrosol, (3) chlorogenic acid, (4) (+)-catechin, (5) procyanidin B₂, (6) resveratrol, (7) quercetin, (8) *p*-coumaric acid, (9) rutin, (10) myricetin.

Table C.2 Retention time and maximum absorbance of sugar compounds

Sugar standards	Retention time (min.)	Maximum absorbance (nm)
Glucose	11.9	210
Fructose	12.6	210
Sucrose	9.5	210
Maltose	10.2	210
Rhamnose	12.9	210

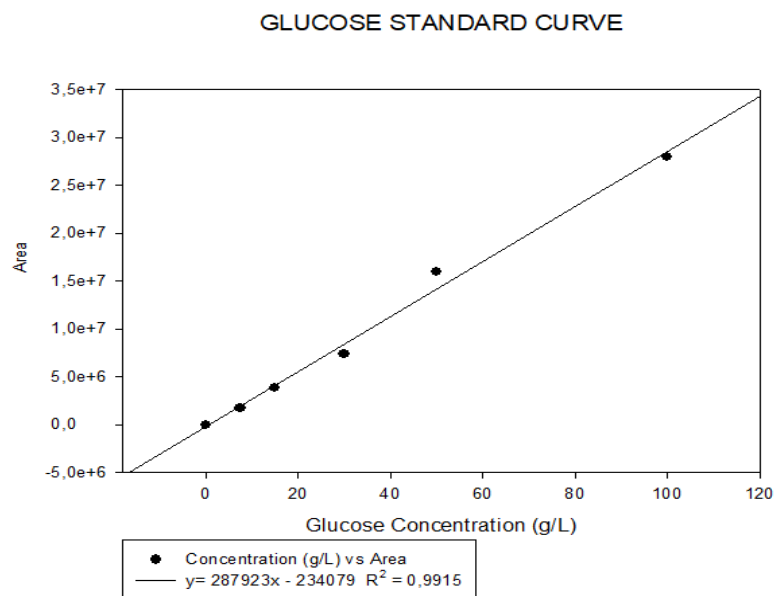


Figure C.4 Standard curve example for sugars

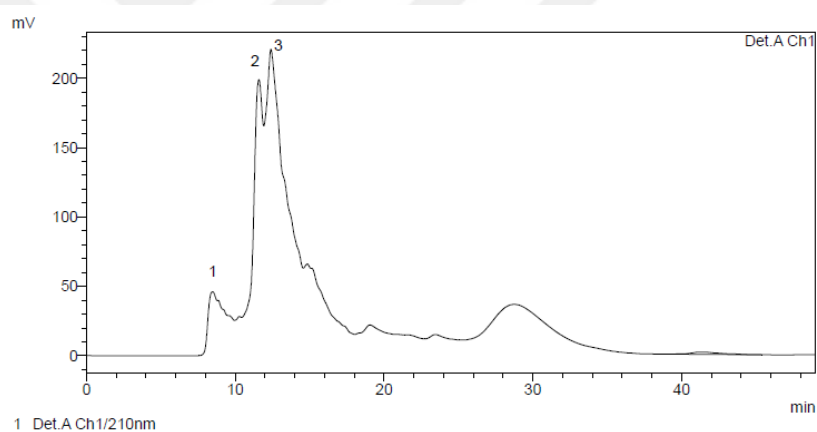


Figure C.5 HPLC chromatograms of sugars at 210 nm. Peaks Assignment : (1) sucrose, (2) glucose, (3) fructose.

Table C.3 Retention time and maximum absorbance of acid compounds

Acid standards	Retention time (min.)	Maximum absorbance (nm)
Tartaric acid	11.1	210
Malic acid	12.0	210
Citric acid	9.8	210
Succinic acid	16.6	210
Lactic acid	6.3	210

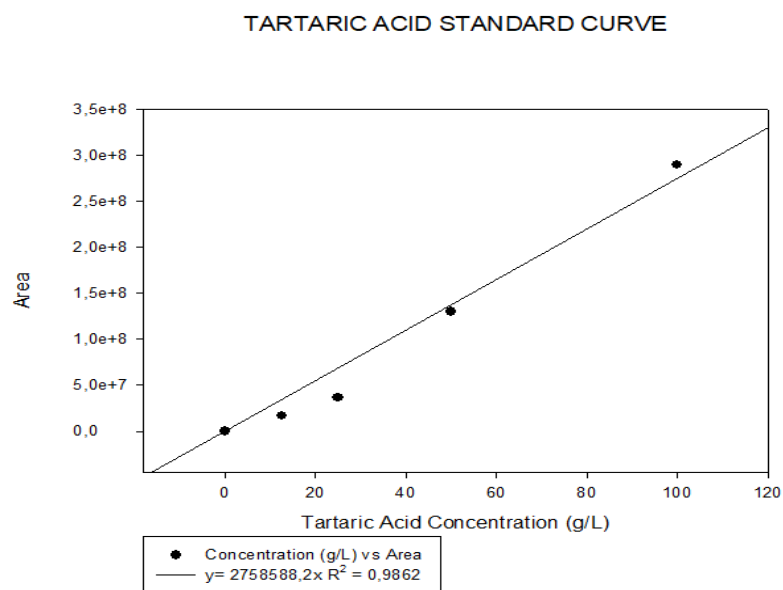


Figure C.6 Standard curve example for organic acids

==== Shimadzu LC solution Analysis Report =====

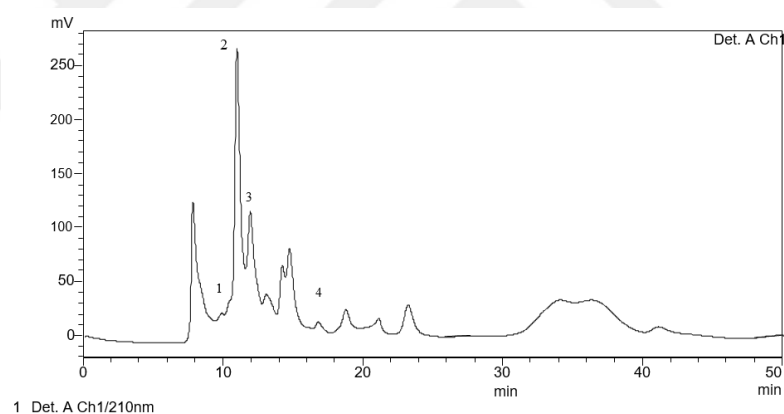


Figure C.7 HPLC chromatograms of organic acids at 210 nm. Peaks Assignment : 1 citric acid, 2 tartaric acid, 3 malic acid, 4 succinic acid.

APPENDIX D THE SENSORY ANALYSIS OF WINES



Figure D.1 Sensory analysis



Figure D.2 Sensory evaluation



Figure D.3 Sensory analysis room

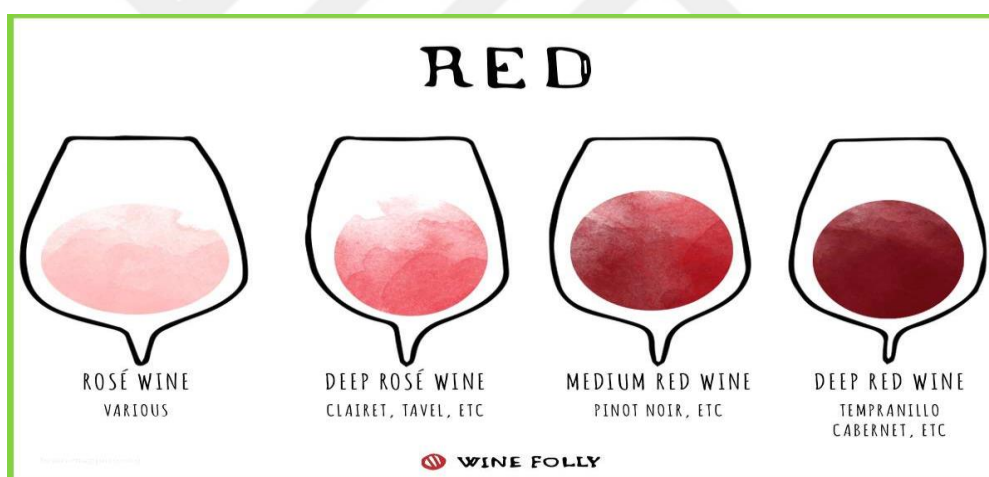


Figure D.4 Color chart for sensory analysis

Figure D.5 Flavor and aroma profile analysis for red wines

Please first evaluate the color of the sample, then smell and evaluate the smell and then taste.

IMAGE

Observe the glass of wine on the table or in the hand, from the top. Please tilt the glass at a 45° angle to the front and / or back to examine the central color, clarity and blur in the center on a white background. After shaking the goblet in a circular motion, examine again at eye level. Please mark your observations by placing a dot in the appropriate arrows below.

Color

Light red

Medium red

Dark red

Violet red



Clarity

Dull

Clear



Turbidity

Available

Absent



NOSE

Smell it immediately after shaking the glass in a circular manner. Smell again and again in different ways for more impressions. If you are having trouble smelling (to awaken the non-smelling nose or to prevent a possible mistake), shake the glass with the palm of your hand and rinse it again and smell it again.

Alcohol Odor

Less

More



Yeast Odor

Less

More



MOUTH

Drink 1-2 sips of water to neutralize the flavors between the two wines. Sip a teaspoon of wine. Spread the wine with some air all over the mouth surface, swallow some and then breathe, move the wine carefully in the mouth and swallow some more. Mark your observations as dots on the following arrows.

Sweetness

Less

More



Acidity

Less

More



Bitterness

Less

More



Tartness

Less

More



Sourness

Less

More



Permanence

Short

Long



General impression

Less

More



APPENDIX F THE STATISTICAL ANALYSIS RESULTS OF WINES

Table F.1 pH and total acidity changes during red wine production*

	pH			Total Acidity		
Time (day)	Wine					
	1	2	3	1	2	3
Must	3.52±0.02 ^{aA}	3.47±0.02 ^{aB}	3.42±0.03 ^{aC}	3.80±0.02 ^{aA}	4.84±0.02 ^{aB}	4.95±0.05 ^{aC}
1	3.63±0.02 ^{bA}	3.59±0.01 ^{bB}	3.62±0.01 ^{cC}	4.01±0.01 ^{bA}	5.71±0.02 ^{bB}	5.56±0.02 ^{bC}
2	3.40±0.02 ^{cA}	3.38±0.02 ^{cB}	3.51±0.02 ^{bC}	4.52±0.02 ^{cA}	4.74±0.02 ^{aB}	4.74±0.04 ^{cC}
3	3.43±0.01 ^{cA}	3.45±0.02 ^{aA}	3.51±0.02 ^{bA}	5.05±0.05 ^{dA}	4.80±0.01 ^{aB}	4.63±0.02 ^{dC}
4	3.43±0.02 ^{cA}	3.49±0.02 ^{aA}	3.45±0.02 ^{aA}	5.78±0.02 ^{eA}	5.36±0.02 ^{cB}	5.67±0.01 ^{eC}
5	3.47±0.02 ^{dA}	3.57±0.02 ^{bB}	3.56±0.03 ^{cC}	6.45±0.02 ^{fA}	6.55±0.02 ^{dB}	6.61±0.02 ^{fC}
Resting with Oak chip (7 days)						
12	3.55±0.01 ^{eA}	3.47±0.01 ^{aA}	3.40±0.01 ^{aB}	6.61±0.01 ^{gA}	7.08±0.56 ^{eB}	7.34±0.01 ^{gC}
Maturation in glass carboy (45 days)						
57	3.67±0.01 ^{fA}	3.62±0.02 ^{dB}	3.63±0.02 ^{dC}	6.01±0.01 ^{hA}	6.30±0.01 ^{dB}	6.21±0.01 ^{hC}
Storage in bottle (90 days)						
147	3.64±0.01 ^{aA}	3.61±0.01 ^{dB}	3.58±0.01 ^{cD}	6.11±0.01 ^{hA}	6.40±0.02 ^{dB}	6.51±0.02 ^{iC}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among pH or total acidity, In the rows, different capitilized letters represent significant differences among wines for pH or total acidity, determined by the least significant difference test at p<0.05

Table F.2 Brix and volatile acidity changes during red wine production*

	Brix (%)			Volatile acidity (g/L)		
Time (day)	Wine					
	1	2	3	1	2	3
Must	22.78±0.21 ^{aA}	22.73±0.39 ^{aA}	22.57±0.45 ^{aB}	-	-	-
1	17.57±0.47 ^{bA}	15.53±0.48 ^{bbB}	15.58±0.11 ^{bbB}	0.34±0.02 ^{aA}	0.31±0.01 ^{aB}	0.36±0.02 ^{aC}
2	10.45±0.16 ^{cA}	7.89±0.03 ^{cB}	8.20±0.04 ^{cB}	0.40±0.02 ^{bA}	0.33±0.01 ^{aB}	0.38±0.02 ^{aC}
3	8.79±0.18 ^{dA}	7.70±0.03 ^{cB}	7.78±0.10 ^{dB}	0.47±0.02 ^{cA}	0.35±0.01 ^{aB}	0.40±0.03 ^{abC}
4	8.10±0.13 ^{eA}	6.78±0.07 ^{dB}	7.70±0.02 ^{dB}	0.47±0.02 ^{cA}	0.40±0.02 ^{bb}	0.41±0.02 ^{abC}
5	7.58±0.35 ^{eA}	6.77±0.02 ^{dB}	7.42±0.03 ^{eB}	0.50±0.02 ^{cA}	0.47±0.02 ^{cB}	0.43±0.01 ^{bcC}
Resting with Oak chip (7 days)						
12	7.71±0.24 ^{eA}	6.44±0.40 ^{deB}	6.64±0.05 ^{fb}	0.54±0.01 ^{dA}	0.48±0.01 ^{cB}	0.44±0.02 ^{cC}
Maturation at glass carboy (45 days)						
57	7.58±0.24 ^{eA}	6.52±0.08 ^{deB}	6.48±0.05 ^{gB}	0.56±0.01 ^{dA}	0.52±0.02 ^{dB}	0.49±0.02 ^{dC}
Storage in bottle (90 days)						
147	6.94±0.12 ^{fA}	6.23±0.05 ^{eB}	6.10±0.02 ^{hB}	0.57±0.02 ^{dA}	0.54±0.02 ^{eB}	0.50±0.02 ^{dC}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among Brix or volatile acidity, In the rows, different capitilized letters represent significant differences among wines for Brix or volatile acidity, determined by the least significant difference test at p<0.05

Table F.3 Alcohol and density changes during red wine production*

	Alcohol (%)			Density (g/mL)		
Time (day)	Wine					
	1	2	3	1	2	3
Must	-	-	-	1.0812±0.15 ^{aA}	1.0785±0.06 ^{aA}	1.0733±0.03 ^{aA}
1	0.01±0.01 ^{aA}	0.25±0.32 ^{aA}	0.05±0.03 ^{aA}	1.0468±0.21 ^{bA}	1.0333±0.04 ^{bA}	1.0489±0.05 ^{bA}
2	7.14±0.20 ^{bA}	9.29±0.15 ^{bB}	8.30±0.12 ^{bB}	1.0319±0.22 ^{bA}	1.0215±0.13 ^{bA}	1.0053±5.20 ^{cA}
3	9.38±0.30 ^{cA}	10.24±0.09 ^{bB}	10.52±0.14 ^{cB}	1.0020±0.02 ^{cA}	0.9989±0.01 ^{cA}	0.9995±0.01 ^{cA}
4	11.33±0.45 ^{cA}	8.25±5.69 ^{bB}	12.18±0.14 ^{dA}	0.9996±0.01 ^{cA}	0.9971±0.52 ^{cA}	0.9979±0.01 ^{cA}
5	13.56±0.11 ^{dA}	13.46±0.09 ^{cA}	13.26±0.22 ^{eA}	0.9975±0.01 ^{cA}	0.9969±0.01 ^{cA}	0.9970±0.01 ^{cA}
Resting with Oak chip (7 days)						
12	13.20±0.46 ^{deA}	13.40±0.02 ^{cA}	13.09±0.09 ^{efA}	0.9957±0.01 ^{cA}	0.9945±0.01 ^{cA}	0.9962±0.01 ^{cA}
Maturation at glass carboy (45 days)						
57	12.91±0.33 ^{efA}	13.10±0.14 ^{dA}	12.85±0.11 ^{fA}	0.9952±0.03 ^{cA}	0.9935±0.01 ^{cA}	0.9960±0.01 ^{cA}
Storage in bottle (90 days)						
147	12.66±0.24 ^{fA}	12.59±0.20 ^{eA}	12.58±0.28 ^{dA}	0.9932±0.01 ^{cA}	0.9927±0.01 ^{cA}	0.9948±0.01 ^{cA}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among alcohol or density, In the rows, different capitilized letters represent significant differences among wines for alcohol or density, determined by the least significant difference test at p<0.05

Table F.4 Total and free SO₂ (mg/L) changes during red wine production*

	Total SO ₂			Free SO ₂		
Time (day)	Wine					
	1	2	3	1	2	3
5	24.67±0.58 ^{aA}	23.33±1.15 ^{aA}	22.00±2.65 ^{aA}	20.56±1.53 ^{aA}	19.27±0.58 ^{aA}	19.58±0.58 ^{aA}
Resting with Oak chip (7 days)						
12	40.00±1.00 ^{bA}	40.67±2.52 ^{bA}	39.67±0.58 ^{bA}	23.00±2.00 ^{bA}	24.00±1.00 ^{bAB}	19.67±1.53 ^{bC}
Maturation at glass carboy (45 days)						
57	54.67±1.53 ^{cA}	53.67±0.58 ^{cA}	52.67±1.53 ^{cA}	23.67±1.53 ^{bB}	25.33±1.53 ^{cAB}	21.00±1.00 ^{cA}
Storage in bottle (90 days)						
147	53.67±0.58 ^{cA}	52.00±2.00 ^{cB}	51.33±1.53 ^{cB}	22.00±1.00 ^{cA}	23.00±1.00 ^{dA}	19.67±0.58 ^{bB}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası,*Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among total or free SO₂, In the rows, different capitilized letters represent significant differences among wines for total or free SO₂, determined by the least significant difference test at p<0.05

Table F.5 L* color analysis results during red wine production^x

L*			
Time	Wine		
(day)	1	2	3
Must	15.25±0.08 ^{aA}	14.99±0.10 ^{aB}	13.82±0.12 ^{aC}
1	13.08±0.10 ^{bA}	16.30±0.11 ^{bB}	18.66±0.27 ^{bC}
2	9.05±0.06 ^{cA}	12.34±0.16 ^{cB}	15.99±0.65 ^{cC}
3	8.80±0.04 ^{dA}	8.88±0.13 ^{dA}	13.28±0.45 ^{aC}
4	7.77±0.02 ^{eA}	6.72±0.18 ^{eB}	8.05±0.25 ^{dC}
5	7.35±0.10 ^{eA}	5.62±0.08 ^{fB}	5.31±0.16 ^{eC}
Resting with Oak chip (7 days)			
12	2.74±0.03 ^{fA}	3.17±0.08 ^{gB}	2.92±0.04 ^{fC}
Maturation at glass carboy (45 days)			
57	2.31±0.02 ^{fA}	2.10±0.05 ^{hB}	2.80±0.05 ^{fC}
Storage in bottle (90 days)			
147	1.26±0.11 ^{gA}	0.96±0.02 ^{iB}	0.94±0.03 ^{gB}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, ^xValues are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among L*, In the rows, different capitilized letters represent significant differences among wines for L*, determined by the least significant difference test at p<0.05

Table F.6 a* and b* color changes during of red wine production^x

	a*			b*		
Time	Wine					
(day)	1	2	3	1	2	3
Must	8.69±0.08 ^{aA}	9.22±0.20 ^{abB}	9.73±0.09 ^{aC}	9.52±0.08 ^{aA}	7.93±0.02 ^{aB}	6.39±0.03 ^{aC}
1	13.66±1.34 ^{bA}	12.88±9.38 ^{abB}	2.13±0.07 ^{bC}	9.17±0.10 ^{aA}	12.63±0.09 ^{bB}	-0.90±0.05 ^{bC}
2	10.69±0.72 ^{cA}	16.55±0.18 ^{bB}	9.74±0.09 ^{aC}	0.61±0.21 ^{bA}	1.59±0.28 ^{cB}	-1.54±0.29 ^{cC}
3	5.60±0.07 ^{dA}	5.58±0.08 ^{cdB}	4.49±0.32 ^{cC}	0.49±0.04 ^{bcA}	-0.65±0.31 ^{eB}	-0.82±0.17 ^{bC}
4	8.14±2.28 ^{aA}	8.81±0.13 ^{caB}	6.64±0.22 ^{dC}	0.82±0.07 ^{dA}	-0.54±0.34 ^{eB}	-1.32±0.07 ^{cC}
5	10.96±0.20 ^{cA}	11.93±0.04 ^{abB}	2.50±0.43 ^{eC}	2.05±0.04 ^{eA}	0.66±0.02 ^{dB}	0.45±0.22 ^{dC}
Resting with Oak chip (7 days)						
12	3.46±0.14 ^{eA}	5.79±0.18 ^{cdB}	0.91±0.07 ^{fC}	1.89±0.08 ^{fA}	0.48±0.02 ^{dB}	-0.63±0.02 ^{bC}
Maturation at glass carboy (45 days)						
57	0.88±0.28 ^{fA}	1.34±0.11 ^{dB}	0.82±0.04 ^{fC}	0.39±0.04 ^{cA}	-0.32±0.24 ^{eB}	-1.24±0.31 ^{cC}
Storage in bottle (90 days)						
147	0.49±0.06 ^{fA}	0.70±0.18 ^{dB}	0.64±0.01 ^{fC}	-0.18±0.06 ^{gA}	-0.36±0.02 ^{eB}	-0.31±0.05 ^{eC}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, ^xValues are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among a* or b*, In the rows, different capitilized letters represent significant differences among wines for a* or b*, determined by the least significant difference test at p<0.05

Table F.7 Chroma (C*) and Hue (H*) analysis for red wine production^x

	C*			H*		
Time (day)	Wine					
	1	2	3	1	2	3
Must	12.89±0.10 ^{aA}	12.55±0.12 ^{aA}	11.22±0.07 ^{aB}	47.61±0.17 ^{aA}	39.18±0.35 ^{aB}	34.72±0.23 ^{aC}
1	17.40±0.04 ^{bA}	21.97±0.37 ^{bB}	23.61±0.60 ^{bC}	132.95±1.69 ^{aA}	50.06±2.65 ^{aB}	337.13±1.20 ^{bC}
2	15.53±0.67 ^{cA}	18.23±0.44 ^{cB}	19.75±0.12 ^{cC}	3.21±0.96 ^{bA}	5.49±1.02 ^{bB}	351.02±1.70 ^{cC}
3	15.68±0.04 ^{cA}	16.64±0.12 ^{dB}	17.94±0.32 ^{dC}	4.97±0.42 ^{bA}	353.37±3.13 ^{cB}	349.60±2.81 ^{cC}
4	12.63±0.31 ^{aA}	15.61±0.26 ^{eB}	16.47±0.54 ^{eC}	6.14±2.03 ^{bA}	356.51±2.18 ^{cB}	348.73±0.56 ^{cC}
5	11.08±0.15 ^{dA}	11.53±0.45 ^{fB}	12.28±0.22 ^{fC}	10.61±0.09 ^{bA}	3.15±0.09 ^{bB}	10.13±5.15 ^{dC}
Resting with Oak chip (7 days)						
12	3.82±0.03 ^{eA}	5.81±0.17 ^{gB}	6.05±0.09 ^{gC}	28.67±1.81 ^{abA}	4.74±0.18 ^{bB}	325.39±1.96 ^{eC}
Maturation at glass carboy (45 days)						
57	0.89±0.05 ^{fA}	1.20±0.02 ^{hB}	1.31±0.02 ^{hC}	24.48±4.93 ^{abA}	347.21±9.12 ^{cdB}	304.10±6.27 ^{fC}
Storage in bottle (90 days)						
147	0.42±0.04 ^{gA}	0.80±0.02 ^{hB}	0.93±0.04 ^{hC}	340.05±3.89 ^{cA}	332.26±5.36 ^{dB}	334.23±3.14 ^{bC}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, ^xValues are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among C* or H*, In the rows, different capitilized letters represent significant differences among wines for C* or H*, determined by the least significant difference test at p<0.05

Table F.8 Yeast count changes in the must and wines (log cfu/mL)*

Days	Wine-1	Wine-2	Wine-3
Must	6.40±0.01 ^{aA}	5.38±1.75 ^{adB}	6.36±0.02 ^{aA}
Fermentation (5 days)			
1	6.61±0.02 ^{aA}	6.56±0.03 ^{bcB}	6.53±0.03 ^{bcC}
2	6.96±0.02 ^{abA}	6.99±0.05 ^{cbB}	6.96±0.07 ^{caA}
3	7.65±0.11 ^{baA}	7.52±0.04 ^{cbB}	7.55±0.09 ^{dbB}
4	7.49±1.12 ^{baA}	6.80±0.03 ^{bcB}	6.71±0.04 ^{ebB}
5	6.55±0.02 ^{aA}	6.50±0.02 ^{bcA}	6.37±0.06 ^{aB}
Resting with oak chip (7 days)			
12	5.61±0.25 ^{caA}	5.81±0.07 ^{acB}	5.77±0.07 ^{fbB}
Maturation at glass carboy (45 days)			
57	4.78±0.60 ^{daA}	4.38±0.07 ^{dbB}	4.29±0.15 ^{gbB}
Storage in bottle (90 days)			
147	2.38±0.10 ^{eaA}	2.34±0.12 ^{ebB}	2.30±0.11 ^{hcC}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among yeast count, In the rows, different capitilized letters represent significant differences among wines for yeast count, determined by the least significant difference test at p<0.05

Table F.9 Flavonoid content of must and red wine samples (mg/L)*

Wine	myricetin	quercetin	rutin	(+)-catch	PB2
Must					
1	0.54±0.01 ^{aA}	0.11±0.01 ^{aA}	0.12±0.02 ^{aA}	60.98±0.64 ^{aA}	45.42±1.80 ^{aA}
2	0.67±0.02 ^{aB}	0.23±0.01 ^{aB}	0.24±0.01 ^{aB}	61.23±0.92 ^{aA}	50.23±1.17 ^{aB}
3	1.11±0.02 ^{aC}	0.28±0.02 ^{aC}	0.28±0.02 ^{aC}	61.49±1.05 ^{aA}	51.16±2.31 ^{aB}
Fermentation (5 days)					
1	4.80±0.02 ^{bA}	3.40±0.03 ^{bA}	1.60±0.02 ^{bA}	63.08±0.07 ^{bA}	274.42±10.63 ^{bA}
2	5.43±0.03 ^{bB}	7.57±0.06 ^{bB}	2.00±0.01 ^{bB}	64.00±0.85 ^{bA}	247.43±10.98 ^{bB}
3	5.62±0.03 ^{bC}	8.52±0.04 ^{bC}	2.21±0.00 ^{bB}	76.26±0.76 ^{bB}	278.53±13.32 ^{bA}
Resting with oak chip (7 days)					
1	4.56±0.04 ^{cA}	3.63±0.03 ^{cA}	1.58±0.01 ^{bA}	72.00±0.48 ^{cA}	531.37±11.82 ^{cA}
2	4.90±0.03 ^{cB}	4.11±0.02 ^{cB}	1.97±0.03 ^{bB}	73.24±0.54 ^{cA}	561.31±27.16 ^{cB}
3	4.94±0.02 ^{cC}	6.93±0.07 ^{cC}	2.13±0.02 ^{cC}	75.53±0.65 ^{cB}	732.34±22.34 ^{cC}
Maturation at glass carboy (45 days)					
1	1.38±0.01 ^{dA}	3.26±0.01 ^{dA}	1.51±0.02 ^{cA}	66.62±1.29 ^{dA}	423.60±11.47 ^{dA}
2	1.42±0.02 ^{dB}	3.94±0.02 ^{dB}	1.83±0.03 ^{cB}	68.10±0.72 ^{dB}	480.69±21.52 ^{dB}
3	1.73±0.02 ^{dC}	6.27±0.02 ^{dC}	2.11±0.02 ^{cC}	72.77±0.99 ^{dC}	643.67±10.39 ^{dC}
Storage in bottle (90 days)					
1	1.12±0.01 ^{eA}	2.86±0.02 ^{eA}	1.38±0.01 ^{dA}	62.12±1.61 ^{abA}	258.30±9.59 ^{eA}
2	1.26±0.01 ^{eB}	3.89±0.02 ^{dB}	1.58±0.02 ^{dB}	65.18±1.69 ^{bA}	273.27±11.51 ^{bA}
3	1.68±0.02 ^{eC}	4.73±0.02 ^{eC}	1.61±0.02 ^{dC}	71.71±1.38 ^{dB}	396.50±10.78 ^{eB}

(+)-catch: (+)-catechin hydrate, PB2: Procyanidin B₂, rutin: rutin hydrate

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among phenolic compound and in the columns, different capitilized letters represent significant differences among wines for phenolic compounds, determined by the least significant difference test at p<0.05.

Table F.10 Non-flavonoid content of must and red wine samples (mg/L)*

Wine	Gallic ¹	coumaric ¹	Chloro ¹	Tyro ¹	Resv ¹
Must					
1	2.80±0.13 ^{aA}	0.05±0.02 ^{aA}	8.42±0.04 ^{aA}	1.05±0.03 ^{aA}	0,05±0.01 ^{aA}
2	4.59±0.05 ^{aB}	0.06±0.02 ^{aA}	8.86±0.10 ^{aB}	2.57±0.12 ^{aB}	0,07±0.01 ^{aB}
3	6.53±0.06 ^{aC}	0.10±0.02 ^{aB}	9.20±0.02 ^{aC}	3.54±0.05 ^{aC}	0,08±0.01 ^{aB}
Fermentation (5 days)					
1	32.77±0.54 ^{bA}	11.27±0.69 ^{bA}	9.71±0.07 ^{bA}	13.75±0.09 ^{bA}	2.03±0.05 ^{bA}
2	23.94±1.49 ^{bB}	11.37±0.80 ^{bA}	10.90±0.07 ^{bB}	19.74±0.10 ^{bB}	2.14±0.03 ^{bB}
3	40.54±1.01 ^{bC}	12.16±0.11 ^{bB}	11.20±0.06 ^{bC}	23.83±0.22 ^{bC}	2.26±0.02 ^{bC}
Resting with oak chip (7 days)					
1	40.39±1.57 ^{cA}	11.21±0.41 ^{bA}	10.50±0.07 ^{cA}	12.86±0.10 ^{cA}	2.63±0.05 ^{cA}
2	39.59±1.11 ^{cB}	11.31±0.43 ^{bA}	11.71±0.07 ^{cB}	19.02±0.30 ^{cB}	2.74±0.10 ^{cA}
3	46.67±1.74 ^{cC}	11.92±0.53 ^{bB}	12.59±0.15 ^{cB}	22.15±0.09 ^{cC}	2.91±0.04 ^{cB}
Maturation at glass carboy (45 days)					
1	21.60±1.07 ^{dA}	7.38±0.05 ^{cA}	7.23±0.03 ^{dA}	10.56±0.09 ^{dA}	2.55±0.03 ^{dA}
2	17.30±0.14 ^{dB}	7.21±0.05 ^{cB}	7.27±0.02 ^{dAB}	11.96±0.12 ^{dB}	2.73±0.07 ^{cA}
3	36.47±1.27 ^{dC}	7.30±0.05 ^{cA}	7.33±0.06 ^{dB}	18.35±0.10 ^{dC}	2.89±0.04 ^{cB}
Storage in bottle (90 days)					
1	11.49±1.49 ^{eA}	7.35±0.06 ^{cA}	7.22±0.09 ^{eA}	6.57±0.04 ^{eA}	2.49±0.10 ^{cA}
2	15.51±1.14 ^{eA}	7.15±0.06 ^{cB}	7.24±0.04 ^{eA}	11.05±0.10 ^{eB}	2.69±0.01 ^{cA}
3	24.78±1.96 ^{eB}	7.33±0.02 ^{cA}	7.32±0.09 ^{dA}	11.49±0.05 ^{eC}	2.87±0.03 ^{cB}

Gallic: gallic acid-1-hydrate, tyro: tyrosol, coumaric: *p*-coumaric acid, chloro: chlorogenic acid, resv: resveratrol

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among phenolic compound and in the columns, different capitilized letters represent significant differences among wines for phenolic compounds, determined by the least significant difference test at p<0.05.

Table F.11 Sugar contents of must and wine samples (g/L)*

Wine	Fructose	Glucose	Sucrose
Must			
1	111.08±0.79 ^{aA}	78.13±1.22 ^{aA}	5,89±1.18 ^{aA}
2	105.41±3.47 ^{aA}	70.97±0.28 ^{aB}	3,92±0.07 ^{aB}
3	93.95±0.50 ^{aB}	71.00±6.10 ^{aB}	2,73±0.21 ^{aC}
Fermentation (5 days)			
2nd day of Fermentation			
1	56.08±1.51 ^{bA}	14.48±1.22 ^{bA}	1,02±0.05 ^{bA}
2	29.20±3.43 ^{bB}	8.50±0.22 ^{bB}	0,93±0.02 ^{bB}
3	16.24±0.79 ^{bC}	8.33±0.08 ^{bB}	0,32±0.03 ^{bC}
End of Fermentation			
1	1.53±0.07 ^{cA}	1.07±0.10 ^{cA}	-
2	1.78±0.04 ^{cB}	0.96±0.03 ^{cB}	-
3	1.97±0.10 ^{cC}	0.93±0.04 ^{cB}	-
Resting of wines with oak chip (7 days)			
1	1.49±0.06 ^{cA}	0.92±0.04 ^{cA}	-
2	1.74±0.04 ^{cB}	0.90±0.01 ^{cA}	-
3	2.02±0.02 ^{cC}	0.79±0.02 ^{cB}	-
Maturation of wines (45 days)			
1	1.47±0.07 ^{cA}	0.79±0.03 ^{cA}	-
2	1.69±0.04 ^{cB}	0.72±0.02 ^{cB}	-
3	2.93±0.08 ^{cC}	0.72±0.02 ^{cB}	-
Storage of wines (90 days)			
1	1.45±0.07 ^{cA}	0.77±0.02 ^{cA}	-
2	1.64±0.05 ^{cB}	0.71±0.03 ^{cB}	-
3	1.84±0.07 ^{cC}	0.70±0.05 ^{cB}	-

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among sugar contents and in the columns, different capitilized letters represent significant differences among wines for sugar contents, determined by the least significant difference test at p<0.05.

Table F.12 Organic acids of must and wine samples (g/L)*

Wine	Tartaric acid	Malic acid	Citric acid	Succinic acid
Must				
1	6.25±0.10 ^{aA}	0.91±0.02 ^{aA}	0.49±0.02 ^{aA}	0.38±0.01 ^{aA}
2	6.27±0.13 ^{aA}	0.86±0.02 ^{aB}	0.47±0.01 ^{aB}	0.39±0.03 ^{aB}
3	6.17±0.06 ^{aB}	0.81±0.02 ^{aC}	0.47±0.02 ^{aB}	0.37±0.02 ^{aC}
Fermentation (5 days)				
2nd day of Fermentation				
1	5.95±0.19 ^{bA}	0.83±0.01 ^{bA}	0.42±0.01 ^{bA}	0.45±0.01 ^{aA}
2	5.65±0.05 ^{bB}	0.80±0.01 ^{bB}	0.42±0.01 ^{bA}	0.41±0.02 ^{aB}
3	5.41±0.11 ^{bC}	0.79±0.02 ^{abB}	0.40±0.02 ^{bB}	0.40±0.01 ^{bB}
End of Fermentation				
1	5.81±0.03 ^{bcA}	0.79±0.01 ^{cA}	0.28±0.02 ^{cA}	0.50±0.02 ^{abA}
2	5.52±0.02 ^{bB}	0.80±0.02 ^{bB}	0.27±0.01 ^{cA}	0.48±0.01 ^{bA}
3	5.31±0.17 ^{bC}	0.78±0.02 ^{abC}	0.24±0.02 ^{cB}	0.45±0.02 ^{cB}
Resting with oak chip (7 days)				
1	5.60±0.14 ^{cA}	0.77±0.03 ^{cdA}	0.26±0.02 ^{cdA}	0.51±0.16 ^{bcA}
2	4.85±0.12 ^{cB}	0.77±0.01 ^{cA}	0.24±0.01 ^{deA}	0.47±0.01 ^{bB}
3	4.78±0.08 ^{cB}	0.72±0.02 ^{acB}	0.21±0.02 ^{cdB}	0.45±0.01 ^{cB}
Maturation (45 days)				
1	4.85±0.13 ^{dA}	0.76±0.02 ^{dA}	0.25±0.02 ^{cdA}	0.57±0.01 ^{cA}
2	4.61±0.07 ^{dA}	0.72±0.02 ^{dB}	0.22±0.01 ^{eB}	0.45±0.01 ^{cB}
3	4.25±0.03 ^{dB}	0.70±0.02 ^{cB}	0.20±0.03 ^{dB}	0.41±0.02 ^{dB}
Storage in bottle (90 days)				
1	4.64±0.10 ^{dA}	0.75±0.02 ^{dA}	0.24±0.02 ^{dA}	0.54±0.01 ^{cA}
2	4.43±0.03 ^{eA}	0.71±0.02 ^{dB}	0.20±0.02 ^{eB}	0.41±0.03 ^{cB}
3	4.19±0.04 ^{dB}	0.70±0.04 ^{bcB}	0.19±0.04 ^{dB}	0.38±0.01 ^{eC}

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the columns, different small letters represent significant differences among organic acid contents and in the columns, different capitilized letters represent significant differences among wines for organic acid contents contents, determined by the least significant difference test at p<0.05.

Table F.13 Sensory analysis results of wines*

Wine	1	2	3
Visual			
Color	6.50±0.10 ^A	6.87±0.06 ^B	7.55±0.30 ^C
Clarity	6.37±0.06 ^A	8.10±0.10 ^B	8.30±0.43 ^B
Turbidity	3.53±0.06 ^A	3.00±0.10 ^A	4.47±0.15 ^B
Odor			
Alcohol odor	5.43±0.06 ^A	4.23±0.06 ^B	5.43±0.06 ^A
Yeast odor	2.30±0.10 ^A	2.30±0.01 ^A	2.43±0.06 ^A
Taste			
Sweetness	4.33±0.06 ^A	3.70±0.10 ^B	4.77±0.15 ^A
Acidity	4.87±0.15 ^A	4.43±0.15 ^A	5.40±0.10 ^B
Bitterness	2.80±0.10 ^A	3.43±1.24 ^A	3.17±0.06 ^A
Sourness	4.60±0.10 ^A	3.33±0.15 ^B	3.20±0.20 ^B
Astringency	5.27±0.06 ^A	5.47±0.15 ^A	6.33±0.15 ^B
Permanency	5.73±0.06 ^A	5.43±0.06 ^A	6.27±0.12 ^B
General impression	6.70±0.20 ^A	5.97±0.15 ^B	7.73±0.15 ^C

Wine-1: 100% Hönüsü, wine-2: 85% Hönüsü + 15% Horoz karası, wine-3: 70% Hönüsü + 30% Horoz karası, *Values are the mean ± SD (n = 3). In the rows, different capitilized letters represent significant differences among wines for sensory analysis, determined by the least significant difference test at p<0.05