EFFECTS OF COMMERCIAL NON-*SACCHAROMYCES* YEASTS ON QUALITY OF WINES PRODUCED FROM EMIR GRAPES

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ABSTRACT

EFFECTS OF COMMERCIAL NON-*SACCHAROMYCES* YEASTS ON QUALITY OF WINES PRODUCED FROM EMIR GRAPES

The aim of this study was to examine the effects of non-Saccharomyces commercial yeast strains on the chemical and organoleptic properties of wine by using them in the production of wines obtained from Emir grapes to contribute to the literature that has limited information on local grapes and to benefit wine producers for the use of these products in the sector.

For this purpose, sequential fermentation was carried out with *Saccharomyces cerevisiae,* each of the active dry commercial yeasts containing *Metschnikowia pulcherrima, Torulaspora delbrueckii*, and *Metschnikowia pulcherrima / Torulaspora delbrueckii* mixture. After 4 months of maturation, chemical (alcohol, total acidity, pH, density, volatile acidity, reducing sugar, total/free SO_2), color, and volatile compound analyses with HS-SPME-GC/MS were performed. The wine fermented with a mixture of *Metschnikowia pulcherrima / Torulaspora delbrueckii* had the best results in sensory analysis. It has been observed that non-*Saccharomyces* yeasts produced lower alcohol and produced isobutanol, isoamyl octanoate, and octanoic acid, which contributed to the aroma complexity of the wine, unlike wine fermented only with *Saccharomyces cerevisiae*. The formation of acetic acid and dodecanoic acid, primarily undesirable in wines from Emir grapes which gives a feeling of oiliness, was detected only in wines fermented with *Saccharomyces cerevisiae*.

At the end of the study, it has been proven that non-*Saccharomyces* yeasts positively affected wine quality by sequential fermentation with *Saccharomyces cerevisiae* as active dry yeast.

ÖZET

SACCHAROMYCES OLMAYAN TİCARİ MAYALARIN EMİR ÜZÜMÜNDEN ÜRETİLEN ŞARAPLARIN KALİTESİ ÜZERİNE ETKİLERİ

Bu çalışmada non-*Saccharomyces* ticari maya türlerinin Emir üzümünden elde edilecek şarapların üretiminde kullanılarak, şarabın kimyasal ve organoleptik özelliklerine etkisini incelemek, henüz bu konuda yerel üzümlerle ilgili kısıtlı bilgi barındıran literatüre katkıda bulunmak ve sektörde bu ürünlerin kullanılması için şarap üreticilerine yarar sağlamak amaçlanmıştır.

Bu amaçla *Metschnikowia pulcherrima*, *Torulaspora delbrueckii,* ve *Metschnikowia pulcherrima / Torulaspora delbrueckii* karışımı içeren ticari aktif mayaların her biri *Saccharomyces cerevisiae* ile sıralı fermantasyon yapılmıştır. Uygulanan 4 ay olgunlaşma süreci sonunda, şaraplarda kimyasal (alkol, asit, pH, yoğunluk, uçar asit, indirgen şeker, toplam/serbest SO_2), renk ve HS-SPME-GC/MS ile uçucu bileşik analizleri yapılmıştır. *Metschnikowia pulcherrima / Torulaspora delbrueckii* karışımı ile fermente edilmiş şarap duyusal analizde en iyi sonucu almıştır. Non-*Saccharomyces* mayaların daha düşük alkol oluşturduğu ve yalnızca *Saccharomyces cerevisiae* ile fermente edilen şaraptan farklı olarak şarabın aroma kompleksitesine katkıda bulunan izobütanol, izoamil oktanoat ve oktanoik asit ürettiği gözlemlenmiştir. Şaraplarda yağlılık hissi gibi olumsuz özellik kazandıran dodekanoik asit oluşumu ise yalnızca *Saccharomyces cerevisiae* ile fermente edilen şaraplarda tespit edilmiştir.

Çalışma sonucunda, non-*Saccharomyces* mayaların aktif kuru maya olarak *Saccharomyces cerevisiae* ile sıralı fermantasyonuyla şarap kalitesi üzerine olumlu etkileri olduğu belirlenmiştir.

to my family

Yusuf, Asuman, Liva, Selda, Sirius and Fume

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CHAPTER 1

INTRODUCTION

Several types of yeast are found in the skin of wine grapes. For this reason, wine from grape juice does not occur only by *Saccharomyces cerevisiae*, known as wine yeast (Arslan, Çelik and Cabaroğlu 2018). In the early stages of natural fermentation, non-*Saccharomyces* (wild) yeasts from the genera *Candida, Hanseniaspora, Torulaspora*, and *Pichia* dominate (Jolly, Varela and Pretorius 2013). With the continuation of fermentation, these yeasts begin to lose their activity due to high alcohol, low pH, SO_2, oxygen an nutrition deficiency, while *Saccharomyces* species become dominant (Arslan, Çelik and Cabaroğlu 2018).

It was not preferred in winemaking due to the unwanted metabolites formed by non-*Saccharomyces* yeast species (Vejarano and Gil-Calderón 2021). However, studies conducted in recent years have shown that these yeast species have positive effects on the organoleptic character, complexity, and chemical-physical stability of wines and increased interest in the subject (Romani et al.. 2020). Non-*Saccharomyces* yeasts in winemaking have been shown to produce more than 1300 volatile compounds and metabolic products (terpenoids, esters, higher alcohols, glycerol, acetaldehyde, acetic acid, and succinic acid) and provide a more complex flavor phenotype (Lai et al.. 2022; Jolly, Varela and Pretorius 2013).

Besides helping to produce lower-alcohol wine, non-*Saccharomyces* yeasts play an essential role in producing glycosidase enzymes. The glycosidase enzyme supports the development of the aromatic profile of wine by hydrolyzing the precursors of nor-isoprenoids, terpenols, and lactones (Mateo and Maica, 2016). In addition, by producing succinic acid, they can balance the total acidity in low-acid wines. Thanks to its proteolytic activities, it decreases protein levels and increases protein stabilization. In addition, it provides a high concentration of glycerol, one of the primary metabolites formed due to fermentation after alcohol. While glycerol regulates the cell's redox potential, it positively affects the taste and viscosity of the wine. However, the production of glycerol also causes an increase in the production of acetic acid, which harms the

quality of the wine. At this stage, non-*Saccharomyces* yeasts are thought to be involved in fermentation with *S.cerevisiae*, and as a result, it was observed that the amounts of volatile acid and acetic acid decreased (Lai et al. 2022).

The biggest problem with using non-*Saccharomyces* yeasts alone is that they cause stuck fermentation due to their low fermentation capacity and alcohol tolerance. For this reason, studies have shown that sequential or simultaneous fermentation with *Saccharomyces cerevisiae* gives better results (Renault et al. 2015; Morata et al. 2019a). The sequential fermentation method, which starts with non-*Saccharomyces* yeast and continues with saccharomyces, is preferred primarily because it gives better results than simultaneous fermentation.

The most frequently used commercial non-*Saccharomyces* yeasts in the market, with a rate of 52%, are stated as *Torulaspora delbrueckii, Lachancea thermotolerans* & *Metschnikowia pulcherrima* (Vejarano and Gil-Calderón 2021). *Torulaspora delbrueckii* produces glycerol, low volatile acid, high terpenol, and 2-phenyl ethanol, especially when subjected to sequential fermentation with *S. cerevisiae* (Romani et al. 2020). Analyses made as a result of fermentation showed that the ethyl propanate, ethyl isobutanate, and ethyl dihydrocinnamate compounds formed came from the characteristic aroma profile of *Torulaspora delbrueckii* (Renault et al. 2015). *Torulaspora delbrueckii* also supports the formation of 3-sulfanyl hexane-1-ol (3SH) compounds that impart grapefruit, citrus peel, and passion fruit flavors, and 3-sulfanyl hexyl acetate (3SHA) compounds that impart passion fruit and boxwood flavors (Morata et al. 2019a).

*Metschnikowia pulcherrima (*anamorph *C.pulcherrima*)*,* one of the most common non-*Saccharomyces* yeast species, affects the concentration of varietal aromas such as terpenes and volatile thiols by producing the extracellular enzyme α-arabinofuranosidase. This yeast species, which is also involved in the formation of high concentrations of esters, especially ethyl octanoate, provides an increase in the production of 2-phenyl ethanol, a vital aroma compound, especially after sequential fermentation with *Saccharomyces cerevisiae* (Morata et al. 2019b).

This study aims to examine the effects of non-*Saccharomyces* active dry commercial yeast species on the chemical and organoleptic properties of the wines obtained from Emir grapes. It is essential for contributing to the literature and the sector since it contains commercial products that are easy to be involved in. Studies conducted around the world to examine the effects of non-*Saccharomyces* yeasts, especially *Torulaspora delbrueckii* and *Metschnikowia pulcherrima,* on wines have focused mainly

on Sauvignon Blanc and Chardonnay from white grapes. In studies on this subject from local grapes, it has been observed that the effects of these two wild yeasts as active dry commercial yeast on Emir grapes have not been examined. The thin-skinned Emir grape, grown around Nevşehir-Ürgüp, is suitable for developing aroma potential and complexity and is an essential option for working in this field. For this purpose, the effect of commercial yeasts *Torulaspora delbrueckii* and *Metschnikowia pulcherrima* on wines obtained from Emir grape by sequential fermentation with *Saccharomyces cerevisiae* will be examined for the first time.

CHAPTER 2

LITERATURE REVIEW

2.1. History of Winemaking

According to Turkish Food Codex Regulation on Wine, wine refers to the product obtained by the partial or complete alcoholic fermentation of grapes, whether crushed or not, or grape must, with or without a geographical indication or a registered name of origin (Republic of Türkiye Ministry of Agriculture And Forestry, 2008).

The archeological findings of winemaking date back more than 7.5 thousand years. Winemaking is thought to be discovered or evolved in southern Caucasia, which today includes areas of northwestern Turkey, northern Iraq, Azerbaijan, and Georgia (Jackson 2008). It is also generally thought that the wine grape (*Vitis vinifera*) domestication ensued in the same area. The earliest wine residues were found in the north of the Zagros Mountains of Iran (Hajji Firuz Tepe) in the early years of the mid-fifth millennium b.c. (McGovern et al. 2017). Also ancient, 8000 years old, Georgian pottery, which belongs to the Neolithic period, gave rise to the idea that wine spread from there to the world. The wine residues are examined by tandem liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS), and the presence of tartaric acid residues identified them. (Guasch-Jané et al. 2004; Jackson 2008)

The ancient Greeks were one of the first civilizations to embrace winemaking. They invented many of the processes and tools employed in winemaking today (using wooden barrels to store and transport wine and methods for aging wine in oak barrels to improve its flavor and texture). The Romans also popularized winemaking throughout their empire. They created numerous innovative winemaking processes (such as using grape presses to extract the juice from the grapes and strategies for improving wine quality).

During the Middle Ages, winemaking flourished in Europe, particularly France, Italy, and Spain. During this time, monasteries had an influential role in developing

winemaking, and many of the methods and practices the monks created are still in use today, such as procedures for grape vine pruning to increase yield and quality as well as techniques for wine maturing in caves and cellars to enhance flavor and texture (Barth 2007; McGovern 2013; Jackson 2008)

2.2. White Winemaking

Wine represents thousands of years of history and a deep cultural significance. Among the wide range of this important beverage, white wine stands out with its special aroma profile, diverse grape species and carefully applied production techniques.

2.2.1. Harvest

The wine grape harvest is a crucial time for winemakers worldwide, marking the beginning of the winemaking process. The success of the wine grape harvest is critical to the quality of the wine produced. The quality of the grapes and other properties, including the weather (precipitation, temperature, and sunlight), the training system, canopy growth, crop load, water management, frequency of insect pests and diseases, and harvest timing, affect the quality of the wine.

Due to the difficulties in determining grape maturity in the vineyard and projecting wine quality, harvest timing is the most crucial and challenging viticultural decision for grape growers and winemakers. The harvest day is mainly affected by the maturity of wine grapes since immature grapes result in less complex wines and more unwanted green notes on the palate in both red and whites. Therefore, grapes are expected to reach phenolic maturity, which can be examined by "qualitative" and "quantitative" factors. Qualitative factors are the appearance of grapes (color intensity and firmness of the skins), stems (color intensity and taste of the seeds), and grape juice and pulp (Adsule, 2014). These are subjective and evaluated by four senses: taste, visual, touch, and smell.

The skin color of colored cultivars changes from green to red, blue, or black. Berries begin to soften, with white cultivars becoming more translucent. Changes in juice color begin when white's turn greenish to whitish, and red's start to take on some skin pigment. Skin tannin polymerization starts to become more desirable. The extractability of undesirable seed tannins decreases while varietal flavor components increase. Quantitative factors consist of fundamental chemical analysis on sugar content (T.S.S.), titratable acidity, and pH, which provide good guidance in determining the harvest time (Goldammer, 2015).

The concentration of total soluble solids (T.S.S.), potential alcohol (°Baumé), or specific gravity are used to express sugar. Gram-soluble solids per 100 g of the solution are the unit of measurement for °Brix. It measures all soluble substances, including sugar, glycerol, acids, and pigments. Typically, 90 to 95% of the total soluble solids in grapes must comprise fermentable sugar. Measuring °Brix approximates only the sugar concentration, therefore, the potential alcohol. One degree of Brix equals 10g/l of sugar, and 1.8° Brix is equivalent to 1% A.B.V. in the finished wine. The two monosaccharides, fructose, and glucose, comprise most of the sugar in grapes. The ratio of these two varies according to the variety and level of fruit maturity, with glucose predominating in the early stages of berry development. Since this measurement is based on the weight-to-weight ratio of sugar to water, it can also change depending on the fruit's physiological conditions (Zoecklein et al.., 1999).

The acid content is essential for fruit and the resultant wine's structural and textural balance. As tartaric acid, titratable acidity (T.A.) in grapes typically ranges from 5.0 to 16.0 g/L; these values can vary by the type & maturity level of the grape, climatic factors, and cultural techniques. Four sources can be used to determine a wine's organic acid content. Tartaric, malic, and, to a much lesser level, citric acid is present in the grape. Citric acid is present in unfermented grapes at a concentration of 0.2–3.0 g/L, while tartaric and malic acids are present at 2.0–10 g/L and 1.0–8.0 g/L, respectively (Ough and Amerine 1988). Lactic acid, acetic acid, and succinic acid are formed during alcoholic fermentation, along with minimal amounts of other acids from the tricarboxylic acid cycle. Bacterial involvement produces significant amounts of lactic and acetic acids and can also produce propionic and butyric acids (McCloskey 1974; Reynolds 2010).

Assessment of pH is also a crucial parameter for determining the optimal harvest time. The pH affects biological stability, physiochemical stability, and sensory characteristics (Zoecklein 1999). Harvesting the grapes below optimal juice pH results in

a sour, vegetative wine with no character. On the other hand, wines with a pH above 3.5 may have a microbial infection and quality deficiency in color and taste (Wolf, 2008).

Besides, climatic conditions in the vineyard, production area, grape type, and wine to be produced should also be considered.

2.2.2. Reception & Pressing

After the harvest, the grapes are wanted to be processed quickly against the risk of oxidation, microbiological infection, and loss of quality. Grapes are pulled out directly to the sorting tables for manual selection, where sorters remove unripe, diseased/ damaged grapes and take them into de-stemmer and crusher machines with fruit elevators. Stems, leaves, and grape stalks are called M.O.G. (material other than grapes) and are preferred to be removed from grapes to prevent the extraction of undesirable phenols. These undesirable phenols give the final wine astringency and bitterness (Kilmartin and Oberholster 2022). The crushing process is done after the destemming process, and these operations can be done together in the same de-stemmer machine.

Grapes removed from M.O.G. and crushed are taken to press to become grape must. This taking process is one of the most critical steps in white winemaking because it directly affects the quality of must and wine. Mistakes at this stage can cause subsequent problems in further stages (fermentation, clarification, filtration, or stabilization), so getting grape must with high quality and satisfactory yield is essential. The winemaker can adjust the time of squeezing (min) and pressure level (mbar) according to the wine that will be produced. Typically employed in vineyards, pneumatic presses use a pocket of air or compressed air to apply horizontal pressure against a pressing cage.

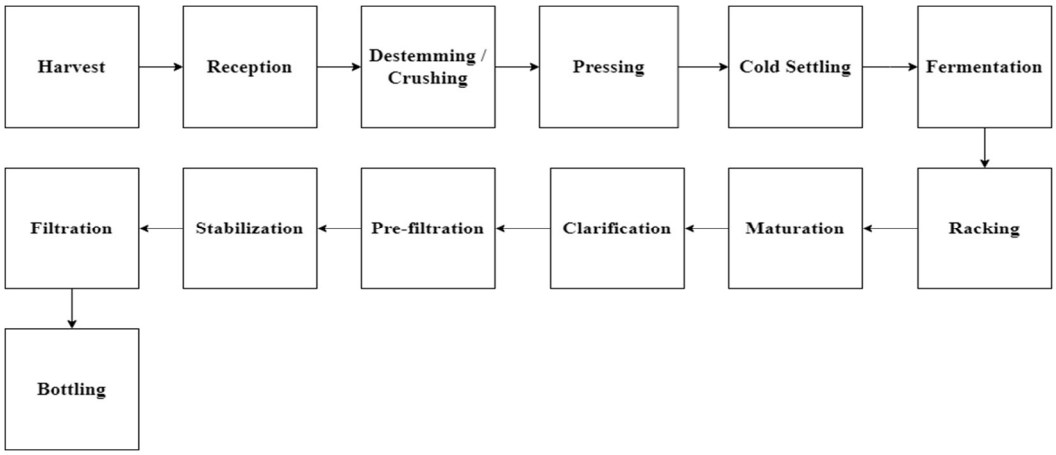

Figure 1. White wine production scheme

2.2.3. Cold Settling

Grape juice obtained after pressing is taken into stainless steel tanks for clarification. Before fermentation, it is necessary to precipitate suspended particles and achieve the desirable turbidity (5—250 NTU) of the winemaker. Enzymatic browning is caused by the oxidative enzyme polyphenol oxidase, which is reduced when extra solids like pulp and skin fragments are removed. Clarifying juice also reduces the growth of volatile sulfur compound odors (such as hydrogen sulfide and similar compounds), reduces herbaceous scents, and can improve delicate/fruity aromas. Reaching a target solids level is crucial to prevent over-clarified juice from lacking the nutrients necessary for a healthy fermentation. Cold settling is the most preferred method, cooling the grape must to 35–40F for 24–48 hours. It can be aided by adding pectinolytic enzymes and clarifying agents - bentonite, gelatine, and silica gel. Solids are sedimented by gravity, while spontaneous yeast fermentation is prevented by cool temperature. The foul lees are then racked off of the clear juice. The capacity and load of the chiller tank, time, and the possibility of oxidation are drawbacks (Reynolds 2010). Using pectolytic enzymes at this stage increases the sedimentation rate while reducing the time. The grape must is then pumped into fermentation tanks or barrels to start fermentation.

2.2.4. Fermentation

The world's most crucial biotechnological process is the fermentation of carbohydrates into alcohol by yeasts. The history of fermentation dates back to ancient civilizations (around 6000BC) brewing beer (Walker 2018). Louis Pasteur's findings on fermentation in 1899 clarified an essential point in winemaking and created a new field of study. He found that the critical microorganism in the winemaking process are yeasts since they conduct alcoholic fermentation. Also, he clarified that certain species of bacteria could grow in wine and cause spoilage (Fleet 1990).

The most well-known ethanol-producing microorganisms are yeasts, used for thousands of years in fermented drinks. *S. cerevisiae* is primarily responsible for the alcoholic fermentation of grape must (Sinha et al. 2012). Selected wine yeast strains are frequently used, typically as active dry yeast (A.D.Y.). They are used to ensure complete fermentation with the proper kinetics and to prevent spoilage caused by the development of unwanted microorganisms. Wine fermentation inoculated with the appropriate A.D.Y. strain also undergoes an initial fermentation step dominated by non-*Saccharomyces* strains. Still, most of the fermentation process is controlled by the inoculated strain. While operating in anaerobic circumstances, *S. cerevisiae* utilizes pyruvic acid produced by sugar catabolism as a sink for the reduced coenzyme NADH. The subsequent phase, catalyzed by alcohol dehydrogenase, transforms pyruvic acid via acetaldehyde to ethanol, allowing glycolysis and ATP synthesis to proceed. While ethanol and carbon dioxide are considered primary metabolites of alcoholic fermentation, acids, higher alcohols, carbonyls, esters, sulfur compounds, terpenes, thiols, tartaric acid, malic acid, and phenolics are secondary metabolites (Cosme et al. 2016). Fermentation kinetics is affected by fermentation temperature, grape juice inoculation, sulfur dioxide addition, settling/clarification of grape must, the composition of the grape must, and interaction between microorganisms (Fleet 1990).

2.2.5. Racking

When the fermentation finishes, wine is transferred from one tank/barrel to another tank/barrel, which is called racking. The purposes are; removing the wine from sediment (dead yeast cells, grape skins, stems, seeds, other impurities) and providing a small amount of oxygen to wine (aeration), improving its flavor and stability. It is also essential to eliminate off-flavors and reductive aromas.

2.2.6. Maturation

Until they are ready to be bottled, many white wines are kept in stainless steel or concrete vats. Keeping oxygen out is crucial; therefore, the vats should be kept whole or covered with N_2 or CO_2. White wines fermented in barrels could later go through barrel maturation, and wines produced in tanks could also be maturated in barrels (Grainger and Tattersall 2016). Producers may choose to mature the wine without racking, called the "élevage sur lie" method, which gives the wine weight, flavor, and/or complexity (Ribéreau-Gayon 2006b). However, alcoholic fermentation can also occur in stainless steel tanks; in this case, wine in oak barrels is only transferred during or after the process is completed (Morata 2021). Also, the blending of wines at this step can be preferred to reach the desired quality.

2.2.7. Clarification

Decantation and settling through the maturation contribute to clarifying wines by getting rid of microorganisms and remaining solids, but not enough to reach the required clarity. Fining explains and modifies wines' sensory or stability attributes (color, flavor). A reactive or adsorptive substance is added to wine to eliminate or reduce the concentration of undesirable components (if necessary, two or three fining agents can be

used simultaneously). This is accomplished by fining agents such as bentonite, albumin, isinglass (fish), caseins, and polyvinylpolypyrrolidone (PVPP), etc. (Reynolds 2010 & Boulton et al. 1999a).

2.2.8. Pre-Filtration

Filtration is based on passing the wine through a filtrate whose pores are tiny, thereby leaving the solids materials in the wine in this filtrate layer. Generally, wines are clarified by resting for some defined time, but it may be slow and insufficient, which requires some methods such as cross-flow filtration, kieselguhr filtration, and plate filtration (Aktan and Kalkan 2000).

2.2.9. Stabilization

Stabilization reduces the possibility of tartrate crystals (potassium or calcium salts of tartaric acid) forming in the wine after bottling. White wines naturally contain higher levels of tartaric acid than red wines. The existence of such tartrates is an undesirable appearance problem for consumers, even if they don't affect the taste (Grainger and Tattersall 2016). The conventional methods are cold stabilization, electrodialysis, and ion exchange treatment. However, these methods are energy and time-consuming polysaccharides (carboxymethylcellulose, mannoproteins, gum arabic, etc.), peptides, or metatartaric acid can be added into wines to prevent precipitation before final filtration and bottling (Guise et al. 2014; Xia et al. 2022; Celotti, Bornia and Zoccolan 1999; Lasanta & Gómez 2012).

2.2.10. Microfiltration and Bottling

Moving the liquid across a porous membrane separates particles with sizes between 0.1 and 10 mm from suspensions during the microfiltration process. Microfiltration provides microbiological stabilization of wine by eliminating yeasts and bacteria that can cause undesirable physical/chemical/organoleptic changes during some defined time (El Rayess et al. 2011). Kaya 2009 stated that microbial load in wines decreased below 10 cfu/ml at the membrane filter outlets, which is the last filtration stage.

Wine bottling involves placing the wine in a glass bottle, capping, and labeling. The shape and color of the bottle, label, design, and closure type differ according to the producer's choice (Reynolds et al. 2018). These external attributes also contribute to wine quality (Orth &Krška, 2001). After bottling, they are left for some defined time aging to express their maximum organoleptic characteristics according to the wine produced (Silva et al. 2011).

2.3. Wine Grapes

A wine grape is a type of grape specifically cultivated and grown for the purpose of making wine. Unlike table grapes, which are often consumed as fresh fruit, wine grapes are chosen for their unique combination of sugar, acidity, flavor compounds, and other attributes that contribute to the production of high-quality wines. These grapes typically have smaller berries, thicker skins, and different chemical compositions compared to table grapes (Upadhyay et al.. 2022). The specific variety of wine grape used can significantly impact the style, flavor, and characteristics of the resulting wine.

2.3.1. *Vitis vinifera*

Vitis vinifera is in the Vitaceae family that belongs to the genus Vitis and originated in Western Asia and southern Europe (Aghbali et al. 2013). It is one of the oldest and most extensively cultivated fruit crops domesticated from *Vitis vinifera* L. subsp. sylvestris (Gmelin) Hegi (McGovern 2013; Grassi and Arroyo-Garcia 2020). There are red, black, and white types that can be seedless and non-seedless. Numerous phytochemical compounds (phenolic compounds, aromatic acids, flavonoids, proanthocyanins, and stilbenoids) can be found in the root, stem, cane, leaf, seed, fruits, pomace, and skin of the grapes (Parihar and Sharma 2021). They are rich in polyphenols, and 90–95% exist in the seeds and skin (Pimple and Badole 2014).

2.3.2. Wine Grapes in the World

Kyoho (table grape), Cabernet Sauvignon (wine grape), and Sultanina (Table, drying, and wine) are the most produced grapes worldwide. As wine grapes, Cabernet Sauvignon, Merlot & Tempranillo are the most planted wine grapes. Table 1 shows grape varieties, colors, and destinations in 75% of the world's area under vine in 2017 (International Organisation of Vine and Wine (OIV) 2017).

Table 1. Grape varieties, color, and destination (International Organisation of Vine and Wine (OIV) 2017)

Grape	Color	Destination
Kyoho	Black	Table
Cabernet Sauvignon	Black	Wine
Sultanina	White	Table, drying, wine
Merlot	Black	Wine

(cont. on next page)

Table 1. (cont.)

Tempranillo	Black	Wine
Airen	White	Wine, Brandy
Chardonnay	White	Wine
Syrah	Black	Wine
Red Globe	Black	Table
Garnacha Tinta /Grenache Noir	Black	Wine
Sauvignon Blanc	White	Wine
Pinot Noir /Blauer Burgunder	Black	Wine
Trebbiano Toscano / Ugni Blanc	White	Wine, Brandy

According to the report State of the World Vine and Wine Sector in 2022 published by International Organisation of Vine and Wine (OIV), Spain has %13 of the world's total surface area planted with vines for all purposes (wine and juices, table grapes and raisins) areas. The list continues as France (11.2%), China (10.8%), and Italy (9.9%), respectively, while Turkey is in the 5th place with 5.6%, with an estimated vineyard surface area of 410 kha in 2022.

2.3.3. Local Wine Grapes in Turkey

Turkey is home to indigenous grape varieties that contribute to Turkish wine's unique character and flavor profile. These local grape varieties thrive in Turkey's diverse terroirs, from the coastal regions to the inland plateaus and highlands.

2.3.3.1. Kalecik Karası

Kalecik Karası is one of Turkey's most famous indigenous red grape varietals cultivated in the Central Anatolia region in Turkey. Wines from Kalecik Karası are known for their vibrant red color, medium body, and elegant, fruity flavors. They often exhibit notes of strawberries, raspberries, blackberries, and cherries. Kalecik Karası wines have a medium acidity and soft tannins, making them approachable and versatile (Çelik et al. 2019).

2.3.3.2. Öküzgözü

Öküzgözü is another important red grape that originated in Elazığ. Wines made from Öküzgözü grapes display a bright garnet color and are characterized by their lively acidity and bright fruitiness. The flavors often include black mulberry, cherry, pomegranate, plum, and violet. Öküzgözü wines are generally medium-bodied with medium tannins, offering a pleasant and refreshing drinking experience. It is a versatile grape that can produce a range of wine styles, from young and fruity expressions to more complex and oak-aged wines (Lemieux 2021; Aktan and Kalkan 2000).

2.3.3.3. Boğazkere

Boğazkere is a red grape varietal originating in Diyarbakır, southeastern Turkey, known for its deep color and bold characteristics. Wines made from Boğazkere grapes are typically full-bodied with high tannins and intense flavors. They exhibit dark fruit notes such as blackberries, black cherries, and plums, along with hints of tobacco, dark chocolate, and spices. Boğazkere wines have great aging potential and develop complexity over time (Lemieux 2021; Aktan and Kalkan 2000).

2.3.3.4. Narince

Narince is a white grape varietal native from Tokat, and it is highly regarded for producing elegant white wines with a crisp acidity and aromatic complexity. Narince wines have pear, honeysuckle, grapefruit flavors, and delicate floral undertones. They have a medium body and a balanced structure, making them versatile for aging and early consumption (Bayram and Kayalar, 2018).

2.3.3.5. Sultaniye

Sultaniye is one of Turkey's oldest grape varietals used in dry and sweet winemaking and marketing as fresh fruit (Ünal, Şener and Şen 2007). Its berries are seedless and medium-sized and have a pale green to yellowish-green color when fully ripe. It is cultivated in various regions, including Thrace and Aegean. Sultaniye wines are light-bodied with moderate acidity and exhibit flavors of subtle fruity notes, including hints of green apple, citrus, and melon. They are often consumed as young, refreshing wines or used to produce aromatic dessert wines.

2.3.3.6. Bornova Misketi

Bornova Misketi is primarily grown in the Aegean region and is known for its aromatic and floral characteristics (Cabaroğlu, Günata and Canbaş 1997). It often exhibits notes of white flowers, bergamot, orange blossom, linden, and tropical fruits such as pineapple and mango. The wines can have a refreshing and lively character. It is often vinified as dry, unoaked white wine; however, making semi-dry and sweet wines is possible.

These indigenous grape varietals reflect Turkey's diverse viticultural heritage and contribute to the country's distinct winemaking identity. The cultivation and utilization of

these local grapes showcase Turkey's potential to offer a wide range of unique and flavorful wines to wine enthusiasts worldwide.

2.3.4. Wines from Emir Grapes

Vitis vinifera L. cv. Emir is a white grape varietal mainly grown in the Cappadocia region of central Anatolia. This region's soil is tuffaceous and primarily composed of volcanic ash (Cabaroğlu, Canbaş and Günata 2002). Wines made from Emir grapes are characterized by their minerality. They also have lime, pear, and daffodil aromas, crisp acidity, and a light body (Lemieux 2021).

Elmacı et al. (2007) studied the effect of using different grape varieties on the sensory characteristics of white wines. Seventeen commercial white wines from 5 grape varieties (Emir-5, Narince-4, Semillon-4, Muscat-2, Chardonnay-2) of 2001 vintage. They've found that alcohol and sulfur aroma attributes and sweet, sour, wet wool, and alcohol flavor attributes were detected in all Emir wines, whereas dust, green apple, raisin, and grape juice aroma notes and salt, metallic, sulfur, medicinal, and raisin flavor characters were detected in the majority of the samples.

With the aim of investigating the effects of selected *S. cerevisiae* strains (indigenous and commercial yeasts) in unpasteurized and pasteurized grape juice to obtain more aromatic cv. Emir wine, Nurgel et al. (2002) analyzed flavor compounds and identified them by GC-FID and GC-MS, respectively. With the addition of native and commercial wine yeasts, it was found that the total concentrations of flavor compounds did not increase where there were differences in the individual volatile compounds. Data from gas chromatography and cluster and factor analyses showed differences in wine volatiles. In contrast, predictions of improving Emir wine quality by inoculating wine strains remained inconclusive. Data from gas chromatography, cluster, and factor analysis indicated differences in wine volatiles, but the prediction of improved Emir wine quality by inoculation with wine strains remained inconclusive.

Cabaroğlu et al. (1997) carried out their study on cv. Emir grapes to examine skin contact effects by analyzing flavor compounds with GC-MS. Identifying seventy-five flavor compounds proved that the white wines made from Emir grapes are rich in volatile

phenols. Wines produced by the skin-contact method had higher total phenol concentrations. Also, 3,5-dimethoxyphenol, 4-vinyl phenol, vanillin, ethylhomovanillate, vanilloyl methyl ketone, 2-(4'-guaiacyl)-ethanol, ethylhomovanillate, and tyrosol showed significant differences. Skin contact before fermentation increased the aroma compounds free and glycosidically bound. It is conceivable to bring the grape juice into contact with the skin before pressing to improve the wines produced from Emir grapes.

Aiming at observing changes in amino acids and phenolic compounds in Emir, Narince, and Sultaniye grapes, high-performance liquid chromatography analysis was performed for two seasons in sequence (2006-2007). Seasonal and varietal variations in amino acid content were observed among the cultivars. In both years, arginine, histidine, and alanine were the mostly found amino acids in all three cultivars. The total amino acid concentration in the Emir cultivar was 1942 mg/L in 2007. The Emir cultivar had the highest histidine in 2006 and 2007 at 229 and 308 mg/L, respectively. The highest alanine concentration in 2006 was also found in Emir at 99 mg/L. The tryptophan level of the Emir cultivar was significantly higher than Sultaniye and Narince cultivars (Ünal et al. 2015).

Ünal and Şener (2006) aimed to fill the gap in the literature on polyphenol oxidase (P.P.O.) in Emir grapes grown in Turkey. For this purpose, they extracted and examined the characteristics of P.P.O. in Emir grapes regarding thermal inactivation, pH and temperature optima, kinetic parameters, and potency of some P.P.O. inhibitors. The optimum pH for grape P.P.O. was 4.2, lower than the other grape varieties, and the temperature was 25°C. Biphasic thermal inactivation behaviour was observed during heat inactivation studies. The most efficient inhibitors were sodium metabisulfite and ascorbic acid, demonstrating that sulfite and ascorbic acid can control enzymatic browning in juice and wine.

Erten et al. (2006) worked on the influence of the addition of commercial wine yeast (*S.cerevisiae*) at inocula of 1×10^4 to 1×10^7 cells /ml in Emir grape must by examining yeast growth, fermentation kinetics, ethyl alcohol, and flavor compound formation. Spontaneous fermentation (without adding commercial yeast) was also performed simultaneously. The results showed that increasing the inoculum level of *S. cerevisiae* causes the earlier disappearance of non-*Saccharomyces* yeasts. Improving the fermentation rate with higher amounts of yeast was observed, but there were no differences in ethanol production. Increasing inoculum levels, especially inoculum sizes of 1×10^6 cells /ml and 1×10^7 cells/ml, causes an increase in the concentrations of higher

alcohols and a decrease in the amount of ethyl acetate, which can be caused by higher persistence of non-*Saccharomyces* yeasts.

2.4. Yeasts in Winemaking

Wine fermentation is a complex biochemical process that involves many microorganisms. Compared to many other food production systems, few efforts are made to eliminate undesirable microorganisms from the raw materials. Grapes naturally contain several genera of yeasts and bacteria. Since different bacteria have different tolerances for inhibiting chemicals and have additional growth requirements, successive growth of those microorganisms occurs during alcoholic and malolactic fermentations (Osborne 2010).

2.4.1. *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is the yeast's primary role in fermented beverage production. It is typically ellipsoid in shape with a large diameter of 5–10 μm and a smaller diameter of around 5 μm. Most *S. cerevisiae* strains grow well at temperatures between 20 and 30 °C and pH 4.5 and 6.5. Considering the oxygen demand, it is sometimes considered a facultative anaerobe; however, it cannot grow in strictly anaerobic circumstances. Oxygen is a crucial growth factor for membrane fatty acids (such as oleic acid) and sterols (such as ergosterol). *S. cerevisiae* is auxotrophic for membrane fatty acids (oleic acid) and sterols (such as ergosterol) under anaerobic conditions. Consequently, supplementing with fatty acids and sterol growth factors (with commercially available yeast nutrients) or adding some oxygen at the beginning of the fermentation process can be required for efficient alcoholic fermentations. (Walker and Stewart 2016).

Choosing yeasts for winemaking involves identifying the cultures that can effectively ferment grape juice and make high-quality wines. The *Saccharomyces* genus is preferred, and the cultures are generally isolated from grape must or wine. Because they are well adapted to the oenological environment, the *Saccharomyces* strains in these substrates can ferment grape must effectively. Table 2. shows the technological characteristics of wine yeast strains.

Table 2. The technological characteristics of wine yeast strains (Rainieri and Pretorius 2000)

Ethanol tolerance	Flocculence
Fermentation vigor	Foam formation
Resistance to SO_2	Film formation
Type of growth in liquid media	Sedimentation speed
Dispersed cells	Growth at high and low temperatures
Aggregates cells	Presence of a killer factor

2.4.2. Non-*Saccharomyces* yeasts

Even though it is expected and preferred for *S. cerevisiae* (inoculated or native) to predominate, wine fermentation is not a single-species fermentation. Non-*Saccharomyces* yeasts are commonly found in grape skin in higher concentrations than *S. cerevisiae,* and they are introduced into the grape must at crushing (Boulton 1999b). These yeasts, which are a component of all wine fermentations and are metabolically active, can impact the quality of the wine through their metabolites. Because they were previously considered spoiling yeasts, the influence of non-*Saccharomyces* yeasts in wine was once limited and eliminated by inoculation with pure *S. cerevisiae* cultures (Jackson 2008). However, during the past three decades, there has been a significant increase in interest in non-Saccharomyces yeasts usage in wine biotechnology (Wang, Mas and Esteve-Zarzoso 2016). Research showed that the harmful metabolic activities of these

yeasts decreased, and beneficial metabolites were produced due to mixed fermentations of *S. cerevisiae* and non-*Saccharomyces* yeast (Ciani and Comitini 2010).

Several strains of different non-*Saccharomyces* species have been extensively studied concerning the formation of some metabolic compounds that impact the bouquet of the wine, contributing to the wine's complexity. Numerous studies on the development and metabolic interactions between non-*Saccharomyce*s and *Saccharomyces* yeasts in mixed cultures have demonstrated their influence on ethanol content, wine flavor, aromatic profile, and quality depending on the strains and the inoculation strategies (Sadoudi et al. 2012). However, finding the correct balance between *S. cerevisiae* and non-*Saccharomyces* species is crucial. If *S. cerevisiae* dominates the non-*Saccharomyces*, it will decrease their impact; conversely, if non-*Saccharomyces* take over *S. cerevisiae*, it may result in stuck or sluggish fermentations (Albertin et al. 2017; Bağder and Özçelik 2008).

The concentration of produced metabolites will determine the contribution of non-*Saccharomyces* yeasts to wine flavor, and it is affected by external factors (environmental circumstances, osmotic pressure, the amount of SO_2 present, alcohol concentration, nutrient levels, etc.) (Jolly, Varela and Pretorius 2013).

The enological features of non-*Saccharomyces* yeast strains and their impact on the complexity of aroma compounds during wine fermentation were examined by Lai et al. in 2022. The experiments are done on forty-two yeast strains isolated from fruits by D.N.A. sequencing. *Hanseniaspora guilliermondii* Ki135, *Pichia kluyveri* Pe114, *Hanseniaspora uvarum* Pi235, and *Saccharomyces cerevisiae* Gr112 were selected. Results showed that *S. cerevisiae* Gr112 showed the best thermal tolerance, ethanol tolerance, and β-glucosidase activity, whereas non-*Saccharomyces* yeast strains produced higher esters, such as ethyl acetate and 2-phenethyl acetate. It is concluded that non-*Saccharomyces* yeast strains provide a more comprehensive range of wine flavors.

Viana et al. (2008) evaluated thirty-eight yeast strains from *Candida, Hanseniaspora, Pichia, Torulaspora*, and *Zygosaccharomyces* yeasts regarding ester formation in a synthetic microbiological environment. *Hanseniaspora* and *Pichia* strains were the best acetate ester producers. *Hanseniaspora guilliermondii* 11027 and 11102, *Hanseniaspora osmophila* 1471, *Pichia* membranifaciens 10113, and 10550 were chosen for further enological characterization based on the ester profile. *H. osmophila* 1471 was a substantial producer of 2-phenyl ethyl acetate. It also consumed more than 90% of the initial must sugars and produced quantities of acetic acid, medium-chain fatty acids, and

ethyl acetate on must that were within the values previously determined for wine. *H. osmophila* 1471 found as a good candidate for mixed starters, and examining the possible interactions with *S. cerevisiae* in further research is suggested.

2.4.2.1. *Torulaspora delbrueckii*

One of the earliest commercially available non-Saccharomyces yeasts was *Torulaspora delbrueckii* (anamorph: *C. colliculosa*). *Torulaspora delbrueckii* is known as *Saccharomyces rosei* and is suggested for producing red and rose wines in Italy with the vinification of grape musts low in sugar and acid (Castelli 1955). It had lower ethanol and higher glycerol and volatile acid levels than *S. cerevisiae* (Arslan, Çelik and Cabaroğlu 2018; Moreno, Klar and Nurse 1991; Renault et al. 2009). The study by King and Dickson 2000 showed that *T.delbrueckii* formed linalool from geraniol, a varietal aroma of the Muscat wines.

Pure and mixed autochthonous *Torulaspora delbrueckii*-214 and *Saccharomyces cerevisiae*-1088 cultures' effects on the fermentation and aroma compounds of Narince wines were examined by Arslan, Çelik and Cabaroğlu 2018. The non-*Saccharomyces T. delbrueckii*-214 yeast slowed down the fermentation process, resulting in higher quantities of glycerol and volatile acid and lower levels of ethanol. Pure culture did not finish fermentation; however, mixed culture with *S. cerevisiae* enhanced wine complexity and aroma intensity by producing higher amounts of alcohol and esters.

Loira et al. (2014) analyzed polyalcohol, aromatic components, and pigment formation and investigated the fermentative behavior of five strains of *T.delbrueckii* in sequential fermentation with *S.cerevisiae*. These five strains produced 7.4-9.0% v/v alcohol and the volatile acid between 0.2-0.7 g/l acetic acid. *T. delbrueckii/S. cerevisiae* fermentations produced 2,3-butanediol, 73% higher than pure culture *S. cerevisiae* fermentation. Higher amounts of production of ethyl lactate, diacetyl, and 2-phenyl ethyl acetate were observed than in pure S. cerevisiae fermentation. 3-ethoxy propanal was produced only in these sequential fermentations. Sequential fermentation had less viticin A and B. Also, 3-ethoxy propanal formation was only observed in these. *T. delbrueckii*

was found to improve the aromatic complexity of wines by contributing to the fruity flavor while maintaining appropriate levels of spoilage features.

In their 2015 study, Azzolini et al. examined the effects of multi-starter fermentation, which involved sequential inoculations of *T.delbrueckii* starter cultures with *S.cerevisiae*, on the fermentation and aroma profile of two different white wine styles: dry and sweet wines. The amount of numerous significant volatile chemicals, including 2-phenyl ethanol, isoamyl acetate, vinyl phenols, C4-C10 fatty acids, and fatty acid esters, was significantly impacted by multi-starter fermentation, according to a chemical examination of Soave and Chardonnay wines (dry wines). Moreover, research using two distinct *T. delbrueckii* strains has demonstrated strain-specific contributions. Vino Santo, a sweet wine, served as additional proof of *T. delbrueckii* activity's beneficial effects on wine quality. This non-*Saccharomyces* yeast is suggested for the fermentation of high-sugar grapes due to its minimal acetic acid production.

Additionally, *T. delbrueckii* affected the amount of several chemical groups, including lactones. Multi-starter-fermented wines have more aromatic diversity and intensity than monoculture-fermented wines concerning sensory properties. These findings highlight the possibility of using *T. delbrueckii* in combination with *S. cerevisiae* to produce a variety of white wines with improved and enhanced flavor.

2.4.2.2. *Metschnikowia pullcherima*

Another commercially accessible yeast is *Metschnikowia pulcherrima* (anamorph *C. pulcherrima*) which is naturally present in grapes, fruits (fresh/spoiled), flowers, nectars, and sap fluxes of trees (Morata et al. 2019b). This commercial strain generates extracellular a-arabinofuranosidase, which affects the level of varietal aromas like terpenes and volatile thiols. It has proteolytic activity, which releases amino acids as a nutrient source for *S. cerevisiae* and controls protein haze formation as a biological fining agent. (Marangon 2012; Romano, Capece and Jespersen 2006). The high capability of producing β-glucosidase enzyme clears off aroma precursors bound to the sugar molecules contributing to wine aroma (Fernández, Úbeda and Briones 2000). It also has high esters, particularly pear-associated ester ethyl octanoate (Jolly, Varela and Pretorius

2013). However, since the fermentative power of *M.pullcherrima* is low (reaching up to 6-7% v/v), it is necessary to use it with other yeast with a high fermentative ability to complete fermentation, such as *S.cerevisiae* & S. *pombe* (Combina et al. 2005; Morata et al. 2019a). Two methods are available for complete fermentation: sequential inoculation, which involves inoculating first with a non-*Saccharomyces* yeast and then *S.cerevisiae* a few days later, and simultaneous inoculation, also known as co-inoculation, with a non-*Saccharomyces* yeast and *S.cerevisiae* strain (Varela et al. 2021).

Ruiz et al. (2018) aimed to examine the sensory effects of selected *M. pulcherrima* strain NS-EM-34 in sequential fermentations with two commercial Saccharomyces cerevisiae strains and its particular impact on the varietal perception of Verdejo white wines. In addition to sensory evaluations of wines, the researchers measured the production of minor (terpenes and thiols) and major varietal volatile components. The levels of the thiol 4-MSP (4-methyl-4-sulfanylpentan-2-one) increased over its sensory threshold, and higher alcohol production decreased when *M. pulcherrima* was used. This result also explains the higher scores in aroma quality, aroma intensity, and overall impression attributes where *M. pulcherrima* was involved in sensory analysis. One of the findings at the end of the study is high glycerol production and low ethanol and acetaldehyde concentration with sequential fermentation.

Dutrative et al. (2019) conducted a study to evaluate the effect of non-*Saccharomyces* yeast on the aroma profile of Riesling grapes wines and find the best strains for this purpose. Fermentation was done with sequential inoculation of four non-*Saccharomyces* yeasts and *S. cerevisiae*. The aroma profile of these wines was compared with the ones fermented with only one *S. cerevisiae* strain. They observed that sequential fermentation of *M. pulcherrima* produced the highest ethyl hexanoate and ethyl octanoate levels, providing apple peel and fruit flavors to the wine. Also, the study done by Sadoudi et al. 2012 showed that *M. pulcherrima*/*S. cerevisiae* co-culture creates a synergistic effect and different aromatic profile than the wines fermented only with *S. cerevisiae* with lower acetic acid production.

Aiming to evaluate the effect of metabolic interactions between Patagonian indigenous *Saccharomyces cerevisiae* MMf9 and β-glucosidase producer *Candida pulcherrima* V6 strains, Rodríguez et al. 2010 examined the fermentation kinetics and sensory quality produced from Muscat d'Alexandrie grape. Simultaneous, sequential, and final inoculation methods were used for laboratory-scale production. The results demonstrated that the best way to combine strains is sequential inoculation. Its kinetic

behaviour resembled a successful spontaneous fermentation; the wine it produced had different aromatic qualities by having the strongest fruity and floral aroma and the highest overall concentration of higher alcohol, esters, and terpenols.

2.4.3. Commercial Active Dry Yeasts

Commercial winemaking yeasts are available in various packaging styles, including fresh cultures, lyophilized yeast, solid culture on agar, and active dry yeast. However, the active dried yeast form dominates the global market, mainly because of its long shelf life and low volume (Maqueda et al. 2010). Inoculating grape must with selected yeasts helps to regulate fermentation, lower the risk of contamination, improve repeatability, and produce certain wine qualities (Pérez-Torrado, Barrio and Querol 2017).

It is estimated that there are 42 commercial products based on non-*Saccharomyces* yeasts now on the market, 79% of which are pure cultures, with the most common strains being *Torulaspora delbrueckii, Lachancea thermotolerans*, and *Metschnikowia pulcherrima*. The others are multi-starters that contain non-*Saccharomyces* yeast species or combinations of *Saccharomyces cerevisiae* and non-*Saccharomyces*. In mixed fermentations, several commercial yeasts have demonstrated sufficient biocompatibility with *S. cerevisiae*. Metabolites of oenological interest (i.e., higher alcohols, glycerol, esters, acids, thiols, and terpenes) are improved. In contrast, acetic acid, volatile phenols, and biogenic amine production are decreased (Vejarano and Gil-Calderón 2021).

CHAPTER 3

MATERIAL & METHOD

3.1. Materials

In this study, the Emir grape (*Vitis vinifera*) harvested in the Nevşehir-Ürgüp region in October 2022 was used. Kavaklıdere Wines supplied the grapes and the equipment used for the winemaking process, and the winemaking process was taken in the Cotes d'Avanos factory. Commercial active dry yeasts were provided from Laffort, France.

3.2. Methods

Chemical and sensory analyses were done in Kavaklıdere Wines and volatile compounds analysis was done in IzTech Integrated Research Centers.

3.2.1. Winemaking

Grapes brought to the factory by a truck in plastic crates were processed on the same day without waiting. They were removed from MOG (material other than grapes) on the sorting table and taken into the de-stemmer and crusher machine with fruit elevators. After adding 2g/hl of SO_2, they were pressed by a pneumatic press. High-quality grape must (free & first press) was taken into the tank for settling. After 18 hours

of cold settling (at 5-7 °C), grape musts were divided into four different tanks for fermentation. Fermentation tanks capacities were 1 ton each. The traditional commercial yeast rehydration procedure was applied to the group selected as the control group (CN). Commercial active dry yeast containing *Saccharomyces cerevisiae* (200 ppm) was added to the bucket, diluted in water 10 times of its weight (37°C), and mixed gently. The starter mixture was left for 20 minutes, and at the end of the period, the starter was acclimated by gradually adding must. The starter was incorporated into the stainless-steel fermentation tank by pumping over. The difference between the temperature of the starter and the grape must that would be inoculated didn't exceed 10°C.

For experimental groups, three commercial active dry non-Saccharomyces yeasts were used, which were *Torulaspora delbrueckii* (ZYMAFLORE® Alpha$^{TD\ n.\ Sacch}$) (NS1), *Metschnikowia pulcherrima* (ZYMAFLORE® KHIO^{MP}) (NS2), and *Metschnikowia pulcherrima & Torulaspora delbrueckii* (ZYMAFLORE® ÉGIDE^{TDMP}) (NS3). The sequential fermentation (*Saccharomyces cerevisiae* inoculation after 24 hours of non-*Saccharomyces* yeast inoculation) method was used. Traditional commercial yeast rehydration procedure was also used with slight modifications. Commercial active dry non-*Saccharomyces* yeast (30 ppm) was added to the bucket, diluted in water 10 times of its weight (25-26°C), and mixed gently. Figure 1. shows yeast and water mixtures right after mixing. The starter mixture was left for 20 minutes, and at the end of the period, the starter was acclimated by gradually adding must. Figure 3. shows their situation after 20 minutes of waiting. The starter was incorporated into the stainless-steel fermentation tank by pumping over. The difference between the temperature of the starter and the grape must that would be inoculated didn't exceed 10°C. After 24 hours, 200 ppm of commercial active dry *Saccharomyces cerevisiae* yeast, also used in the control group, was added to the 3 experimental group tanks following the traditional commercial yeast rehydration procedure.

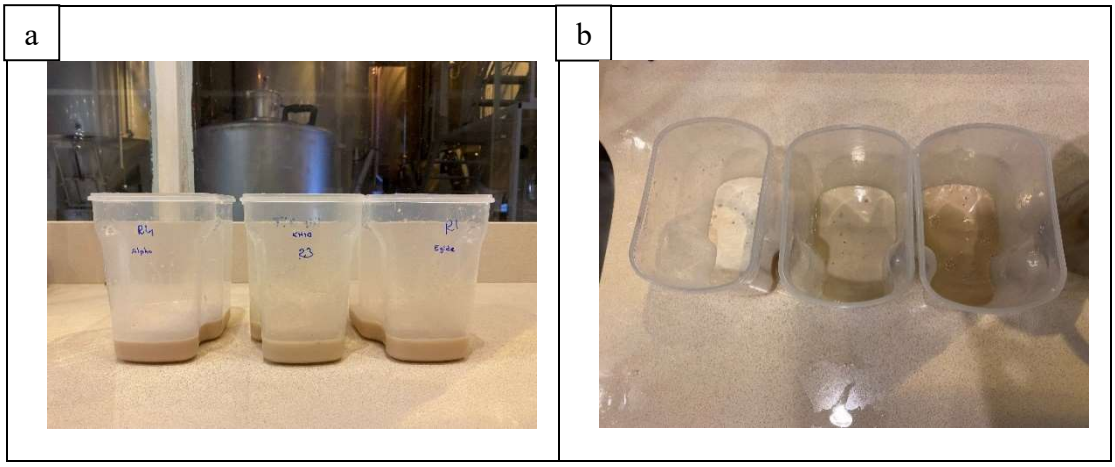

Figure 2. (a) Yeast and water mixtures immediately after mixing and (b) swelled yeast after 20 minutes of waiting

Controlled fermentation was ensured by measuring the density temperature in the morning and evening. Tanks inoculated at 16°C reached a maximum of 21°C during fermentation. Reducing sugar analysis was performed daily when the density of the grape musts decreased below 1.000 g/ml. When the reducing sugar dropped below 4 g/l, the wines were separated from the yeast residue by transferring the wine to a stock tank (racking). 25 mg/l SO_2 was added to the wine to prevent oxidation and left to rest. Volatile acid, SO_2, and sensory analysis were conducted to avoid deformation in wine quality. After four months of maturation, wines were bottled in February 2023 without any clarification, filtration, and stabilization process for chemical and sensory analysis. Figure 3 shows the wine samples that were analysed.

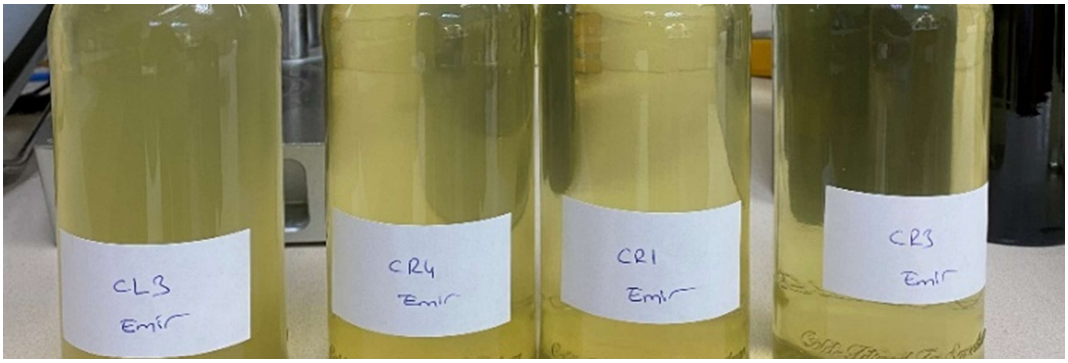

Figure 3. The wine samples that were analysed

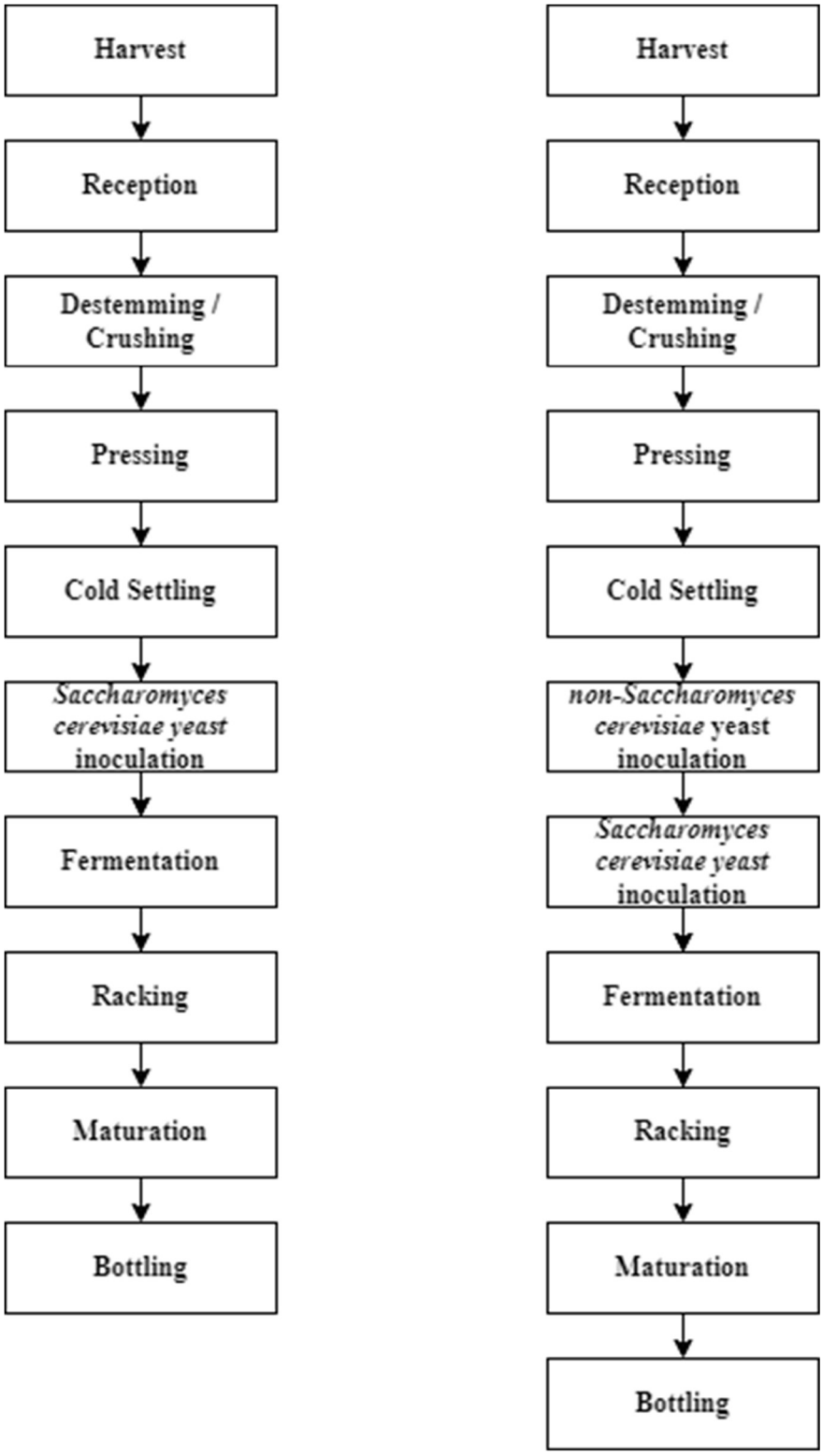

Figure 4. Flow chart of the winemaking process in the study

3.2.2. Analyses of Grape Must and Wines

Total dry matter, density, total acidity, pH, reducing sugar, free SO2, and volatile acidity analyses were made in the grape must. In the wine samples, in addition to these analyses, total SO_2, alcoholic strength by volume, color, and volatile compound analysis were also conducted.

3.2.2.1. Total Dry Matter (%Brix) Analysis

The water-soluble dry matter in grape must as % Brix (at 20 °C) was determined by refractometer (International Organisation of Vine and Wine (OIV) 2022).

3.2.2.2. Density Analysis

Density in the grape must was determined by an electronic hand densimeter at 20°C. The density in wine was measured with the method "density at 20 °C and specific gravity at 20 °C measured by pycnometry" (International Organisation of Vine and Wine (OIV) 2022).

3.2.2.3. Total Acidity

Total acidity analysis was done using potentiometric titration to pH 7 against a standard alkaline solution. (Method OIV-MA-AS313-01) (International Organisation of Vine and Wine (OIV) 2022).

As a pre-treatment, approximately 50 ml of the sample is stirred under a vacuum with the help of a magnetic stirrer in a 500 ml flask using the apparatus to remove CO_2. A sample of 10 ml of pre-treated wine and 10 ml of distilled water were added into a beaker. After immersing the probe of the pH meter in the beaker, a magnetic stir bar was also thrown into it. Then it was placed on the stirrer. Then, 0.1M sodium hydroxide solution was added very slowly to the beaker while stirring continuously until the pH is 7.0 at 20°C. The used volume of 0.1M sodium hydroxide was recorded as “n”.

The total acidity expressed in milliequivalents/liter is given by:

$A = 10\ n.$

It is recorded to one decimal place.

The total acidity expressed in grams of sulfuric acid/liter is given by:

$A' = 0.049 \times A$

3.2.2.4. pH

The pH value was measured using a pH meter.

3.2.2.5. Alcoholic Strength by Volume

Alcoholic strengths by volume (v/v) analysis of the wine were done by distillation of wine samples and measurement of alcohol by volume (v/v) of the obtained distillate with the help of an alcoholmeter (Method OIV-MA-AS312-01 / Type IV) (International Organisation of Vine and Wine (OIV) 2022).

3.2.2.6. Reducing Sugar

Wine samples were treated with lead acetate or zinc 2-hexacyanoferrate for clarification. Clarified wine reacted with a certain amount of alkaline copper salt solution, and the excess copper ions were determined iodometrically. (Method OIV-MA-AS311-01A) (International Organisation of Vine and Wine (OIV) 2022).

3.2.2.7. Volatile Acidity

Volatile acidity analysis was done by separating volatile acids from wine samples by steam distillation and titration of the obtained distillate. (Method OIV-MA-AS313-02) (International Organisation of Vine and Wine (OIV) 2022).

3.2.2.8. Free Sulfur Dioxide Analysis

As a pre-treatment, the wine samples were stored in a closed and full bottle at 20°C for two days before their measurements. The analysis was conducted by using the apparatus in Figure 5.

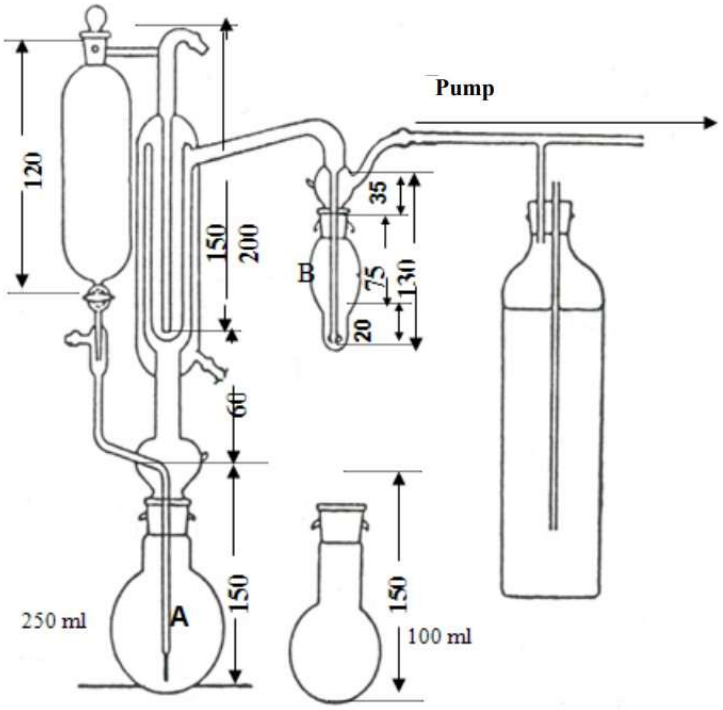

Figure 5. Sulfur dioxide measurement apparatus (International Organisation of Vine and Wine (OIV) 2022)

Three ml of hydrogen peroxide solution and two drops of indicator reagent were transferred to flask B. The hydrogen peroxide solution was neutralized with 0.01M sodium hydroxide solution (the initially blue-purple color turns green after neutralization). Bubbler B was attached to the apparatus.

Fifty ml of wine sample and 15 ml of phosphoric acid were taken into flask A, and the flash was attached to the apparatus. Nitrogen was passed through the instrument to form bubbles for 15 minutes. The free sulfur dioxide is oxidized to sulfuric acid. Flask A was removed from the apparatus, and the acid formed in bubbler B was diluted with 0.01 M sodium hydroxide solution. The volume spent was recorded as n ml.

Free sulfur dioxide was "6.4 * n" in mg/l and written as an integer (Method OIV-MA-AS323-04A) (International Organisation of Vine and Wine (OIV) 2022).

3.2.2.9. Total Sulfur Dioxide Analysis

The sample's estimated total sulfur dioxide concentration was less than 50 mg/l; therefore, 50 ml of the sample and 15 ml of phosphoric acid were placed in a 250 ml flask A. The flask was attached to the apparatus.

Three ml of hydrogen peroxide solution was transferred to bubbler B, and a burner was placed under the bubbler, which gave a small flame at a height of 4-5 cm to boil it.

The nitrogen flow didn't interrupt during boiling. Within 15 minutes, the total sulfur dioxide was transported and oxidized, and sulfuric acid formed. The amount of sulfuric acid was determined by titration with a 0.01 M sodium hydroxide solution. The volume spent was expressed as "n."

Total sulfur dioxide was "6.4 * n" in mg/l and written as an integer for samples that have sulfur dioxide less than 50 mg/l (Method OIV-MA-AS323-04A) (International Organisation of Vine and Wine (OIV) 2022).

3.2.2.10. Color Analysis

The sample cup was filled with equal volumes of wine samples (about 20 mL). Using a CR-400 Chroma meter (Konica Minolta, UK), values for L* (lightness), a* (red/green value), and b* (blue/yellow value) were assessed to determine the color of the samples. The instrument's details include the observer angle of 2° and the illuminant D65.

3.2.2.11. Sensory Analysis

A sensory analysis of wines was carried out by blind tasting with 12 wine experts in a tasting room with large windows that let in sunlight. Wines were served numbering randomly as 976, 673, 973 and 376 on a wine tasting table lined with white cover. The

wines were analyzed in 3 main categories: appearance, smell, and taste. Appearance consists of color intensity, color tonality & shade, transparency, and brightness. Condition, intensity, fragrance on the nose and sweetness, alcohol, acidity, body, minerality, flavor intensity, persistence, and fineness on the palate were analyzed. Also, the panelist evaluated the aroma profile in both nose and palate (fruits, flowers, spices, and vegetables) and, finally general impression. The grades were given out of 5 (1: lowest and 5: highest). Table 3. shows the sensory evaluation form used in the analysis.

Table 3. Sensory evaluation form

	976	**673**	**973**	**376**
Appearance				
Color Intensity				
Color Tonality & Shade				
Transparency				
Brightness				
Smell				
Condition				
Intensity				
Fruits				
Flowers				
Spices				
Vegetables				
Fragrance				
Taste (Palate)				
Acidity				
Body				
Alcohol				
Sweetness				
Flavor Intensity				
Fruits				
Flowers				
Spices				
Vegetables				
Persistence				
Fineness				
Quality				
General Impression				

3.2.2.12. Volatile Compound Analysis

The volatile compounds analysis was conducted using the headspace solid-phase microextraction (HS-SPME) coupled with GC–MS following the method of Hu et al. 2019 with slight modifications. 5 mL of wine sample was added to a 20 mL glass vial containing 1 g NaCI and 10 µL internal standard (16 µg/L, 2-octanol) and then equilibrated at 40 ◦C for 15 min. The 50/30 µm DVB/CAR/PDMS fiber (Sigma Aldrich, 2 cm length, 50/30 µm thickness was immersed in the headspace, stirred at 40 ◦C, 600 rpm for 30 min, and then desorbed using a Restek Stabilwax DA column in a GC injection port at 230 ◦C for 5 min. The carrier gas was helium (99.999%), and the flow rate was 1.3 mL/min. The program of GC was as follows: 40 ◦C for 3 min, raised to 160 ◦C at 4 ◦C/min and then raised to 220 ◦C at 7 ◦C/min for 8 min. The GC and MS transfer line temperature was 250 ◦C, and the ion source was 230 ◦C. Electron ionization (EI) mass spectrometric data from m/z 35 to 350 were scanned at 0.2 s intervals. The compounds were identified qualitatively by comparing their area% with pure standards and the NIST 05 mass spectrum library.

3.2.3. Statistical Analysis

The obtained data were analyzed using the Minitab statistical software program (v.19.1, Minitab Inc., Pennsylvania, USA). One-Way ANOVA and Tukey's Multiple Range Test were performed on the data at $p<0.05$ to determine any significant differences between wine samples. Chemical and sensory properties were analyzed by applying Principal Component Analysis (PCA) on SIMCA software (version 14.1, MKS Umetrics, Malmo, Sweden).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Chemical Properties

The Emir grape must analysis before the fermentation is given in Table 4. As expressed by Brix, the total dry matter in grape must was 19.4, where the corresponding potential alcohol value was 11% (v/v), and density was 1080 with 11.26% (v/v) potential alcohol. Total acidity and pH were found as 2.95 and 3.46, respectively. Since sulfur dioxide is toxic to non-*Saccharomyces* yeast (Henick-Kling et al. 1998), the levels are put at lowest and free SO_2 and total SO_2 were found as 14 and 115 mg/l, respectively.

Table 4. Pre-fermentation analysis of grape musts from Emir grape

Analysis	Content
Brix / Brix Alcohol	19.4 / 11% (v/v)
Density / Density Alcohol	1080 / 11.36% (v/v)
Total acidity (g/L)	4.05
pH	3.46
Free SO_2 / Total SO_2 (mg/L)	14 / 115

Table 5. Daily (morning/evening) density/temperature changes for grape musts from Emir grapes

Days	Hours	CN		NS1		NS2		NS3	
Day 1	08:30	1080	16.4	1079	16.9	1078	17.2	1079	17.2
	16:30	1080	16.2	1077	17.4	1077	17.6	1077	17.7
Day 2	08:30	1077	16.8	1073	18.7	1072	18.5	1071	19.2
	16:30	1075	16.2	1067	20	1068	19.7	1064	20.4
Day 3	08:30	1064	16.1	1047	20.9	1048	20.5	1044	20.3
	16:30	1059	17.3	1042	21	1041	20.6	1040	20.1
Day 4	08:30	1045	16.9	1026	21	1024	20.7	1024	21
	16:30	1039	16.8	1021	20.6	1021	21	1022	21
Day 5	08:30	1030	17.5	1012	20.8	1013	20.6	1012	20.6
	16:30	1027	17.5	1009	20.9	1009	20.8	1008	20.5
Day 6	08:30	1020	16.9	1003	20.4	1003	20.3	1004	20.2
	16:30	1017	17.7	1002	20.1	1001	21	1001	20.1
Day 7	08:30	1011	17.9	998	19.6	998	19.5	999	19.7
	16:30	1009	18.6						
Day 8	08:30	1004	18.4						
	16:30	1002	18.2						
Day 9	08:30	999	18.6						
	16:30								

As shown in Table 5, fermentation of the control group (CN) wines was finished in 9 days, with an average decrease of 7-15 units per day in their densities at 16-19 °C. The measurement of density/temperature of NS1, NS2, and NS3 was started after adding non-*Saccaromyces* yeast and *Saccharomyces cerevisiae*, while it was measured after adding *Saccharomyces cerevisiae*. Experimental groups NS1, NS2, and NS3 completed their fermentation in 7 days. The tanks were also kept at a temperature between 16-21°C, and 6-14 units decrease in the densities was observed. The temperature directly affects the fermentation kinetics, ethanol yield, and production of other fermentation by-products; therefore, monitoring is essential (Şener, Canbaş and Ünal 2007).

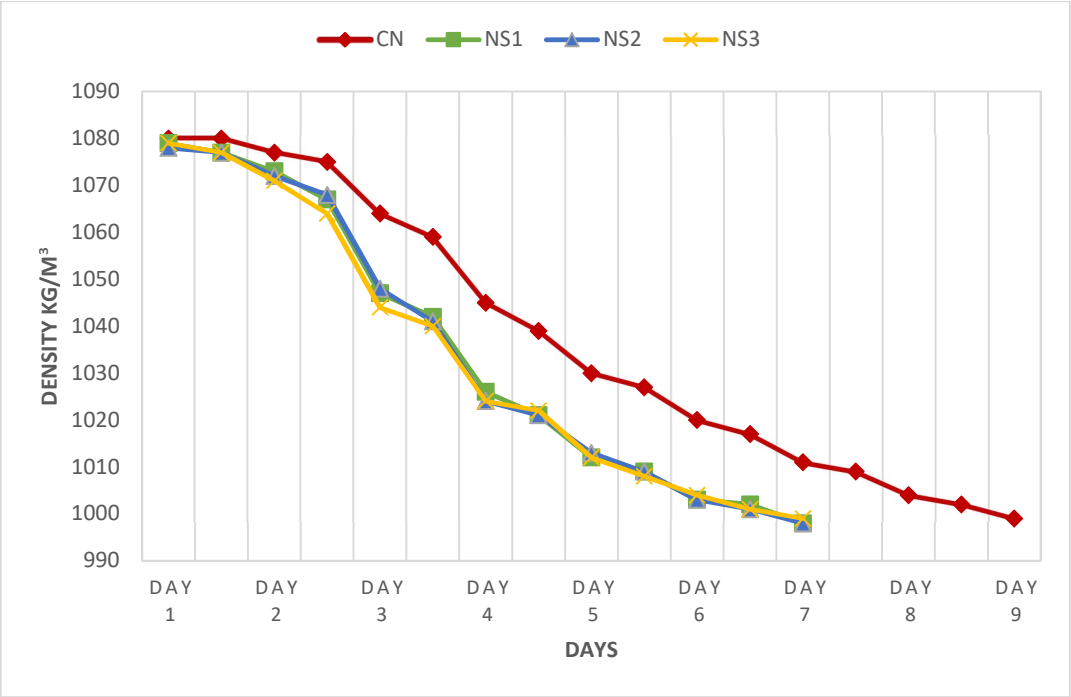

Figure 6. The density decreases of the grape musts during fermentation

Figure 6 shows the density decrease during the fermentation of wines. Beginning densities are 1080, 1079, 1078, and 1079 for CN, NS1, NS2, and NS3, respectively. The reduction in the densities for NS1, NS2, and NS3 is similar, whereas CN differs. All groups gradually declined during fermentation; however, NS1, NS2, and NS3 decreased sharply (20-24 units) only in the third and fourth days. The regular decrease is preferred for reaching maximum aroma complexity, which is the result of yeast addition (Heard and Fleet 1985)

The reducing sugar analysis is done when the density of the grape must during fermentation below 1000 as an indication of the end of fermentation. According to the Turkish Food Codex (2008), the sugar is expected to be ≤ 4 g/L for dry wine. Table 5 shows CN had 3.90 g/l reducing sugar, while NS1, NS2, and NS3 had 4.00, 4.00, and 3.90 g/L sugar, respectively.

Table 6. End-of-fermentation analysis of wines

Sample	Alcohol % (v/v)	Total Acidity* (g/L)	pH	Volatile Acidity** (g/L)	Reducing sugar (g/L)
CN	11.9 ± 0.0 [a]	4.30 ± 0.03[c]	3.16 ± 0.01[c]	0.26 ± 0.01 [a]	3.90 ± 0.01 [a]
NS1	11.8 ± 0.07 [b]	4.40 ± 0.0 [a]	3.20 ± 0.01 [a]	0.3 ± 0.01 [a]	4.00 ± 0.01 [a]
NS2	11.8 ± 0.0 [ab]	4.40 ± 0.03[bc]	3.25 ± 0.0 [b]	0.2 ± 0.01 [a]	3.90 ± 0.01 [a]
NS3	11.9 ± 0.0[a]	4.50 ± 0.0 [ab]	3.23 ± 0.01 [a]	0.25 ± 0.01 [a]	4.00 ± 0.01 [a]

* expressed as sulfuric acid

** expressed as acetic acid.

a-c: Significantly different results are indicated by various superscripts ($p<0.05$).

Table 7. Chemical analysis of wines produced from Emir grapes after four months of resting

Sample	Alcohol % (v/v)	Total Acidity* (g/L)	pH	Volatile Acidity** (g/L)	Reducing Sugar
CN	12.0 ± 0.0[a]	3.9 ± 0.0[a]	3.15 ± 0.0[a]	0.32 ± 0.01[a]	1.5 ± 0.07[b]
NS1	11.7 ± 0.0 [d]	3.8 ± 0.0[a]	3.16 ± 0.0[a]	0.30 ± 0.01[a]	1.1 ± 0.00[bc]
NS2	11.9 ± 0.0 [b]	3.9 ± 0.7[a]	3.19 ± 0.04[a]	0.31 ± 0.01[a]	2.1 ± 0.14[a]
NS3	11.8 ± 0.0 [c]	3.5 ± 0.0[b]	3.15 ± 0.02[a]	0.32 ± 0.01[a]	1.1 ± 0.14[c]

* expressed as sulfuric acid

** expressed as acetic acid.

a-d: Significantly different results are indicated by various superscripts ($p<0.05$).

Table 6 shows the end-of-fermentation analysis of wines, and Table 7 shows the total acidity, alcohol, volatile acidity, pH, and reducing sugar analysis results done for the wines after four months of resting and bottling. The density of CN was 0.990, NS1 and NS2 were 0.991, and NS3 was 0.990, and they were found as significantly different from each other ($p<0.05$). Similar results were found for Emir wines in studies done by Cabaroğlu 1995; Cabaroğlu et al. 1997; Cabaroğlu et al. 1999, and Bağatar 2011.

The total acidities of CN (3.9), NS1 (3.8), and NS2 (3.9) wines were not significantly different from each other; however, NS3 had the lowest acidity (3.5), and it substantially differed ($p<0.05$). When the pre-fermentation acidity and end-of-fermentation acidity were compared, it was observed that the acidity increased during fermentation. In contrast, when the wine was left to rest, the total acidity decreased for all the groups.

Non-*Saccharomyces* yeasts can produce wines with reduced ethanol concentration when sequentially inoculated with *S. cerevisiae* (Contreras et al. 2014). The results showed that CN had the highest ethanol concentration (12% v/v), and NS1 had the lowest (11.7% v/v), where NS2 had 11.9 % v/v, and NS3 had 11.8% v/v. It can be said that the use of non-Saccharomyces yeast in sequential fermentation with *Saccharomyces cerevisiae* caused a decrease in the alcohol content of wines, as expected. Similar results for the alcohol content of wines from Emir grapes were found in the literature (Balıkçı et al. 2016; Cabaroğlu et al. 1997)

The pH of the wines produced from Emir grapes was examined. The average pH values for CN, NS1, NS2, and NS3 were found as 3.16, 3.17, 3.15, and 3.19, respectively. The highest pH was found in NS3 (3.19), and the lowest pH was in NS2 (3.15), where all the results didn't significantly differ from each other ($p>0.05$).

The acetic series of acids, present in wine free and combined as salts, give the wine its volatile acidity (International Organisation of Vine and Wine (OIV) 2023). Wines are always wanted to have a low volatile acidity level. When there is an excessive amount, it is a sign of wine deterioration because they give the beverage an unpleasant vinegar flavor and smell (Vilela-Moura et al. 2010). The maximum acceptable legal limit of volatile acidity for white wines is 1.08 g/L (18 meq/L) as acetic acid on Turkish Food Codex. For this study, CN had 0.32 g/L, NS1 had 0.30 g/L, NS2 had 0.31 g/L, and NS3 had 0.32 g/L as acetic acid. The results were below the determined by limit Turkish Food Codex, and they were not significantly different from each other ($p>0.05$). Ruiz et al. 2018 stated that *Metschnikowia pulcherrima* could decrease volatile acidity; however, in this study it was not observed.

After four months of resting, the reducing sugar analysis results were 1.5 g/L, 1.1 g/L, 2.1 g/L, and 1.1 g/L for CN, NS1, NS2, and NS3, respectively. NS2 had the highest reducing sugar, and NS3 had the lowest and the results were significantly different. NS1 was not different from NS3 and CN ($p>0.05$).

Table 8. Free and total sulfur dioxide analysis results in wines from Emir grapes

Sample	Free SO_2 (mg/L)	Total SO_2 (mg/L)
CN	24.5 ± 0.71^{a}	109 ± 0.7^{a}
NS1	21.0 ± 1.41^{ab}	95 ± 1.4^{b}
NS2	19.5 ± 0.71^{b}	98 ± 0.0^{b}
NS3	21.5 ± 0.71^{ab}	99.50 ± 2.1^{b}

a-b: Significantly different results are indicated by various superscripts ($p<0.05$).

Sulfur dioxide is the most important additive for winemaking since no other additive has the same dual properties of anti-oxidation and preservation. All forms of microorganisms, including yeast, lactic acid bacteria, and to a lesser extent, acetic acid bacteria, are inhibited by this substance from growing. Its activity stops Brettanomyces' growth, yeasts' development, and yeast haze generation. In addition to having antiseptic properties, SO_2 in wine protects against oxidation in a significant way (Santos et al. 2011). However, excessive SO_2 usage should be avoided for both health and enological reasons, as it might affect the finished product's organoleptic properties by producing aroma defects (Ribéreau-Gayon et al. 2006a).

Therefore, OIV's maximum concentration permitted in wines is currently 150 mg/L for red wines and 200 mg/L for white and rosé wines (containing a maximum of 4 g/L of reducing substances) (International Organisation of Vine and Wine (OIV) 2022). Since the end-of-fermentation analysis was done right after the fermentation was finished, it was customary to observe the lowest values. Table 8 shows free and total sulfur dioxide analysis results in wines from Emir grapes. The free & total SO_2 values for CN, NS1, NS1 and NS3 were 1.0 mg /L & 30.0 mg/L, 0 mg/L & 19 mg/L, 1 mg/L & 18 mg/L and 1 mg/L & 20 mg/L respectively.

After four months of resting, the analysis showed that CN had the highest total SO_2 concentration ($p<0.05$) but didn't exceed the legally determined value. NS1 had 95 mg/L total SO_2, NS2 had 98 mgL and NS3 had 99.50 mg/L. During maturation and storage, 30 mg /L of free SO_2 for white wine is also recommended (International Organisation of Vine and Wine (OIV) 2022). In this study, CN had 24.5 mg/L of free SO_2, where NS1 had 21.0 mg/L, NS2 had 19.5 mg/L, and NS3 had 21.5 mg/L.

4.2. Color Characteristics

Table 9. Color measurement results of wines

Sample	L*	a*	b*
CN	27.41 ± 0.02^{a}	-2.53 ± 0.04^{b}	6.00 ± 0.04^{a}
NS1	27.34 ± 0.2^{a}	-2.41 ± 0.05^{a}	5.71 ± 0.09^{b}
NS2	27.92 ± 0.65^{a}	-2.50 ± 0.08^{ab}	5.37 ± 0.13^{c}
NS3	28.04 ± 0.62^{a}	-2.48 ± 0.03^{ab}	5.81 ± 0.15^{ab}

L*: lightness, a*: redness/greenness chromaticity, b*: yellowness/blueness chromaticity

a-c: Significantly different results are indicated by various superscripts ($p<0.05$).

The wines' L*, a*, and b* color values were measured with a Hunter Lab color measuring device. L*, a*, and b* values are given with a 3-dimensional coordinate system, and in this coordinate system, the L* value indicates the transition from brightness to darkness on the vertical axis. +a* indicates redness, -a* greenness, +b* yellowness, and -b* blueness. Table 9 shows the color measurement results of wine samples. L* values of CN, NS1, NS2, and NS3 were found as 27.41, 27.43, 27.92, and 28.04, respectively, and were not significantly different. All the samples had a negative a* value, meaning they were greenish. NS3 (-2.48) and NS2 (-2.50) were considered as same statistically ($p>0.05$); however, CN (-2.53) and NS1(-2.41) were significantly different ($p<0.05$). It can be said that CN was the most greenish among these samples. When comparing the yellowness/blueness of wine samples, CN had the highest b* value (6.00), and NS2 had the lowest (5.37) b* value, which differed significantly from each other the other groups ($p<0.05$). The b* value of NS1 was 5.71 and 5.81 for NS3.

4.3. Volatile Compound Profiles

The analysis was done using HS-SPME–GC/MS method, and 25 volatile compounds were detected. Table 10 shows the volatile compounds of the samples with their area% and potential effect on the wine and Table 11 shows heat map of these volatile compounds. Octanoic acid, ethyl ester (ethyl octanoate), known as giving the wine the aromas of green apples, pears, and pineapple, was present at the highest concentration in all of the compounds but mostly in NS2 (30.98%). Cabaroğlu et al. 1997 also detected these compounds in Emir wines. Balıkçı et al. 2016 stated that the sequential fermentation of *L.thermotolerans* decreased ethyl octanoate. For this study, the use of *Metschnikowia pulcherrima and Metschnikowia pulcherrima* / *Torulaspora delbrueckii* caused an increase in the related compound.

NS2 was rich in decanoic acid, ethyl ester (ethyl decanoate) (22.46%), positively contributing to young wines' flavor by introducing floral and fruity notes. The concentrations were 20.91%, 22.40%, and 21.63% for CN, NS1, and NS3, respectively. It was also observed in the studies done on Emir wines in the literature (Cabaroğlu et al. 1997; Nurgel et al. 2002)

Hexanoic acid, ethyl ester (ethyl hexanoate) which is related to green apple flavor was highly found in NS2 (7.86%) and less in CN (7.02%). Octanoic acid is responsible for grass acid- like aroma and its concentration in CN were higher than other samples. NS3 also has the highest amounts of a main contributor and flavor enhancer in wines, 1-Butanol, 3-methyl- (isoamyl alcohol) (9.46%). For CN, NS1 and NS2 were 8.98%, 7.99%, and 8.77%, respectively. In the study done by Nurgel et al. 2002 it was observed that isoamyl alcohol is one of the compounds with the highest concentration compared to other higher alcohols. Also, Vilanova et al.., 2012 found 3-methyl-1-butanol to contribute to aroma intensity with hexanoic acid and octanoic acid.

Dodecanoic acid only observed in CN at a lower concentration (0.27%). Nurgel et al. 2022 reported that dodecanoic acid was observed in Emir grape wines with spontaneous fermentation. The dodecanoic acid concentration was higher in Emir grapes' free-run juice in the study by Selli et al. 2011. In contrast, 1-Propanol, 2-methyl- (isobutanol) and Octanoic acid, 3-methyl butyl ester (isoamyl octanoate) were found in NS1, NS2, and NS3, not CN. It can be concluded that these compounds are formed with

non-Saccharomyces yeasts. The study by Carpena et al. 2020 stated that *M. pulcherrima* caused a high production of higher alcohols such as isobutanol.

Isoamyl acetate, one of the important esters in wine, was highly found in NS3 which contains *Metschnikowia pulcherrima* in common. Dodecanoic acid, ethyl ester was observed with only the presence of *Torulaspora delbrueckii*. NS1 was found rich in phenylethyl alcohol (gives honey, spice, rose, lilac aromas), ethyl 9-decanoate (gives a pleasant odor) and 1-octanol (contributes to green aroma) concentrations (Reynolds 2021; Kafkas et al. 2005; Katarína et al. 2014). Ethyl acetate were found in CN, NS1, NS2 and NS3 at the levels of 3.64%, 2.47%, 2.88% and 2.92%, respectively. High levels of this compound are indicative of microbial spoilage, but at low levels it can enhance fruitiness & add complexity to wine (Cliff and Pickering 2006; Jackson 2020). Methyl octanoate, methyl decanoate, hexanoic acid were present in all of the samples at similar levels. Diethyl succinate (melon aroma), and ethyl butyrate (sour fruit, fruity aroma) were highly found in CN which contributes positively to the wine quality however n-decanoic acid was also high in CN and gives unwanted soapy aroma to the wine (Lasik-Kurdyś, Majcher, and Nowak 2018; Li et al. 2007; Cosme et al. 2016).

4.4. Sensory Properties

The results were analyzed using ANOVA and a radar chart in Excel. Table 12 and Figure 7,8,9 shows the results of sensory analysis. NS3 had the highest scores for color intensity, color tonality & shade, and brightness; however, the differences were insignificant ($p>0.05$). NS3 also had the highest score in transparency, whereas CN3 had the lowest with a significant difference ($p<0.05$). Condition parameter was put in to examine whether there was an unwanted smell (rancid, rotten eggs, burning tires, spoiled, unappetizing), and all the samples were found clean. Also, the intensity of aroma and fragrance in the nose were similar statistically ($p<0.05$). CN was evaluated as weak by fruity and floral aromas, while it had the highest score in vegetable aroma. NS3 was the fruitiest wine, and NS2 smelled more of the flower than the other samples on the nose and palate. According to chemical analysis, NS2 had the highest reducing sugar ($p<0.05$) and the highest score for the sweetness parameter in sensory analysis. According to the

laboratory analysis results, NS3 had the lowest acidity; however, the panelist didn't observe any significant difference in acidity in the palate between the samples ($p<0.05$).

Table 10. The volatile compounds of the samples with their area% and potential effect on the wine from literature

Compounds	NS1	NS2	NS3	CN	Effect
Ethyl Acetate	2.47	2.88	2.92	3.64	high levels: microbial spoilage; low levels: enhance fruitiness & add complexity to wine (Cliff an Pickering 2006; Jackson 2020)
Butanoic acid, ethyl ester (ethyl butyrate)	0.32	0.39	0.33	0.56	has a positive contribution to wine quality, gives sour fruit, strawberry, fruity aroma (Li et al 2007)
1-Propanol, 2-methyl- (isobutanol)	0.32	0.35	0.41	0.00	involved as ester precursors which are important contributors of wine aroma (Carpena et al 2020)
1-Butanol, 3-methyl-, acetate (isoamyl acetate)	7.02	7.59	7.71	7.64	one of the most important esters in wine, gives a banana smell (Plata, Mauricio and Ortega 2003)
1-Butanol, 3-methyl- (isoamyl alcohol)	7.99	8.77	9.46	8.98	the major higher alcohol and a main contributor and flavor enhancer in wines (Blanco, Sáenz-Navajas and Ferreira 2016; Chambers and Koppel 2013)
Hexanoic acid, ethyl ester (ethyl hexanoate)	7.75	7.86	7.73	7.02	gives green apple flavor to the wine (Gil et al 2006)
Acetic acid, hexyl ester (hexyl acetate)	0.90	0.93	0.00	0.81	provides apple, fruit, herb, sweet or waxy aromas to the wine (Carpena et al 2020)
Octanoic acid, methyl ester (methyl octanoate)	0.13	0.11	0.11	0.12	considered as unwanted odorants however they contribute significantly to the complexity to the flavor of wine (Zhao et al 2017
Octanoic acid, ethyl ester (ethyl octanoate)	30.32	30.98	29.49	27.23	gives the wine the aromas of green apples, pears, and pineapple (Avram et al 2015)
1-Octanol	1.56	1.22	1.18	1.44	contributes the green aroma of the wine (Katarína et al 2014)
Decanoic acid, methyl ester (methyl decanoate)	0.11	0.10	0.10	0.11	one of the common volatile fatty acids in wine, provides fatty, rancid, and cheese aromas (Wang, Mas and Esteve-Zarzoso 2016)

(cont. on next page)

Table 10. (cont.)

Decanoic acid, ethyl ester (ethyl decanoate)	22.40	22.46	21.63	20.91	as highly positive contribution to flavor of young wines by introducing floral and fruity notes (Wada and Shibamoto 1997)
Octanoic acid, 3-methylbutyl ester (isoamyl octanoate)	0.19	0.18	0.23	0.00	contributes to flowery-fruity aromatic profile of wines (Jurado et al 2007)
Ethyl 9-decenoate	0.64	0.17	0.29	0.51	provides a very pleasant odour, and ethyl cinnamate has been described as exhibiting a fruity–honey-like odour (Kafkas et al 2005)
Acetic acid, 2-phenylethyl ester (2-phenyl acetate)	0.00	2.30	2.41	2.37	gives a fruity and flowery flavour with a honey note (Rojas et al 2003)
Hexanoic acid	0.96	0.91	0.89	1.20	gives sweaty, cheesenotes to wines (Cosme et al 2016)
Phenylethyl Alcohol	4.37	3.68	3.81	3.95	provides honey, spice, rose, lilac (Reynolds 2021)
Octanoic Acid	5.95	5.24	5.33	7.30	gives grass acid- like aroma profile to the wine (Cosme et al 2016)
Hexadecanoic acid, ethyl ester (ethyl palmitate)	0.09	0.08	0.00	0.16	contributes to flavor notes of the wines with fruity and floral scents (Duan et al 2018)
Acetic acid	0.13	0.13	0.12	0.27	higher levels: microbial spoilage; low levels: enhance fruitiness & add complexity to wines (Jackson 2020)
n-Decanoic acid	4.09	3.36	3.45	4.81	gives soapy aroma to the wine (Cosme et al 2016)
Butanedioic acid, diethyl ester (diethyl succinate)	0.00	0.29	0.28	0.42	gives fruity melon notes to wine aroma (Lasik-Kurdyś, Majcher, and Nowak 2018)
Dodecanoic acid	0.00	0.00	0.00	0.33	provides floral, fruity, candy, waxy, soap flavor to the wine (Summerson et al 2021)
1-Hexanol	0.00	0.00	0.20	0.24	provides higher pleasant fruit perception to the wine (Ling et al 2021)
Dodecanoic acid, ethyl ester	2.30	0.00	1.93	0.00	gives floral and sweet odor to final aroma (Park et al 2013)

Table 11. Heat map of volatile compounds

Compounds	NS1	NS2	NS3	CN
Ethyl Acetate				
Butanoic acid, ethyl ester				
1-Propanol, 2-methyl-				
1-Butanol, 3-methyl-, acetate				
1-Butanol, 3-methyl-				
Hexanoic acid, ethyl ester				
Acetic acid, hexyl ester				
Octanoic acid, methyl ester				
Octanoic acid, ethyl ester				
1-Octanol				
Decanoic acid, methyl ester				
Decanoic acid, ethyl ester				
Octanoic acid, 3-methylbutyl ester				
Ethyl 9-decenoate				
Acetic acid, 2-phenylethyl ester				
Hexanoic acid				
Phenylethyl Alcohol				
Octanoic Acid				
Hexadecanoic acid, ethyl ester				
Acetic Acid				
n-Decanoic acid				
Butanedioic acid, diethyl ester				
Dodecanoic acid				
1-Hexanol				
Dodecanoic acid, ethyl ester				

Table 12. Sensory analysis results

	CN	NS1	NS2	NS3
Appearance				
Color Intensity	3.6 ± 0.9[a]	3.8 ± 1.1[a]	3.8 ± 1.1[a]	4.0 ± 1.0[a]
Color Tonality & Shade	2.5 ± 1.2[a]	2.5 ± 0.8[a]	2.6 ± 0.8[a]	2.9 ± 1.1[a]
Transparency	3.3 ± 1.0[b]	4.1 ± 0.8[ab]	3.7 ± 0.8[ab]	4.5 ± 0.8[a]
Brightness	3.3 ± 1.2[a]	4.1 ± 1.0[a]	3.7 ± 1.1[a]	4.3 ± 0.9[a]
Smell				
Condition	4.8 ± 0.4[a]	4.2 ± 1.5[a]	4.2 ± 1.5[a]	4.8 ± 0.6[a]
Intensity	3.2 ± 0.8[a]	3.3 ± 0.8[a]	3.8 ± 0.6[a]	3.9 ± 0.5[a]
Fruits	2.7 ± 0.7[b]	3.1 ± 0.7[ab]	2.8 ± 0.7[ab]	3.7 ± 1.1[a]
Flowers	2.7 ± 0.8[a]	3.0 ± 1.0[a]	3.6 ± 0.8[a]	3.4 ± 0.9[a]
Spices	2.3 ± 1.0[a]	1.9 ± 0.8[a]	2.2 ± 1.0[a]	2.2 ± 1.1[a]
Vegetables	3.0 ± 1.2[a]	2.3 ± 1.1[a]	2.8 ± 1.2[a]	2.3 ± 1.2[a]
Fragrance	3.1 ± 0.9[a]	2.9 ± 0.5[a]	3.7 ± 0.9[a]	3.8 ± 0.8[a]
Taste				
Sweetness	3.0 ± 0.9[a]	2.9 ± 0.8[a]	3.5 ± 0.8[a]	2.9 ± 0.7[a]
Alcohol	3.2 ± 0.8[a]	2.8 ± 0.8[a]	2.8 ± 0.8[a]	2.8 ± 0.9[a]
Acidity	3.6 ± 0.7[a]	3.6 ± 0.9[a]	3.4 ± 0.7[a]	3.9 ± 1.0[a]
Body	3.0 ± 0.9[a]	3.2 ± 0.7[a]	3.2 ± 0.8[a]	3.4 ± 0.7[a]
Minerality	3.5 ± 1.2[a]	3.3 ± 1.0[a]	3.8 ± 0.6[a]	3.9 ± 1.0[a]
Flavor Intensity	3.3 ± 0.5[a]	3.5 ± 0.7[a]	3.8 ± 0.5[a]	3.7 ± 0.8[a]
Fruits	3.0 ± 0.7[a]	3.2 ± 0.8[a]	3.3 ± 0.8[a]	3.8 ± 0.9[a]
Flowers	2.7 ± 0.9[b]	3.0 ± 1.0[ab]	3.7 ± 0.7[a]	3.5 ± 0.9[ab]
Spices	2.1 ± 1.1[a]	2.0 ± 1.0[a]	2.1 ± 1.2[a]	1.8 ± 1.0[a]
Vegetables	2.9 ± 1.0[a]	2.4 ± 1.1[a]	2.5 ± 1.4[a]	2.7 ± 1.5[a]
Persistence	3.4 ± 0.7[ab]	3.3 ± 0.5[b]	3.7 ± 0.5[ab]	4.1 ± 0.9[a]
Fineness	3.1 ± 0.9[b]	3.2 ± 0.7[ab]	3.6 ± 0.5[ab]	3.9 ± 0.7[a]
Quality	3.2 ± 0.9[b]	3.3 ± 0.6[ab]	3.6 ± 0.7[ab]	4.1 ± 1.0[a]
General Impression	2.9 ± 1.0[b]	3.0 ± 0.6[ab]	3.8 ± 0.5[ab]	4.3 ± 0.9[a]

a-b: Significantly different results are indicated by various superscripts ($p<0.05$).

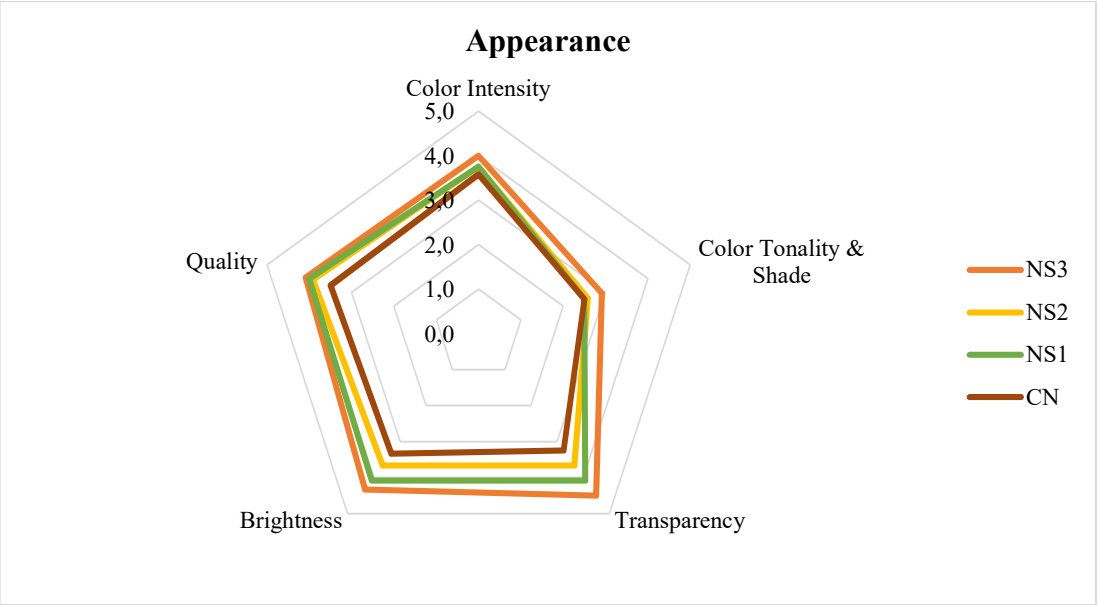

Figure 7. Radar chart of the sensory scores for appearance

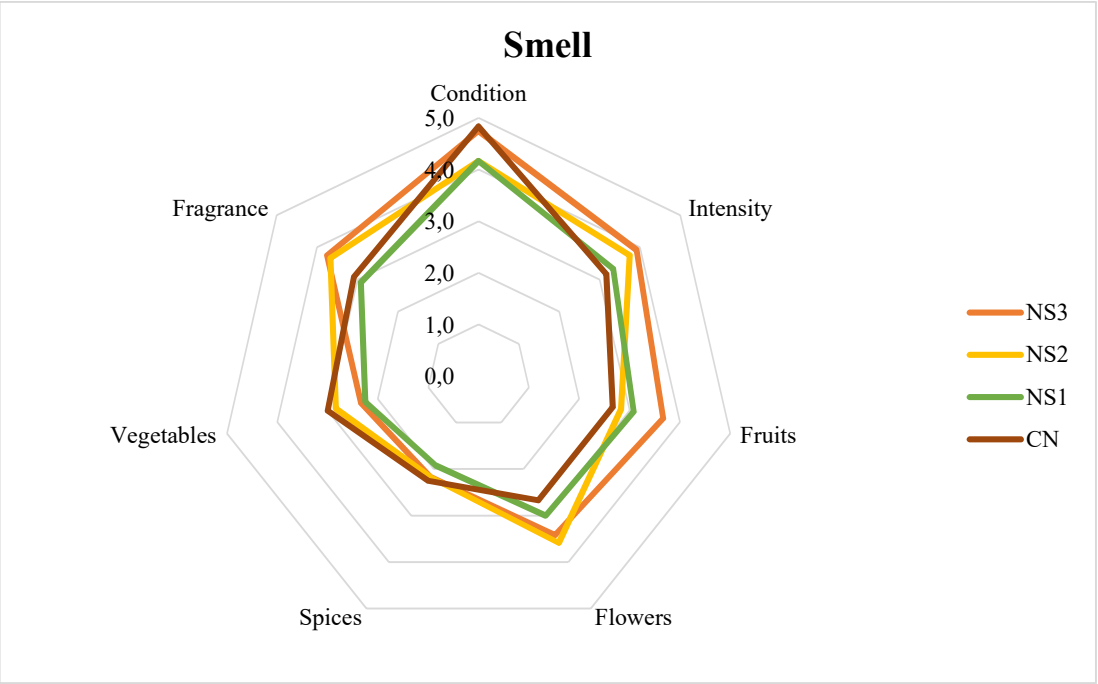

Figure 8. Radar chart of the sensory scores for the smell

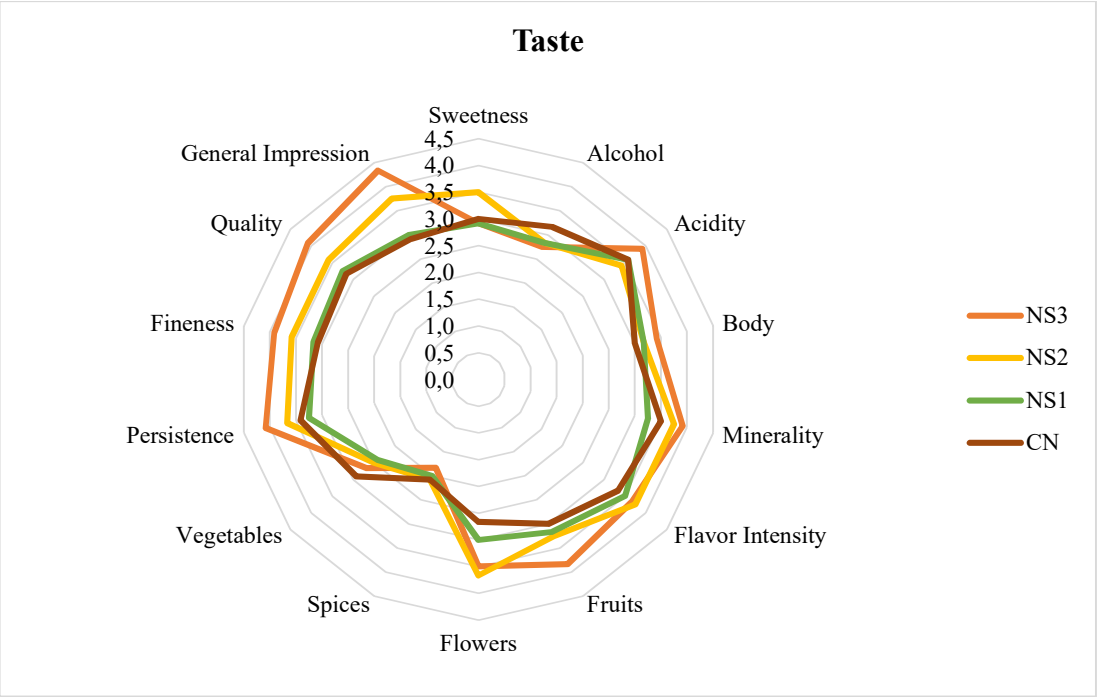

Figure 9. Radar chart of the sensory scores for taste

Minerality is the most characteristic feature of Emir grapes, and NS3 tasted more mineral than the other samples. CN had the lowest score in minerality, but the results were not statistically different ($p>0.05$). Spices were the least observed flavor in the nose and palate and highly in CN. Persistence, fineness, and quality scores at their highest in NS3 significantly. The general impression of CN was the lowest while NS3 had the highest; NS1 and NS2 were similar. It can be concluded that using non-*Saccharomyces* yeast, especially a mixture of *Metschnikowia pulcherrima* / *Torulaspora delbrueckii*, significantly affects the final wines' quality and general impression.

4.5. PCA of Chemical and Sensory Properties of Wines

Principal Component Analysis was used to examine the chemical properties of wines with score and loading plots, as shown in Figures 10 and 15. PC1 (51.7%) and PC2 (29.8%) explain 81.5% of the data variation. The wines with fermented *Metschnikowia*

pulcherrima (NS2), *Torulaspora delbrueckii* (NS1), and the mixture of *Metschnikowia pulcherrima / Torulaspora delbrueckii* (NS3) were separated from the sample fermented with *Saccharomyces cerevisiae* (CN) and according to PC1. Besides, no difference between CN and NS1 regarding PC2 was observed.

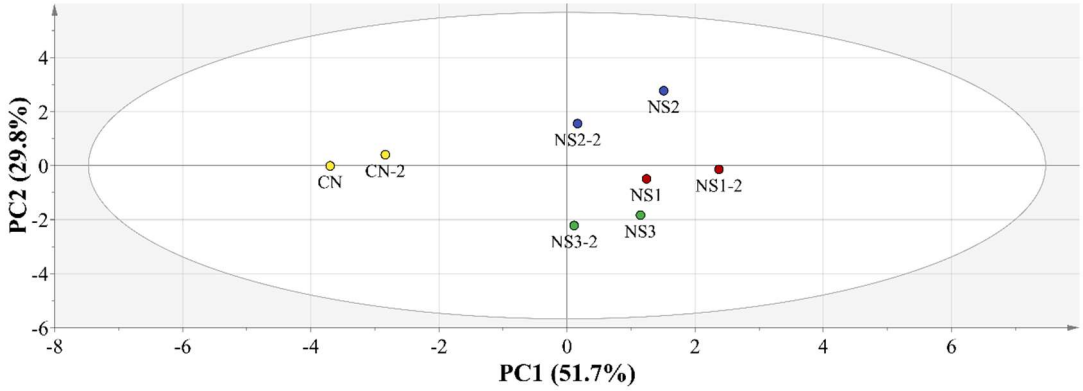

Figure 10. PCA score plot of the chemical properties of wines (R^2= 91.4% and PC=3)

Based on the effects of their chemical properties, samples were separated by PC1 and PC2, as shown in the loading plot (Figure 11). Density and pH were essential in separating the samples to the positive part of PC1. In contrast, samples with higher levels of remaining properties except reducing sugar tended to be located on the negative part of this axis (i.e., CN). Volatile acidity and free SO_2 were also found as negatively affiliated with PC2.

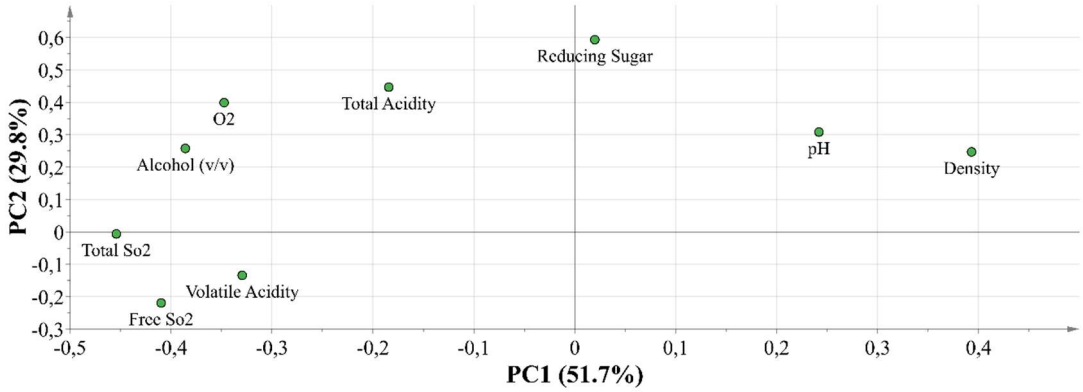

Figure 11. Loading plot of chemical properties of wines

Figure 12 represents the data variation of sensory properties of the samples. The model explains 99.8% of the variations in the data, where this value is 99.5% and 0.3% for PC1 and PC2, respectively. CN diverged from NS1, NS2, and NS3 on the PC2 axis.

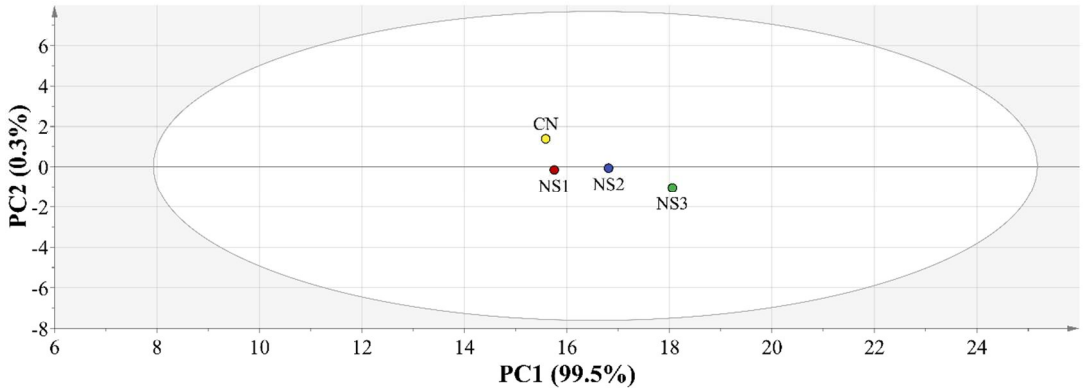

Figure 12. PCA score plot of the sensory properties of wines (R2= 99.8% and PC=2)

Figure 13 indicates the loading plot of sensory properties. It was observed that color intensity, brightness, and transparency affected PC1 more significantly than color tonality & shade, whereas the latter two negatively influenced PC2.

The condition has the most significant effect on PC1 among the smell properties. On the other hand, vegetable and spice aromas on the nose cause the samples to be located on the positive side of the PC2. Higher scores on fragrance, intensity, flowers, and fruits shift the samples to the negative side of the PC2.

In the palate, contribution level 0.2 was chosen as the threshold for the impact strengths of the properties. On PC1, spice flavor has a weaker effect than the other properties. Among the properties that affect PC1 more strongly, acidity, flavor intensity, minerality, persistence, fineness and quality were the most significant. Alcohol has the strongest effect, followed by vegetable flavor on PC2, while persistence, fineness, fruit and flower flavor, and quality have negative and weaker effects.

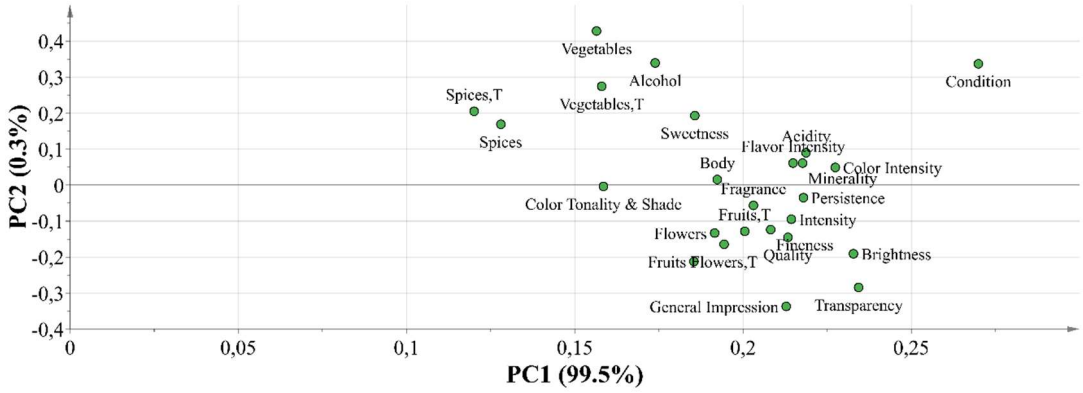

Figure 13. Loading plot of sensory properties of wines

Figure 14 shows the score plot of aroma compounds. The model showed that CN was separated from NS1, NS2 and NS2 according to PC1. CN was diverged from NS3, NS2 and NS1 while NS3 and NS2 were same and NS1 was tended to be located on the negative side on the PC2 axis.

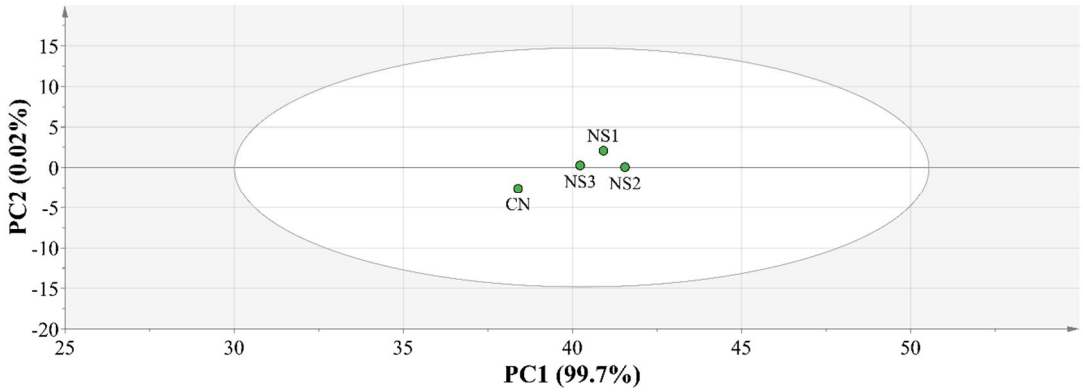

Figure 14. PCA score plot of the volatile compounds of wines (R2= 99.8% and PC=2)

Figure 15 represents the loading plot of volatile compounds. The model explains 99.9% of the variations in the data, where PC1 is 99.7% and PC2 is 0.2%. Ethyl decanoate and ethyl octanoate, followed by isoamyl alcohol, significantly affected PC1 among other volatile compounds. Isoamyl acetate and ethyl hexanoate; n-decanoic acid and phenyl ethyl alcohol; 2-phenyl acetate, 1-octanol, dodecanoic acid, ethyl ester had similar positive effects on this axis. Octanoic acid had a more significant influence than ethyl acetate, where they were both more effective than the rest of the compounds.

According to PC2, dodecanoic acid, ethyl ester caused the samples to be on the positive side mostly whereas 2-phenyl acetate were the strongest ones that caused samples to be located on the negative side. The positive effect of ethyl octanoate was higher than ethyl hexanoate, phenylethyl alcohol and ethyl decanoate. Also, ethyl acetate and isoamyl alcohol had the highest negative effects after octanoic acid. Octanoic acid was found to be more essential than ethyl acetate, n-decanoic acid, and isoamyl acetate. The rest of the volatile compounds had similar effects on PC2.

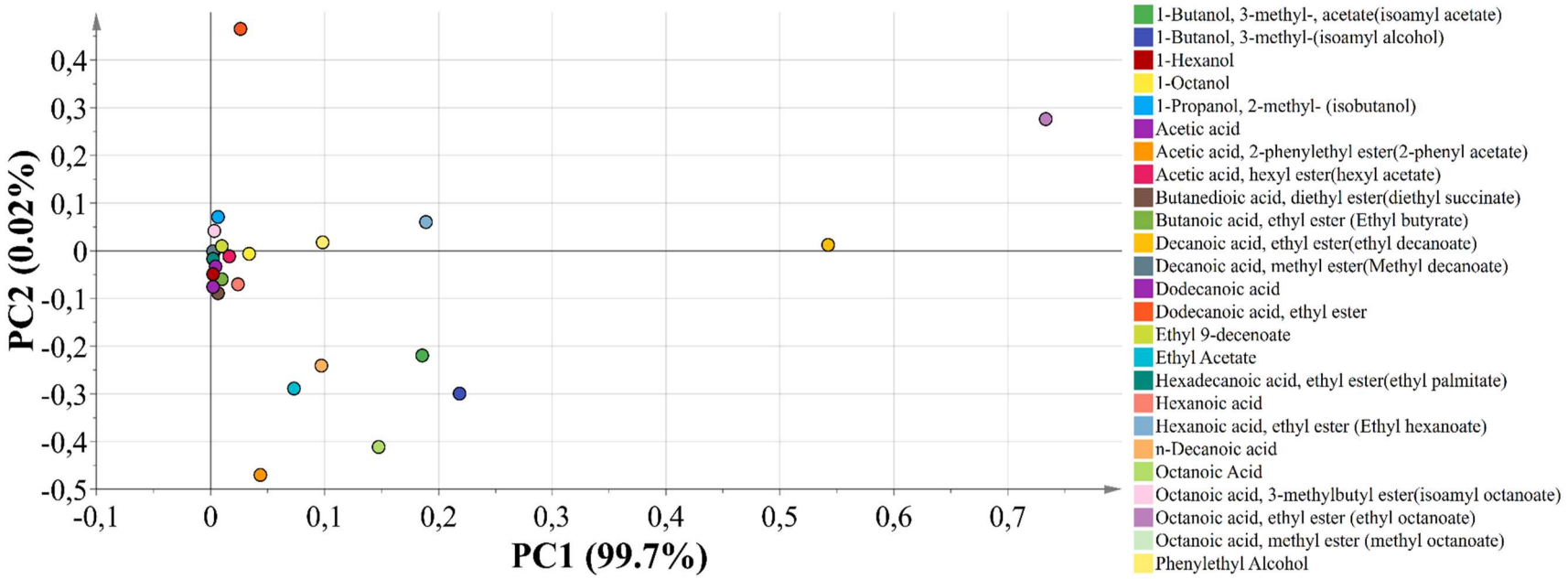

Figure 15. Loading plot of volatiles compounds

CHAPTER 5

CONCLUSIONS & FURTHER STUDIES

Non-*Saccharomyces* yeasts were previously considered spoiling yeasts, and their use was limited/eliminated by inoculation with pure *S. cerevisiae* cultures. However, during the past three decades, there has been a significant increase in interest in non-Saccharomyces yeasts usage in wine biotechnology. Numerous studies on the development and metabolic interactions between non-Saccharomyces and Saccharomyces yeasts in mixed cultures have demonstrated their influence on ethanol content, wine flavor, aromatic profile, and quality depending on the strains and the inoculation strategies.

Wines were produced from Emir grapes harvested in the Cappadocia region due to the sequential fermentation of non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae*. The study was carried out with commercial active dry yeasts containing *Metschnikowia pulcherrima*, *Torulaspora delbrueckii,* and the mixture of *Metschnikowia pulcherrima / Torulaspora delbrueckii* to compare with wine fermented with only *Saccharomyces cerevisiae*.

The chemical and sensory analysis was done after 4 months of maturation in the stainless-steel tanks. The results showed that wine with *Saccharomyces cerevisiae* had the highest ethanol concentration (12% v/v) while non-Saccharomyces wines had lower than that (11.7% v/v, 11.9 % v/v, 11.8% v/v). The use of non-*Saccharomyces* yeast in sequential fermentation with Saccharomyces cerevisiae caused a decrease in the alcohol content of wines, as expected. According to sensory properties, the mixture of *Metschnikowia pulcherrima / Torulaspora delbrueckii* is preferred mainly by the panelists. Volatile compounds analysis with HS-SPME-GC/MS also showed that isobutanol, isoamyl octanoate, and octanoic acid produced with the presence of non-*Saccharomyces* yeast, which contributes to aromatic complexity by giving a flowery-fruity aroma to the wines. Acetic acid (higher levels indicate microbial spoilage) and

dodecanoic acid (waxy, soap flavor) were only observed in wines with *Saccharomyces cerevisiae,* decreasing the quality and general impression.

In conclusion, winemakers can prefer non-Saccharomyces yeasts to improve wine aroma complexity and decrease ethanol levels. By considering all chemical and sensory properties, *Metschnikowia pulcherrima* & *Torulaspora delbrueckii* (ZYMAFLORE® ÉGIDE^{TDMP}) gave the best results for Emir grapes.

Further studies can focus on other commercial active dry non-Saccharomyces yeasts and their effect on other local grapes found in Turkey to contribute to the wine industry and the literature. It may also provide a terroir approach to catch the characteristic of these grapes.

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