

**ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES**

MSc THESIS

Rasha DALLOUL

**A STUDY ON THE PARTIAL REPLACEMENT OF SODIUM
CHLORIDE WITH DIFFERENT CHLORIDE SALTS IN
FERMENTED CRACKED GREEN TABLE OLIVES FROM
CV. SARI ULAK**

DEPARTMENT OF BIOTECHNOLOGY

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We certify that the thesis titled above was reviewed and approved for the award of degree of the Master of Science by the board of jury on 01/02/2018.

.....
Prof. Dr. Hüseyin ERTEN
Supervisor

.....
Prof. Dr. Turgut CABAROĞLU
Member

.....
Prof. Dr. Haşim KELEBEK
Member

This MSc Thesis is written at the Food Engineering Department of Institute of Natural and Applied Sciences of Çukurova University.

Registration No:

**Prof. Dr. Mustafa GÖK
Director
Institute of Natural and Applied Sciences**

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ABSTRACT

MSc. THESIS

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Rasha Dalloul

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Supervisor : Prof. Dr. Hüseyin ERTEN

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Jury : Prof. Dr. Hüseyin ERTEN

: Prof. Dr. Haşim KELEBEK

: Prof. Dr. Turgut CABAROĞLU

Sodium chloride is essential ingredient during the table olive fermentation, however lowering the sodium intake is advised by authorities due to high sodium intake causing some health problems. The aim of this study was to evaluate the influence of partial replacement of NaCl with different chloride salts of KCl, MgCl₂ and CaCl₂ on the physicochemical, microbiological and sensory characteristics of fermented cracked cv. Sarı Ulak green table olives. The values of pH and total acidity as lactic acid of cracked table olives were in the range of 4.00-4.58 and 5.34-6.07 g/kg respectively. The numbers of lactic acid bacteria, yeasts, non- *Saccharomyces* spp. and total mesophilic aerobic bacteria increased during the fermentations. Coliform bacteria did not survive after two weeks. Sensory analysis showed that the most preferred trail is with NaCl brine followed by NaCl+KCl combination and then NaCl+MgCl₂ salt treatment. The trail which conducted by CaCl₂ along with NaCl, was not preferred because of the bitter taste.

Keywords: Sarı Ulak, cracked table olive, fermentation profile, chloride salts, lower sodium chloride.

ÖZ

YÜKSEK LİSANS TEZİ

**SARI ULAK ÇEŞİDİNDEN SOFRALIK KIRMA YEŞİL ZEYTİN
ÜRETİMİNDE SODYUM KLORÜRÜN FARKLI KLORÜR
TUZLARI KULLANARAK AZALTILMASI ÜZERİNE BİR
ÇALIŞMA**

RashaDalloul

**ÇUKUROVA ÜNİVERSİTESİ
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Jüri : Prof. Dr. Hüseyin ERTEN

: Prof. Dr. Haşim KELEBEK

: Prof. Dr. Turgut CABAROĞLU

Sodyum klorür sofralık zeytin fermantasyonunda elzem bir ingredienttir. Bununla birlikte, yüksek miktarda sodyum tüketiminin bazı sağlık sorunlarına sebep olması nedeniyle, otoriteler sodyum tüketiminin azaltılmasını tavsiye etmektedir. Bu çalışmanın amacı Sarı ulak çeşidinden sofralık fermente kırma yeşil zeytin üretiminde sodyum klorürün farklı klorür tuzları kullanarak azaltılmasının sofralık kırma zeytinin fizikokimyasal, mikrobiyolojik ve duyuşal özellikler üzerine etkisinin incelenmesidir. Sofralık kırma zeytinlerin pH değeri 4.00-4.58 arasında ve toplam asitlik, laktik asit cinsinden, 5.34-6.07 g/kg arasında değişmiştir. Fermantasyon sırasında laktik asit bakterileri, toplam maya, *Saccharomyces* spp. dışında maya ve toplam mezofilik aerobik bakteri sayıları artmıştır. Koliform bakteriler iki hafta sonra ortamdan izole edilememiştir. Duyusal analiz sonuçlarına göre en fazla beğenilen örnek NaCl tuzu kullanılarak üretilen zeytinler olmuş ve bu örneği sırasıyla NaCl+KCl ve NaCl+MgCl₂ tuz kombinasyonları izlemiştir.

Anahtar Kelimeler: Sarı Ulak, Kırık sofralık zeytin, Fermentasyon profili, Klorür tuzları, Düşük sodyum klorür

EXTENDED SUMMARY

Olive has been produced and consumed in the Mediterranean region since 5000 years ago. It is said “olive tree is the first of all trees and considered as a peace symbol. Olive is an important part of human diet and it plays an essential role in the economy of producing countries. Among the Mediterranean countries that consider a big producer of olives is Turkey. It had 169 million olive trees in the 2014/15 season. According to the International Olive Council in 2018, Turkey produces 455,000 tons and consumes about 355,000 tons of olives. The main varieties of table olives in Turkey are Ayvalık, Domat, Gemlik, Yağlık, Memecik, Memeli, İzmir Sofralık, Çilli Çelebi, Uslu, Edincik Su and Sarı Ulak. Olives harvesting time start in November until March, when its color is changing firstly from green to violet then to black in common according to variety. In Turkey, 76 % of the harvested olives are pressed for oil and the rest for table olive. According to the international standards, table olives are classified as green olives, black olives and turning color olives. The commercially preparation types of olives are whole olive, stoned, stuffed, pitted, and olive paste. Olive is considered a functional food because it contains bioactive compounds which benefit to the human health furthermore reduce the risk of some chronic diseases. These compounds are: essential fatty acids (unsaturated fatty acids especially oleic acids), vitamins (A, E and C) which considered as antioxidants, Minerals (iron, potassium, calcium,...etc), polyphenols and flavonoids. In addition, olives contain small amount of proteins and carbohydrates. The main way in producing table olives is fermentation, which considers one of the oldest and traditional way for food processing. It involves placing the olive fruits in brine solution made by water and a variable concentration of the common salt, NaCl. This process carry outs spontaneously by the dominants microflora (LAB and yeast). As mentioned above, the main component of the fermentation is NaCl salt due to its role in decreasing the water activity thus inhibit the growth of unwanted pathogens keeping the product safe. In addition, it

improves the taste and the flavor of olive. In spite of its important role in olive fermentation, it has been detected that its presence in the diet and overall consumption leading to some chronic diseases like high blood pressure and heart diseases, so that, recently diet low in sodium has been recommended. One approach to accomplish that in the production of table olive that is most consumable food is to use different chloride salts along with NaCl, which have benefit role in the health, these chloride salts like KCl, $MgCl_2$ and $CaCl_2$.

In this study, the fermentation of cracked green table olives from Sarı Ulak variety was done by using KCl, $MgCl_2$ and $CaCl_2$ salts with NaCl in the fermentation brine in order to partially reduce the NaCl salt. The physiochemical properties, which detect the quality of the product, examined and determined in all over the process. The pH value for fermented olives was ranged from 4.00 to 4.58, total acidity for fermented olives was detected as 5.34- 6.07 g/mL. Total dry matter for fermented olives was between 30.31-32.85 % and the total ash was between 3.86-6.42 %. During the fermentation, the microbiological analyses was done to determine the effects of using different chloride salt mixtures on the number and activity of microorganisms that responsible of the fermentation these were LAB, mizophilic bacteria, yeast, non-*Saccharomyces* yeast and the coliform bacteria. The addition of other chloride salts increase the activity of LAB to produce the lactic acid and inhibit the growth of pathogens, increasing the safety of the product. Yeast play fundamental role in the taste and flavor of the product. These salt had the same effects of NaCl and did not disrupt the process or make unusual fermentation. Sensory analyses tests, which considered very important for the overall acceptance of the products, were conducted on 11 panelists. The most preferred olives were NaCl and KCl brined olives. $MgCl_2$ brined olives also accepted to some extent by the panelists and it had been detected that it produce olives with a slightly different acceptable taste. Whereas the fermented olives in $CaCl_2$ brine was not accepted due to the bitter taste.

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CONTENTS	PAGE
ABSTRACT	I
ÖZ.....	II
EXTENDED SUMMARY	III
ACKNOWLEDGMENT	V
CONTENTS	VI
LIST OF TABLES	VIII
LIST OF FIGURES.....	X
LIST OF ABBREVIATIONS	XII
1. INTRODUCTION.....	1
2. LİTERATURE REVIEW	7
3. MATERIAL AND METHODS	23
3.1. Material	23
3.1.1. Raw Material	23
3.1.2. Tools and Equipments Used in Experiments and Analyzes	23
3.2. Method.....	23
3.2.1. Harvest.....	23
3.2.2. Olives Fermentation	23
3.3. Analyses	26
3.3.1. Physical and Chemical Analyses.....	26
3.3.1.1. Maturity Index	26
3.3.1.2. The Number of Olive Fruits Per Kg	27
3.3.1.3. The 1000 Olive Weight	27
3.3.1.4. Fruit Dimensions	27
3.3.1.5. Flesh/Stone Weight Ratio.....	28
3.3.1.6. Color Analyses	28
3.3.1.7. Total Dry Matter Determination.....	28
3.3.1.8. Total Ash Determination for processed olives	28

3.3.1.9. pH Determination	29
3.3.1.10. Determination of Total Acidity	29
3.3.1.11. The Concentration of Sodium Chloride.....	29
3.3.2. Microbiological Analyses.....	29
3.3.3. Sensory Evaluation	29
3.3.4. Statistical Analyses.....	30
4. RESULTS AND DISCUSSION.....	33
4.1. General Composition of Olives Used in Experiments.....	33
4.1.1. Physiochemical Properties of Cracked Green Olives Used As Raw Material	33
4.2. Chemical and Microbiological Analyses During the Fermentaion Process of green cracked Sarı Ulak olives.....	35
4.2.1. The pH and the Total Acidity of the Brines of Sarı UlakOlives Variety during the fermentation	35
4.2.2. Microbiological Analyses During the Fermentation Process of Sarı Ulak Olives Variety.....	38
4.2.2.1. The Number of LAB	38
4.2.2.2. The Number of Total Mesophilic Aerobic Bacteria.....	41
4.2.2.3. The Number of Yeasts.....	42
4.2.2.4. The Number of Non- <i>Saccharomyces</i> Yeasts.....	44
4.2.2.5. The Number of Coliform Bacteria.....	45
4.3. Physiochemical Analyses of Proccessed Cracked Green Table Olives.....	46
4.4. Sensory Analyses.....	47
5. CONCLUSION	51
REFERENCES	53
CURRICULUM VITAE	63

LIST OF TABLES	PAGE
Table 2.1. Main Varieties of Olive Cultivated in the Regions of Turkey	12
Table 4.1. Physicochemical properties of the Sarı Ulak olives variety	33
Table 4.2 The physiochemical analyses of fermented Sarı Ulak vriety of cracked table olives at different salt brines (A: 8% NaCl, B : 4%NaCl+ 4% KCl, C: 4 % NaCl+ 4% CaCl ₂ , D: 4% NaCl+ 4% MgCl ₂)	46
Table 4.3. The results of preference test for fermented Sarı Ulak olives (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4% NaCl+ 4% CaCl ₂ , D: 4% NaCl+ 4% MgCl ₂)	48



LIST OF FIGURES	PAGE
Figure 1.1. <i>Olea europea</i> L tree	2
Figure 1.2. The top twenty countries in 2014 for the production of olives harvested	3
Figure 1.3. Sarı Ulak fruits.....	5
Figure 2.1. The parts of the fruit of olive	7
Figure 2.2. Olive production in Turkey.....	11
Figure 2.3. The stage of spanish- style olive production.....	15
Figure 2.4. Stages of oxidized black olives processing.....	17
Figure 2.5. Natural black table olives production	18
Figure 3.1. Fermentation of cv. Sarı Ulak.....	24
Figure 3.2. Processess applied in the production of natural green table olives.....	25
Figure 3.3. Maturity index of olive fruits.....	27
Figure 3.4. Sensory analyses form (Hedonic scale)	32
Figure 3.5. Form of preference test	32
Figure 4. 1. The pH of the brines during the fermentation (A: 8 % NaCl, B: 4 % NaCl+ 4 % KCl, C: 4 % NaCl+ 4 % CaCl ₂ , D: 4 % NaCl+ 4 % MgCl ₂).....	36
Figure 4.2. Changes in total acidity during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4%KCl, C: 4% NaCl+4 % CaCl ₂ , D: 4% NaCl+ 4% MgCl ₂)	36
Figure 4.3. The changes of the number of LAB on MRS agar during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4% NaCl+ 4% CaCl ₂ , D: 4% NaCl+ 4% MgCl ₂).....	39
Figure 4.4. The changes of the number of LAB grown on M17 agarduring the fermentation (A: 8% NaCl, B: 4%NaCl+ 4% KCl, C: 4% NaCl+4% CaCl ₂ , D: 4% NaCl+ 4% MgCl ₂).....	41

Figure 4.5. The changes of the number of mesophilic aerobic bacteria grown on PCA agar during the fermentation (A: 8% NaCl, B: 4% NaCl + 4% KCl, C: 4% NaCl+4 %CaCl ₂ , D: 4% NaCl +4 % MgCl ₂).....	42
Figure 4.6. The changes of the number of yeasts grown on PDA agar during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4 % NaCl+ 4 % CaCl ₂ , D: 4 % NaCl+ 4 % MgCl ₂).....	43
Figure 4.7. The changes of the number of non- <i>Saccharomyces</i> yeast grown on L-lysine agar during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4% NaCl+ 4% CaCl ₂ , D: 4% NaCl+ 4% MgCl ₂).....	45
Figure 4.8. The results of sensory analyses for fermented Sarı Ulak olives	48

LIST OF ABBREVIATIONS

NaOH	: Sodium hydroxide
NaCl	: Sodium chloride
EU	: European Commission
KCl	: Potassium chloride
CaCl ₂	: Calcium chloride
MgCl ₂	: Magnesium chloride
Cm	: Centimetre
C	: Centigrade degree
CO ₂	: Carbon dioxide
g	: Gram
kg	: Kilogram
L	: Litre
ml	: Millilitre
cfu	: Colony-forming Unit
mg	: Milligram
mm	: Millimetre
log	: Logarithm
N	: Normality
Spp.	: Species
Subsp.	: Subspecies
C	: Chroma
LAB	: Lactic acid bacteria



1. INTRODUCTION

Olive trees are among the oldest species of trees that are cultivated in the world (Fadıloğlu and Göğüş, 2009). According to archaeological studies the edible olives have been founded since about 5000 to 6000 years ago, returning to the early Bronze Age (3150 to 1200 BCE). Olive's tree were first domesticated in the Mediterranean region what is now Southern Turkey, Syria, Lebanon and Palestine (Vossen, 2007). During the middle ages olives plants continue to distribution and the first distribution was through Egypt, Tunisia and Morocco. The second was in Anatolia throughout the Aegean Islands, Greece, Italy and Spain. The third distribution was in Iran via Pakistan and China (Fadıloğlu and Göğüş, 2009). Spaniards have taken the olives to the North and South America thus completed their expansion to all regions that suitable for their growth (Valero et al., 2016).

Olive considered as a fruit, belong to the family of Oleaceae, the genus is *Olea* and the species are *europaea*. The scientific name for olive tree is *Olea europaea* L (Alevs et al., 2012). The scientists use the nomenclature of *Olea europaea* L to distinguish it from the wild olives subspecies that called *oleaster* or *acebuche*. (Vossen, 2007). Figure 1.1. represents *Olea europaea* L tree.

Olive tree has a long life period and it has the ability to withstand under all critical climate conditions, therefore, it is also used as landscape (Ayoub and Ajlouni, 2014).

Olive production is carried out in 37 countries around the world for economic reasons. It is observed that % 95 of the 9.8 million hectares of olive production areas in the world is located in the north of mediterranean region. Approximately % 86 of the 13 million tons of the world's olive productions is obtained in six typical mediterranean countries. 9 % of this production is in Turkey, 26 % in Spain, 23 % in Italy, 15 % in Greece, , 8 % and 5 % is provided by Tunisia to Morocco (Figure 1.2) (Anon, April 2014).



Figure 1.1. *Olea europaea* L tree (Biopix, 2003)

Turkey with average production of 1.77 million tons of olives from trees grown in 826.092 hectares is the fourth country in fresh olives production (Abuzayed et al., 2018). The world production of olives in 2017/2018 is approximately 2.854 million tons (www.oliveoiltimes.com).

After harvesting, olive drupes cannot be eaten because of the bitterness compound mainly oleuropein. Olive fruit contains a low portion of sugar ranging from 2.6 % to 6 %, while other drupes contain 20 % or more (Haggag et al., 2014). These are characteristics which make it inedible directly from the tree so, there should be several processes to remove this bitterness; moreover, these processes differ from one area to another and which are related to variety. There is an exclusion to this law in some olives, as they get sweet when mature and could be edible, in most cases this is due to fermentation. (Uylaser et al., 2008). In Turkey, there is a variety of naturally debittered olives cultivated in Karaburun peninsula (Sozbilen et al., 2016).

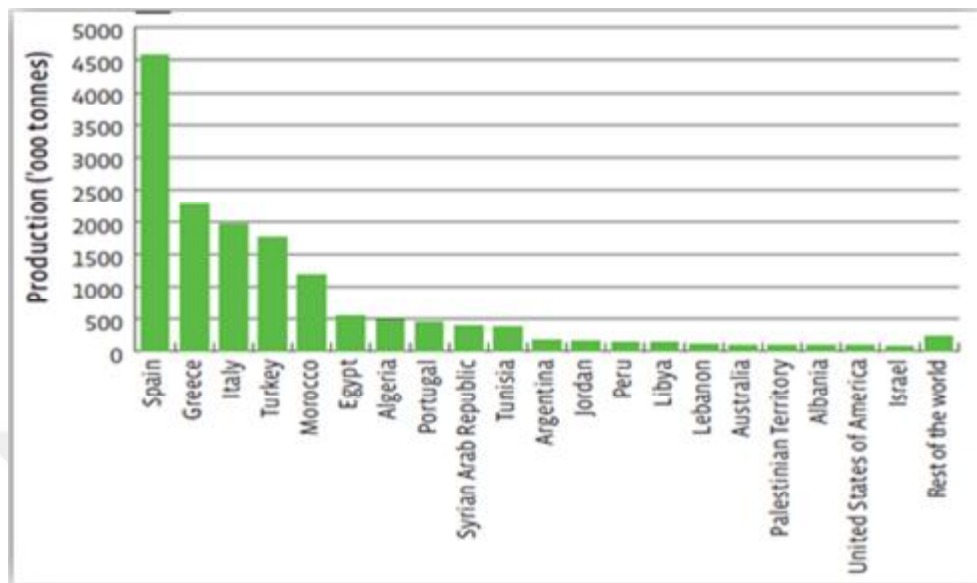


Figure 1.2. The top twenty countries in 2014 for the production of olives harvested (Sergeeva, 2017)

Table olives are the most important fermented vegetables in the world (Hurtado et al., 2012). The mediterranean diet pyramid advises to consume this fruit because of its nutritional benefits as minerals, vitamins, terpenes, monounsaturated fatty acids and phenolic compounds. Moreover, several research classifies table olives as a probiotic food due to the ability of microorganisms for forming biofilms over the fruit's epidermes (Mateus et al., 2016).

Fermented vegetable preparation depends on the use of NaCl which also known as the common salt. It is considered as the main ingredient of the brine. NaCl in fermented vegetables play an important roles like increasing the ionic strength of the solution as well as reducing the activity of water and keeping the final product safe and free from undesirable pathogens (Panagou et al., 2011).

In recent years, health-related authorities have recommended reducing sodium intake in the diet due to its major role in increasing blood pressure as well as cardiovascular diseases that consider the major cause for morbidity and mortality especially in Europe (Panagou et al., 2011; Rodríguez-Gómez et al., 2012). Today

more than 75 % of sodium intake is from processed or restaurant foods. 10- 12 % is from sodium that naturally occurring in the diet and the remaining is from salt used in home made foods (Panagou et al., 2011). In USA, the daily recommended sodium intake is 2.3 g (Anderson et al., 2015), but in recent studies of sodium uptake in urinary excretion for heart failure patients it was 3.6 to 4.2 g Na/ day (Butler et al., 2015). In Europe nearly the same amount of sodium consumption was concerned especially in Spain, the intake of sodium was 9.8 g/ day. Recently, a strategy has been done to reduce the salt levels of foods that contribute to salt intake in Europe and elsewhere (Rodríguez-Gómez et al., 2012). In Turkey, the daily salt intake was determined to be greater than in other countries. Both daily average salt consumption and blood pressure values of Turkish society are higher than other countries. The average recommended daily amount of salt about 5 g per person per day while the average salt intake for healthy adults is about 18 g in Turkey (Anon, 2011).

It is possible to decrease the negative effects of high sodium chloride intake by replacing this salt with other beneficial chloride salts. These salts are potassium chloride (KCl), calcium chloride (CaCl_2), and magnesium chloride (MgCl_2) (Panagou et al., 2011). For a lower blood pressure, it is a recommendation to have a low sodium (Na) diet (Leiba et al., 2005). The European Union gives the permission for consumers to use two specific minerals to prepare fortified food, these minerals are Ca and K (Bautista-Gallego et al., 2010).

In this study, cv. Sarı Ulak olive from Tarsus region was used. It is grown in Tarsus city located on the Mediterranean parts of Turkey. It is mainly grown in the foothills of the Tarsus Mountains, however, cv. Sarı Ulak is grown in other districts of the region. The fruit of cv. Sarı Ulak has a medium size. Fruit shape is long and cylindrical. Generally, the flesh is 72 % and the kernel is around 28 %. It is generally regarded as black and green table olives (Kaya, 2017). Sarı Ulak fruit is given in Figure 1.3. The flavor is good. It is able to provide a very high yield with its resistance to hot weather on the foothills of the Tarsus Mountains. It does

not need excess water but it shows periodicity. There is a demand in the market to cv. Sarı Ulak in the production of green table olives. Sarı ulak is important variety for the producer because it is fruitful as a tree, and is also delicious as a green olives ([http:// www.ciftcikulubu.net](http://www.ciftcikulubu.net)).



Figure 1.3. Sarı Ulak fruits (www.haberler.com, 2015)

As for as it is known, the studies on the reduction of NaCl for cracked green table olives are limited, therefore, the objectives of this study was to determine the effect of the partial replacement of sodium chloride by different chloride salts (potassium chloride, calcium chloride and magnisium chloride) on the physiochemecal, microbiological and sensory propereties of cracked cv. Sarı Ulak fermentation.



2. LITERATURE REVIEW

The International Olive Council (IOC), define the table olives as “ the healthy fruits of specific varieties of cultivated olives, picked at the right point of ripeness and of such quality that, undergo several process to become an edible food with good quality” (Ciafardini, 2015).

The dimentions of olive fruits ranged from 2-3 cm in long and 1-2 cm in wide. The weight of olive fruits varies between 0.5- 20 g, but in general it is from 3-10 g. The olive fruit has a bitter taste depending on the variety and the degree of ripening (Tuna, 2006).

There are two main parts that forming the fruit of olive, the pericarp which represents 66- 85 % of the fruits weight, and the endocarp, it is also known as the pit or the kernel which contains the seed. The seed represents < 3 % of the fruits weight (Figure 2.1). The pericarp is composed of two parts, the epicarp (the skin) and the mesocarp (the pulp) (Kiritsakis and Paiva-Martins, 2017).

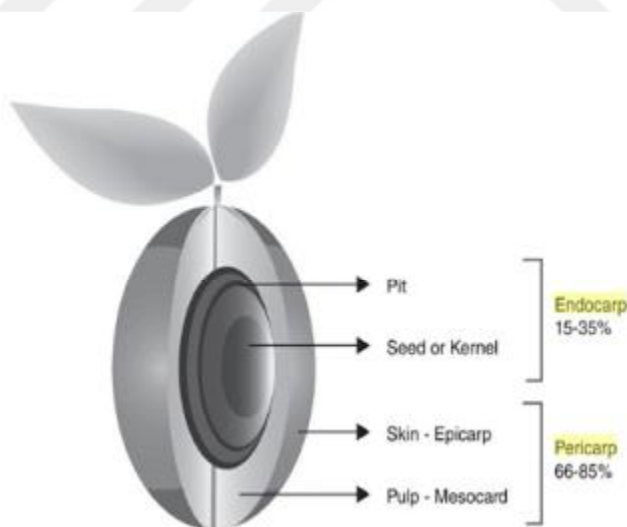


Figure 2.1. The parts of the fruit of olive (Kiritsakis and Paiva-Martins, 2017)

The fruit of olives distinguishes from the other drupes in a low content of sugar (2.6- 6 %) and a high content of oil (20- 35 %), while the other drupes have about 12 % sugar or more and a low content of oil (1-2 %) (Irmak et al., 2010).

The chemical composition of olive fruits varies depending on the amount of oil contents, the degree of ripening, the environmental factors and the growing conditions (Tokuşoğlu and Artık, 2010). The average composition is 35-42 % water, 25-28 % fat, 1.9-2.5 % crude protein, 1.7-2.0 % crude cellulose, 6-9 % ash (mineral matter), 2.8-6.2 % sugar (glucose, fructose and sucrose), less than 1% of polyphenols, less than 1% of aromas substances, less than 1% of coloring matter, less than 1% enzymes, less than 1% alkaloids and less than 1 % pectin (Aktan and Kalkan, 1999).

The majority of sugars in the olive fruits is reducing sugars (Tuna, 2006), like glucose and fructose. Sucrose, maltose, isomaltose, mannose and galactose are also present in some varieties (Kiritsakis and Paiva-Martins, 2017). These reducing sugars are converted to lactic acid by homofermentative LAB and to lactic acid, acetic acid and CO₂ by heterofermentative LAB. Diacetyl is also formed as secondary product. As a result, during fermentation, the acidity of the medium increases, while the pH decreases, so that the environment becomes safer for the product (Tuna, 2006).

The protein content of olive fruit is low, whereas the quality is high. It ranges from 1.5 to 3.0 % related to the degree of ripening. The two main amino acids that commonly found in olives fruits are aspartic and glutamic acids (Kiritsakis and Paiva-Martins, 2017). During the fermentation, the most abundant component of protein in the brine is nitrogen which is utilized by the microorganisms (Tuna, 2006).

Other important constituents of olive fruits are phenolics because of their ability to become antioxidants and free radical scavenger, which are important to human health. Phenolic compounds also affect the color of olives and the antimicrobial activity against specified microorganisms. The most important of

these compounds is oleuropein (Erten et al., 2016). The amount of oleuropein is 14 % of dry matter in young olives (Fadıloğlu and Göğüş, 2009), after ripening of olives it decreases about 2 % (Kara and Özbaş, 2013). Oleuropein is the ester of 3,4- dihydroxyphenyl and elenolic acid. Its concentration can reach up to 140 mg/g on a dry matter basis in olives (Erten et al., 2016).

Phenolic compounds especially oleuropein and its hydrolyzed derivatives have antimicrobial activities against various microorganisms including lactic acid bacteria (LAB) (Romeo, 2012). *L. plantarium* is the most important LAB species that have the capability to degrade oleuropein in olives. Several studies detected the ability of oleuropein to decrease the blood pressure, so that it gives the processed olive its nutritional values (Ghabbour et al., 2011). The oleuropein biodegradation is the main step in the green table olives production (Lorizzo et al., 2016). Polyphenols inhibit the growth of lactic acid bacteria in the production of natural fermented table olives. In such cases where the yeast predominates, low lactic acid production can be achieved, which means that the characteristic taste of the olive does not occur and therefore the shelf life is shortened (Bautista-Gallego et al., 2010). This can be explained by the fact that yeasts produce CO₂ gas to cause fish eye flaws and reduce the quality of the final product by causing the fruit to be soft. Therefore, lactic acid bacteria during olive fermentation play an important role in limiting the negative effects of yeast on final product quality (Özay and Borcaklı, 1996). On the other hand, yeast can play an important role in table olive fermentation, it produces compounds significant for preservation of table olives such as organic acids, alcohol and esters for aroma and taste (Kailis and Kiritsakis, 2017).

Organic acids are a minor component of flesh of olive fruits and their amount is 1.5 %. They are formed during the disintegration and formation of other components in olive fruits, such as carbohydrates, which play an important role in metabolic activity. Malic and citric acids are the main organic acids found in olive

fruits. They have an effect on the olives colour and have an important role in preserving the texture of the olives (Ergönül and Nergiz, 2010).

The flesh of olive fruits has a concentration of some minerals like calcium and iron which are considered the main minerals in the flesh, whereas other minerals like potassium, phosphorus, manganese and copper are found in less concentrations (Fernandez-Hernandez et al., 2010). The copper content of the fruit increases considerably during ripening. Potassium (322.69-457.19 mg / 100g) and potassium phosphorus (51.13 mg / 100g), calcium (33.15 mg / 100g) and sodium (3.20 mg / 100g) were found to be the most abundant minerals in the composition of olive fruit (Tokuşoğlu and Artık, 2010).

The table olives varieties in different regions of Turkey are given in Table 2.1. The most common variety for production of black table olives in Turkey is the cultivar (Cv.) Gemlik. 50 % of the table olives market in Turkey are from Gemlik. Olives are cultivated in five different regions of Turkey, which Aegean region is the first in production of olives, then, Marmara, Mediterranean and Southeastern Anatolia. There are some cultivars of olives grown in some parts of the region of Black sea (Figure 2.1) (Erten et al., 2016).

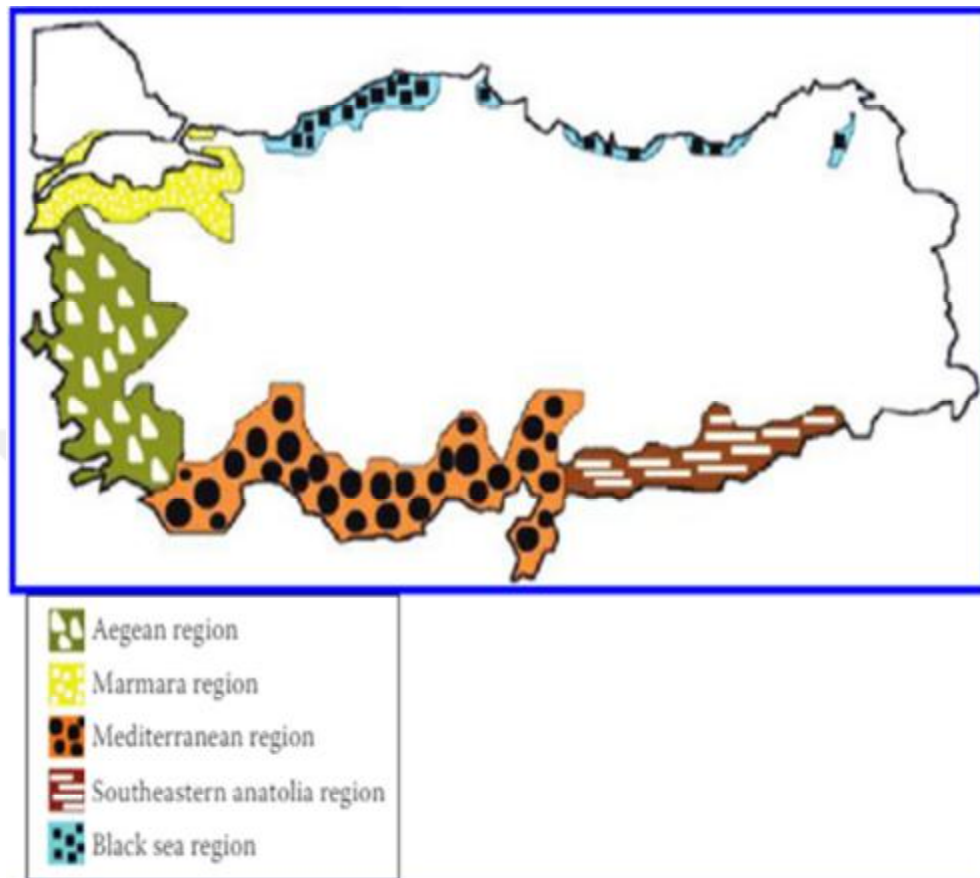


Figure 2.2. Olive production in Turkey (Erten et al., 2016)

Table 2.1. Main Varieties of Olive Cultivated in the Regions of Turkey (Erten et al., 2016)

Region	Varieties
Aegean	Gemlik
	Memeli
	Memecik
	Ayvalık
	Domat
	Uslu
	Yamalak
	Çilli
Marmara	Manzanilla (Spanish variety)
	Gemlik
	Edremit
	Edincik Su
	Karamürsel Su
	Samanlı
Mediterranean	Tavşan Yüreği
	Sarı Ulak
	Kan Zeytini
	Gemlik
Southeastern Anatolia	Halhalı
	Kan Çelebi
	Eğriburun

The main olive cultivars evaluated as black table olives in Turkey; Gemlik, Uslu, Edincik Su, Kan Çelebi, Halhalı, Tavşan Yüreği and Çelebidir, while Domat and Yalamak Sarısı are green olive varieties. The varieties of Ayvalık and Memecik are evaluated as green, pink scratch, black and for olive oil. Sarı Ulak is

among the olive varieties of Mediterranean region, which is cultivated in Tarsus, Seyhan, Kozan and Yumurtalik. It constitutes about 6 % of the tree of olives in the Mediterranean region. It contains 19 % oil and used for the production of green and black table olives (Efe et al., 2013).

The Turkish Codex Food Standard states that, the table olives are the products of healthy fruits of cultivated varieties of olive tree (*Olea europaea* L), after several process of treatments to remove its bitterness, with or without fermentation, with or without lactic acid and /or other additives, in order to ensuring the stability of the product during the storage and maintain the product free from spoilage (Erten et al., 2016). Therefore, olives are one of the rare fruits that can not be consumed straight after harvesting, because of the oleuropein and the other phenolics that give the olive its bitter taste, so that they need to be reduced for improving the taste of olives (Boskou, 2017). All types of olive fruits can be processed as table olives. The olives for production of table olives are divided into three types regarding to the ripeness degree of the fresh fruits. These types are:

- Green olives, the fruits harvested before coloring during the period of ripening and when the size of fruits become normal.
- Turning- color olives, the fruits harvested at color change prior to the complete ripeness stage.
- Black olives, when the fruits fully ripe or slightly before reaching full maturity (Erten et al., 2016).

Usually the olive processing based on the fermentation of the olives in brine solution, this method is old and traditional method with low consumption of energy and not expensive (Rodríguez- Gómez et al., 2012). The salt of the brine is very important, it reduces the water activity and thus maintaining the stability of

the final product by inhibiting the growth of pathogens, also affects the taste of the final product (Panagou et al., 2011).

According to the trade preparations of table olives production, there are three main production methods :

- Spanish- style olives: fruits of olives (green olives, black olives and turning-color olives) undergo alkali treatment (treated olives).
- Californian- style: fruits are oxidized by air to become dark (green olives or olives turning colour) with or without alkali treatment (Bruno et al., 2015).
- Greek- style or natural olives (green olives, black olives and turning- color olives) directly brined (untreated olives).

The spanish- style table olives are one of the most commercialized type of table olives especially in western countries, due to the unique flavor of the product. It based on treatment of the olive fruits with an alkaline solution (lye treatment) (1.82- 5 % w/v NaOH). The stages of production are shown in Figure 2.3. In order to remove the bitterness of the olives fruits, the alkaline solution is used to destroy the glucoside oleuropein, then the excessed alkali is eliminated by washing the fruits one or more times, then the fruits undergo lactic acid fermentation process in brine solution containing NaCl (10- 13 %) (Sánchez et al., 2018).

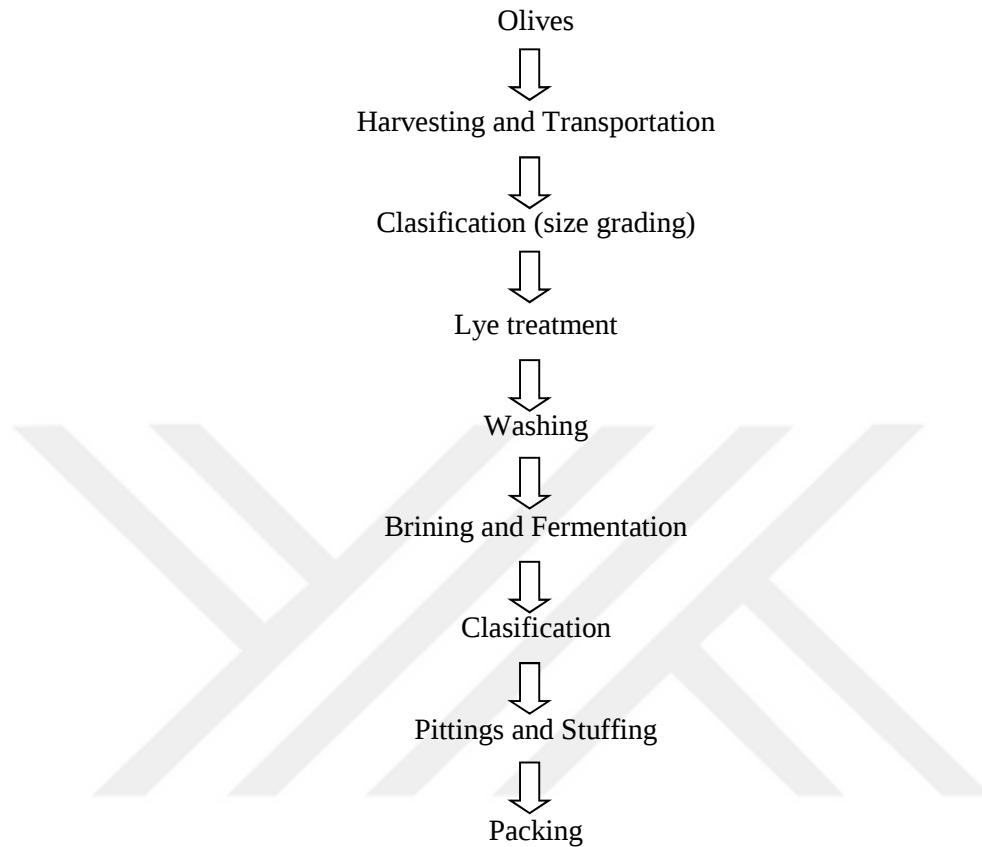


Figure 2.3. The stage of spanish- style olive production (Cillidag, 2013)

The optimum temperature for fermentation process is 20- 27 C°. The fermentation temprature, the amount of salt along with the type and the number of LAB determine the time of the fermentation process that it generally takes 3- 4 weeks. At the end of the process, the total acidity reach 0.8- 1.2 % and the pH should be ≤ 4.3 in order to maintain the safety and the stability of the final product (Erten and Tangüler, 2014). After the fermentaion proccess is completed, the fruits of olive stored or conditioned before the final packaging and storage (Kiritsakis and Paiva-Martins, 2017).The alkali treatment may be take about 5- 7 h (Bruno et al., 2015). The early phase of the fermentation process, the prodominant microorganisms are Gram-negative bacteria (*Enterobacter*, *Citrobacter*,

Aeromonas, *Escherichia*, and *Klebsiella*). When the pH decreased to 6.0, the second phase of the fermentation starts. In this phase the Gram-negative number decreases and then fully disappears. The main microorganisms in this phase are *Pediococcus* and *Leuconostoc*. They produce lactic acid thus further decreasing the pH and this promotes the beginning of the third phase. In the third phase the predominant LAB is *Lactobacillus plantarum* which is homofermentative lactobacilli with preferred pH between 5.5 and 5.8. In this phase yeast (*Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, *Pichia membranifaciens*, *Kluyveromyces lactis*, *Debaryomyces hansenii*, *Pichia anomala*, *Pichia guilliermondii*) are also present with LAB and it plays a significant role in enhancing the sensory properties of the final product (Lanza, 2013).

In Californian-style, olives are darkened by oxidation using ferrous-gluconate or lactate solution. This process is used commonly in United States. It consists about 30% of the world produced table olives. Firstly the fruit of olives are kept in acidic solution for long period, around 6-18 months or more, then alkali treatment is done, after that the fruits washed in water for 24-30 h. Color fixation is occurred by ferrous-gluconate, then the fruits are washed again with water then prepared for packaging or storage. The pH in this process is close to be neutral. The microorganism in this process are yeast and LAB. The advance of this process over the traditional ones is that the final product rich in bioactive compounds (Medina et al., 2018). Figure 2.4. Illustrates the stages of the process

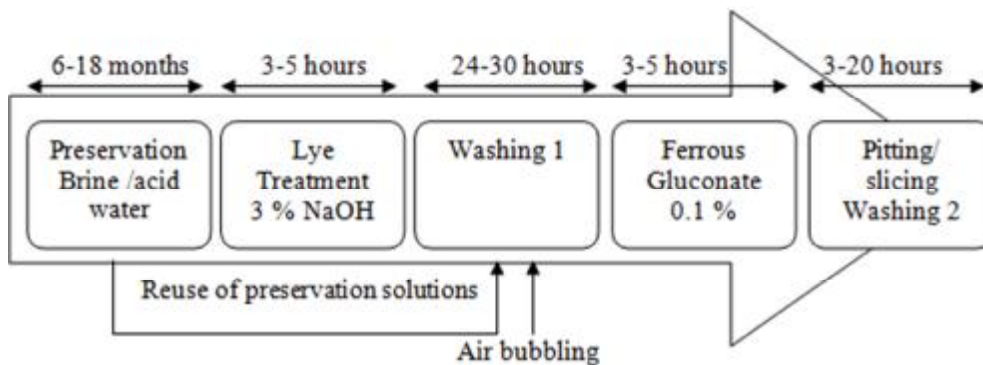


Figure 2.4. Stages of oxidized black olives processing (Medina et al., 2018)

In Greek- style or natural fermented olives, it is considered as traditional process of table olives in Turkey. Two types of olives are produced, natural black and natural green table olives. In natural black table olives production the olive fruits are harvested with the desired color, then they put into brine solution (8-10% NaOH) and the fermentation starts. The fermentation process takes along period about 8- 12 months this may be due to the high concentration of salt. The predominant microorganisms in this process are yeast, but the Gram-negative and LAB also detected. The stages of this process are shown in Figure 2.5. During the fermentation the salt concentration and the pH must be controlled. Secondary fermentation may be occurred in this process because the bitterness and the reducing sugars are not full disappeared from the final product. After the ending of the fermentation, the fruits are packed for marketing (SI, 2013). In Turkey, other type of natural black olives production are used, it is called Gemlik- style, that depends on Gemlik variety of olives. It is the most common variety of cultivated olives in Turkey. This variety differ from the others black olives in the thickness of the flesh of olives.

The generally used processing methods are Greek-style, Spanish- style, dry-salted olives, Gemlik- style and scratched and cracked green olives for the production of table olives in Turkey. The dry-salted olives style which is very common in Turkey, Gemlik variety. This style is also called (Sele). The olive fruits

are harvested when it is fully ripened without lye solution (Alak and Uylaşer, 2017). The process includes putting the fruits in a high concentration of salt (15-20 % NaCl) in order to remove water and bitterness compounds. The process takes about 40- 60 days, then the fruits are washed and dried for direct consumption (Ramirez et al., 2013; Erten et al., 2016).

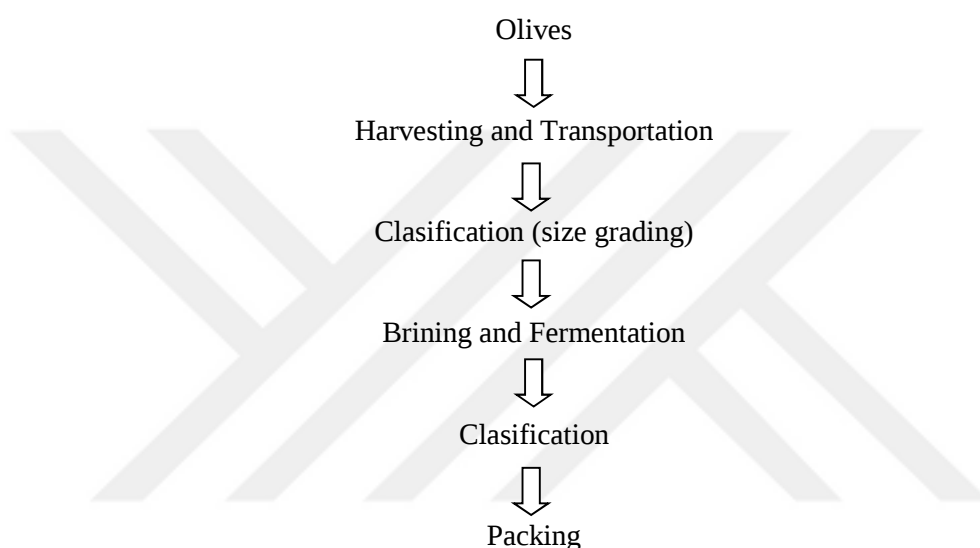


Figure 2.5. Natural black table olives production (Cillidag, 2013)

Regarding cracked green table olives, these type products are very preferable by the consumers in Turkey. Cv. Ayvalık is used for the production of scratched and cracked table olives. Others varieties used for production are: Domat, Memecik, Yamalak and Sarı Ulak. It is natural process in brine without lye treatment. The fruit of olives, after harvested and sorting are washed then cracked or scratched and putted into fermentation brine with salt. The process takes about 3-4 weeks to be ready for direct consumption or packaging. It is very important to change the brine solution periodically during the fermentation time, in order to get rid of all of bitterness (Cillidag, 2013; Erten et al., 2016).

The microorganisms play a major role in the fermentation of table olives, especially LAB and yeast with less extend. Each stages of fermentation characterized by specified species of microorganisms (Mateus et al., 2016). The fermentation process depends mainly on the metabolic activity of the LAB that utilizes the fermentable sugar and converts them into lactic acid which decreases the pH of the fermentation brine, thus prevents the development of other pathogens. LAB both homofermentative like *Lactobacillus*, *Streptococcus* and *Pediococcus* and heterofermentative as *Leuconostoc* and some members of *Lactobacillus*, were observed during the fermentation process. In naturally brined black and green table olives fermentation, the phenolic compounds partially inhibit the growth of LAB, so in this process yeast species can become predominant and have a major role in organoleptic characteristics of the fermented table olives. *Candida*, *Pichia*, *Saccharomyces* are the common yeast genera in the fermentation of olives, while others are detected in some olives varieties as *Debaryomyces*, *Issatchenkia*, *Zygorhiza*, and *Wickerhamomyces* (Heperkan, 2013).

Bavaro et al (2017) studied the molds that developed during the Greek-style fermentation of some Greek and Italian black olives varieties of Cellina di Nardò, Leccino, Kalamàta, and Conservolea. About 60 fungal strains were detected. They observed that these molds have the ability to produce mycotoxins as secondary products, some others can be used as co-starter culture with LAB and yeast in future of olives fermentation.

In spite of the main role of NaCl in the fermentation of table olives, it has a strong relationship with many health problems, so that some researches and studies have been carried out in order to replace it partially with other chloride salts. Some of the conducted previous studies can be summarized as follows:

Zinno et al (2017) detected that the partial replacement of NaCl with KCl had not activate the pathogens growth during the fermentation of Nocellara del Belice table olives according to Spanish – style method.

Bautista-Gallego et al (2016) studied the effects of salt mixtures on the fermentation of green Manzanilla table olives which produced by Spanish- style method. They used KCl and CaCl_2 salts beside NaCl. They studied the different parameters of colour and sensory attribute and thire releation to the initial composition of the brine. They observed that the better color corresponded to the higher CaCl_2 concentration in the brines. Moreover, the CaCl_2 has reduced the saltiness but increased the bitterness.

Leventdurur et al (2016) studied the microbial profile of yeast in the natural fermentaion of black table olives which obtained from the Gemlik variety grown in three diffirent region of the Cukurova in Turkey. The fermentation process ended in 180 days. Three different concentrations of NaCl (10 % w/v, 8 % w/v and 8 % w/v with glucose 5 %) were used. They detected a wide range of yeast which includes eight genera and nine species, the predominant yeasts were *Candida boidinii* (41%), *Wickerhamomyces anomalus* (32%) and *Saccharomyces*

sp. (18%). In NaCl 8 % brine, the main species was *W.Anomalus*, while in the other brines the most species was *C. Boidinii*.

Mateus et al (2016) studied the fermentation of cracked green table olives from Maçanilha Algarvia variety under five different salt concentrations of brines. Brine 1 (8% NaCl), brine 2 (4% NaCl+ 4% KCl), brine 3 (4% NaCl+ 4% CaCl_2), brine 4 (4% KCl+ 4% CaCl_2) and brine 5(2.7% NaCl+ 2.7% KCl+ 2.7% CaCl_2). The yeast microbiota and other Gram- negative bacteria had been detected. The predominant yeast speacies were *Pichia membranaefaciens*, *Candida boidinii*, *Zygosaccharomyces mrakii*, *Priceomyces carsonii*, *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus* and the yeast-like fungus *Galactomyces geotrichum*. Brine 1, 2 and 3 had a high yeast population, while brine 4and 5 had the lowest yeast population. There was not any effects of different salts concentration on the yeast growth.

Bautista-Gallego et al (2015) stated that the NaCl could be reduced partially by using a mixture of KCl with CaCl_2 in the fermentation of Spanish-style

Manzanilla green olive. Adding CaCl_2 in the brine decreased the pH and if the concentration of CaCl_2 exceed the 20-30 g/L it might increased the number of *Enterobacteriaceae*. On the other hand, the presence of KCl decreased the total acidity of the brine. Brines obtained with the mixture of KCl and CaCl_2 had an important role in increasing the LAB.

Tataridou and Kotzekidou (2015) studied the effect of using oleuropeinolytic starter culture on table olives fermentation in different salt brines. Kalamata variety olives were used as black olives, whereas Chalkidikis variety for green olives. The olive fruits were putted into two different types of brine solution. A brine contained (2.3% NaCl, 32.2 mM Ca-acetate and 33.9 mM Ca-lactate). B brine contained (4% NaCl). The pH was 5.0. The starter culture was from the *Lactobacillus plantarum* species. The resultant olives have rich in biophenolic compounds like tyrosol, thus had an antimicrobial activity against pathogens.

Bautista-Gallego et al (2012) studied the fermentation profile of the Spanish- style green table olives using different salt mixtures. They used fresh Gordal variety of olives. They putted the olives in brines solutions containing different concentrations of salts (NaCl 40-100 g/L, KCl 0-40 g/L and CaCl_2 0-60 g/L). They observed that the CaCl_2 inhibit the sugars diffusion thus hindered the gaz pocket spoilage development. The KCl had activate the lactic acid production.

Panagou et al (2011) studied the effect of NaCl reduction in the fermentation of Conservolea natural black olives by using different salt mixures, these were (8% NaCl as a control), (4% NaCl with 4% KCl), (4% NaCl with 4% CaCl_2), (4% KCl with 4% CaCl_2) and (2.6% NaCl+ 2.6% KCl+ 2.6% CaCl_2). The resultant total acidity was 0.70- 0.86 g/100 mL, while the pH was 3.9-4.2. The acceptability of the final product depeneded mainly on the sensory evaluation. The processed olives by CaCl_2 brines had been low accepted by the panelists. The olives that have been brined in KCl salt were the most preferred by the panelists.

Bautista-Gallego et al (2011a) studied the impact of using different chloride salt mixtures on minerals and selected feutures of the fermented cracked

Alorena table olives. The total concentration of salts was ($\text{NaCl} + \text{KCl} + \text{CaCl}_2 = 0.11 \text{ kg/L}$). The most preferred quality was obtained in the mixture of 0.037 kg/L NaCl and 0.073 kg/L KCl . The best color of fruit was observed in these concentration 50% KCl - 50% NaCl vey 50% KCl -50% CaCl_2 . The initial concentration of CaCl_2 had the total acceptance. The amount of minerals in the fermented fruit were dependent on the initial mineral level and the interaction between NaCl - CaCl_2 and CaCl_2 - KCl . According to the results obtained, it has been determined that NaCl can be replaced with KCl or CaCl_2 in olive production.

Bautista-Gallego et al (2011b) studied the fermentation profile of Gordal variety of green table olives using different salt mixtures (NaCl with KCl and/or CaCl_2). The presence of calcium caused a decrease in the brine pH, the pH after processing and the diffusion of sugar into the brine thus decreasing the overall growth of LAB. It also reduced the number of *Enterobacteriaceae*. KCl salt activate the growth of microbes. Using these salts with NaCl had a remarkable effect on the characterization of final product.

In another study by Kanavouras et al (2005) reported that NaCl level can be decreased by adding acetic acid and calcium acetate to the brine. Those also improved the physiochemical and sensory attributes and kept the final product free from pathogens.

3. MATERIAL AND METHODS

3.1. Material

3.1.1. Raw Material

In this research green olives of Sarı Ulak variety were obtained from Tarsus District, after harvesting on November 2016.

3.1.2. Tools and Equipments Used in Experiments and Analyzes

The fermentation were carried out in 5-litre food grade plastic containers. The “Sanyo MIR-153”, “Aqua Lytic AL654 and “Velp Scientifica FTC 90E” brand incubatores with adjustable temperature were used for the growth of microorganisms. “Hirayama (Japan)”brand autoclave was used for sterilization. For pH determination “Mettler Toledo Ion/ S220” brand pH meter was used. For dry matter determination, “ Venticell 222” brand fan oven was used. For ash analysis, “Protherm PLF 110/15” model oven was used. Color measerments for L*, a*, b*values, were determined with color measurement device “Minolta marka CR100”.

3.2. Method

3.2.1. Harvest

Olives were harvested by hand in November 2016 when they reached harvest maturity (yellow-green) for green olives. Approximatly 20 kg of olives were transported to the Cukurova University Faculty of Agriculture Department of Food Engineering as soon as possible.

3.2.2. Olives Fermentation

Olive fruits were sorted to remove damaged, crushed, soft and different coloured olives. Olives were cracked by stone. Then olives were fermented in four different brines containig different chloride salts (Figure 3.1). They were 8 % NaCl,

4 % NaCl + 4% KCl, 4 % NaCl + 4 % CaCl_2 and 4 % NaCl + 4 % MgCl_2 . Brine including 8 % NaCl salt alone was used as control (Figure 3.2).



Figure 3.1. Fermentation of cv. Sarı Ulak

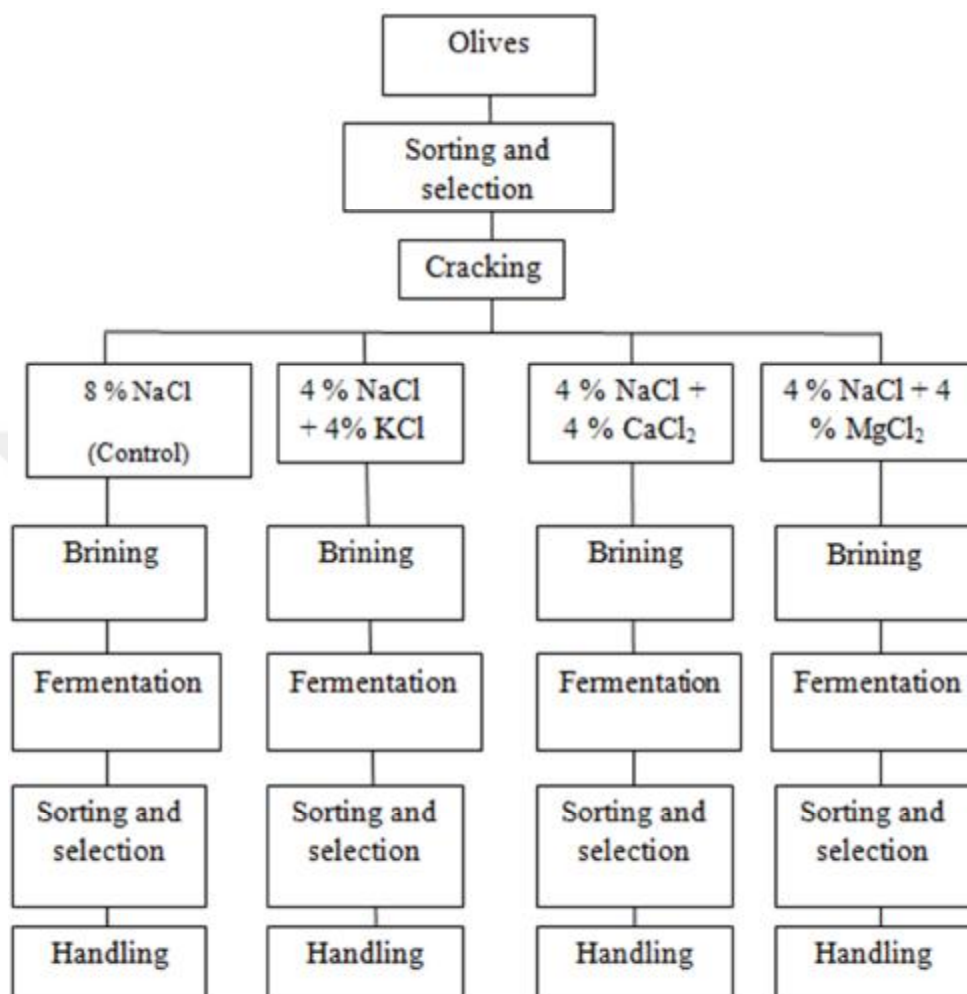


Figure 3.2. Processes applied in the production of natural green table olives

Each brine was given a code number as follows:

- A code for control brine containing 8 % NaCl
- B code for brine containing 4 % NaCl + 4% KCl
- C code for brine containing 4 % NaCl + 4 % CaCl₂
- D code for brine containing 4 % NaCl + 4 % MgCl₂

The results are evaluated by those codes. Olivefruits were putted into plastic containers, then filled with suitable brines and covered with food grade perforated disks in plastic disks to block the olives from floating and closed loosely to let CO₂ gas out. Fermentations were carried out in duplicate at room temprature. The amount of chloride salts in brines were always kept at 8 %. The salinity of brines during the fermentation was controlled by Bome and if necessary different chloride salts were added to the brines. For monitoring the fermentation developement, pH and total acidity of the brines were followed. When the fermentation process was completed, table olives were packaged and stored at +4°C for physical, chemical and sensory analyses.

3.3. Analyses

3.3.1. Physical and Chemical Analyses

3.3.1.1. Maturity Index

It was used to determine the degree of ripeness of the olive fruits, thus they can be categorized. It was used also to determine the exact harvesting time of the different fruits olives varieties. To determine the maturity index (MI), randomly selected 100 grains of olive fruits were taken. Then the olives fruits were separated into eight groups according to their skin colours as following (Figure 3.3):

Group 0: green skin

Group1: yellow- green

Group 2: green and reddish dotted, half the surface start to colour change

Group 3: fruit surfacereddish or bright violet

Group 4: fruit surface black and flesh white

Group 5:fruit surface black and flesh less than 50% purple

Group 6: fruit surface black and flesh more than 50% purple

Group 7: fruit surface black and flesh 100% purple (Guzman et al., 2015).

Maturity index is the total of the number of olives in each group have been multiplied by the score, with the sum, then have been divided by 100 (Sibbet and Ferguson, 2005).

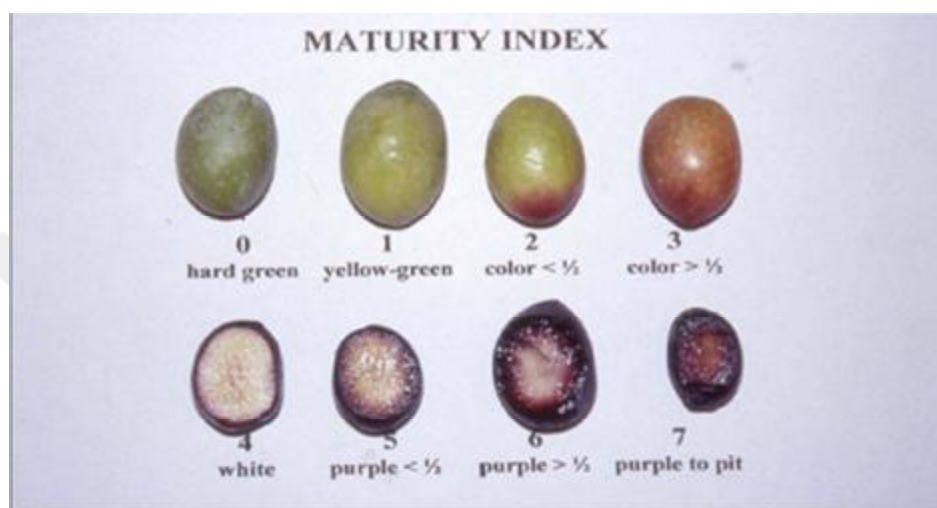


Figure 3.3. Maturity index of olive fruits (Hermoso et al., 1991)

3.3.1.2. The Number of Olive Fruits Per Kg

100 g of olive samples was weighed and the number of fruits in kg was determined by counting how many fruits were in this amount (Özdemir et al., 2011).

3.3.1.3. The 1000 Olive Weight

Randomly selected 100 olives were weighed and multiplied by 10 to calculate the weight of 1000 olives.

3.3.1.4. Fruit Dimensions

The width and height of 20 randomly chosen olive fruits were measured with a caliper with a sensitivity of 0.1 mm (Anon., 1997).

3.3.1.5. Flesh/Stone Weight Ratio

100g of randomly chosen olive fruits were weighed, then their stones were removed with olive pitting machine. The removed stones were weighed separately. The percentages of the weights were calculated, then the % flesh was divided by % stone (Yazıcıoğlu, 1966).

3.3.1.6. Color Analyses

The color of the olive samples was measured with a Konica Minolta Chroma Meter CR-400 (Japan) colorimeter. L *, a *, b * values were determined. L *, "a *" and "b *" values were obtained during the color measurement and the values of "L" darkness to brightness, "+ a *" redness, "-a *" greenness, "+ b *" "- b *" indicates going to the yellowness and blueness respectively (Gould, 1977). Hue (Color tone) and C the chroma values were calculated with the following formula (Artes et al., 2002).

$$\text{Hue} = [\arctan (b^*/a^*)]$$

$$C = [(a^{*2} + b^{*2})^{1/2}]$$

3.3.1.7. Total Dry Matter Determination

Dry matter determination in olives was made according to Cemeroğlu (2010).

3.3.1.8. Total Ash Determination for processed olives

Ash in processed olives were determined at 500 ° C in an ash oven (Cemeroğlu, 2010).

3.3.1.9. pH Determination

The pH level of brines and olive samples were analysed using pH meter (A.O.A.C., 1990).

3.3.1.10. Determination of Total Acidity

Total acidity expressed as lactic acid was determined by titrating the sample up to pH 8.2 with 0.1N NaOH (Anon, 1990).

3.3.1.11. The Concentration of Sodium Chloride

The sodium chloride concentration was analyzed by titration with 0.1 N silver nitrate solution. (Anon, 1997).

3.3.2. Microbiological Analyses

Microbiological analyses were done in olives brines. Samples were serially diluted with 0.85 % NaCl. Total mesophilic bacteria were determined on plate count agar (PCA) medium which incubated at 30°C for 48 h. For LAB, MRS agar containing 0.5 g/L of cycloheximide to prevent the growth of yeast were used and plates were incubated at 30° C for 2-4 days under anaerobic conditions. M17 agar was used to count cocci LAB, incubated at 30°C for 48 h. For coliform microorganisms, violet red bile agar medium (VRBA) was used, incubated at 37°C for 24 h. To count total number of yeasts, potato-dextrose agar medium (PDA) which contained 0.1g/L oxytetracycline to prevent the growth of LAB was used and plates were incubated at 25°C for 2-4 days. To count non- *Saccharomyces* yeasts, L-lysine agar medium was used, incubated at 25°C for 2-4 days (Campaniello et al., 2005 ; Settanni et al., 2011).

3.3.3. Sensory Evaluation

In sensory tests, the method given by Marsilio et al (2000) was used. 11 selected panelists evaluated the fermented green cracked table olives for their odor,

taste, salinity, color, bitterness, hardness, color of flesh and general acceptance. For this purpose, 9-point hedonic scale was used. According to this scale, 9 "Highest" (like extremely) and 1 "Lowest" (dislike extremely) has been evaluated. Form used in the analyses was given in Figure 3. 4. In sensory analysis, the "Preference (ranking) Test" (Figure 3. 5) was applied to determine the most preferred method among different fermentation technological processes within different chlorine salt applications. In the preference test, olive samples were presented to the panelists and ranked from the most favored to least preferred (Altuğ and Elmacı, 2005).

3.3.4. Statistical Analyses

The results of physicochemical properties of raw material and chemical, sensory characteristics of processed olives was compared statistically by IBM SPSS Statistics 22 program. Duncan multiple comparison test was applied to significant differences. The level of significance was assessed as $P = 0.05$ (Özdamar, 2015).

DUYUSAL ANALİZ FORMU

Ad/Soyad:

Ürün Kodu:

Tarih:

Kabuk RengiKabuk ParlaklığıMeyve Eti RengiKoku ve AromaAcılık (işlenmemiş zeytin acılığı)TuzlulukAsitlik

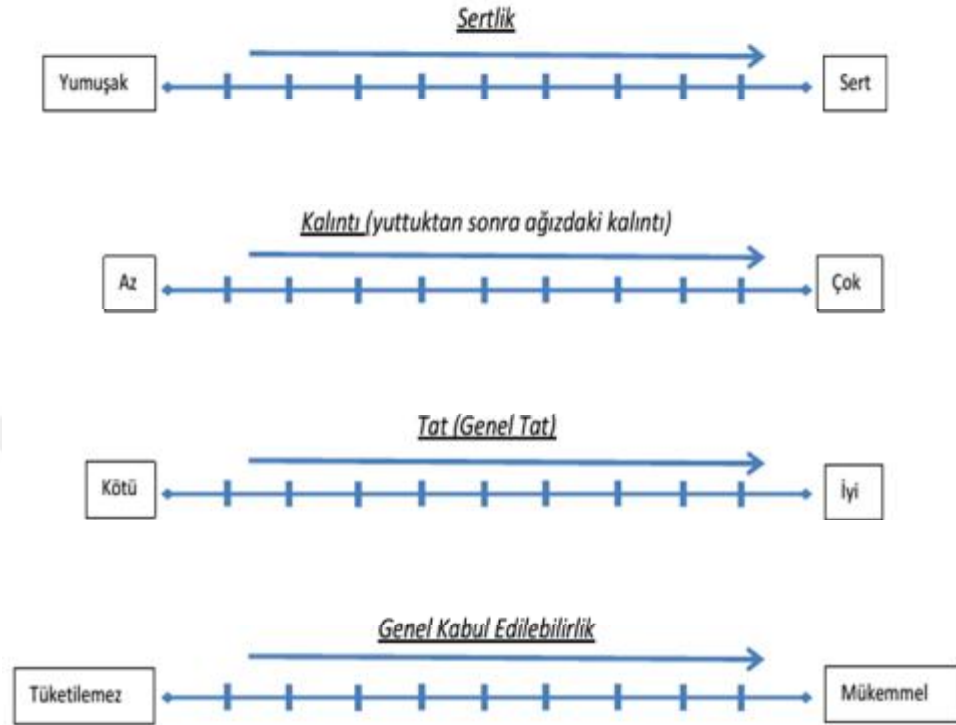


Figure 3.4. Sensory analyses form (Hedonic scale)

<u>Tercih (Sıralama) Testi</u>	
Ürünlerin tüm özelliklerini göz önüne alarak en fazla beğenilenden en az beğenilene doğru sıralayınız.	
<u>Sıra</u>	<u>Ürün Kodu</u>
1.	
2.	
3.	
4.	

Figure 3.5. Form of preference test

4. RESULTS AND DISCUSSION

4.1. General Composition of Olives Used in Experiments

4.1.1. Physiochemical Properties of Cracked Green Olives Used As Raw Material

In processed table olives, the size and the general appearance are very important characteristics for quality determination. Determination of the quality and the price of the table olives are made according to physical characteristics such as the number of olives per kg, flesh/ stone ratio, color and maturity index etc. (Uylaşer et al., 2008). Some physiochemical properties of the Sarı ulak olives variety that used in this study are given in Table 4.1.

Table 4.1. Physicochemical properties of the Sarı Ulak olives variety

Maturity index	0.48 ± 0.000
The number of olive fruits per kg	198 ± 12.020
The 1000 olive weight (kg)	4.81 ± 0.193
Flesh/ Stone ratio	4.37 ± 0.141
The average fruits length (mm)	1.53 ± 0.354
The average fruits width (mm)	0.88 ± 0.173
pH	5.13 ± 0.049
Color	
L* values	69.79 ± 3.178
a* values	-10.93 ± 5.465
b* values	+38.25 ± 5.099
Hue values	74.05
C values	39.78
Total acidity (g/ kg)	0.2 ± 0.000
Total dry matter (%)	34.39 ± 0.388

The maturity index of the used cracked green olives was determined to be 0.48 ± 0.000 (Table 4.1). In another study on Gemlik variety of black table olives the maturity index was 5.36, very high (Erdogan, 2014).

As shown in Table 4.1, the number of olive fruits per kilogram is 198. Özay and Borcaklı (1996) and Şahin et al (2000) detected that the number of olive fruits from Gemlik variety per kilogram was 318 and 304 pieces. Uylaşer et al (2008) reported that the number of olive fruits, also from Gemlik variety was 272 pieces/ kg. The number of olive fruits per kilogram in this study were lower than the values reported in other studies. In present study the 1000 olive weight was 4.81kg, while in another study on Gemlik variety of black olives it was 4.25 kg (Erdogan, 2014). The flesh /stone ratio was 4.37. In other studies it was between 4.6 and 5.5 (Kumral, 2005; Uylaşeret al., 2008). The fruit length of the Sarı Ulak olive variety used in this experiment was measured as 1.53 mm and the fruit width was measured as 0.88 mm. Kumral (2005) and Uylaşer et al (2008) detected that the fruit length of the fresh olives from Gemlik variety was 20.40 and 21.37 mm and the fruit width was 14.40 and 16.68 mm. The fruit length and fruit width values obtained in this study were much lower than those obtained from other studies. In this study, the pH of Sarı Ulak olives was 5.13 ± 0.049 (Table 4.1). The pH of this study was similar to some previous studies like the study made by Lopez-Lopez et al (2004) determined the pH of green olives as 3.69, Uylaşer et al (2008) found that the pH of the Gemlik olive variety grown in the district of Bursa was 5.23 ± 0.06 , the pH of olive grown in İznik province was 5.27 ± 0.15 , the pH of olives grown in Mudanya province was 5.50 ± 0.29 and the pH of olives grown in Orhangazi province was 5.34 ± 0.13 .

As a result of the color measurements made on the fruits of Sarı Ulak olive variety, L *, a *, b *, Hue and C values were 69.79 ± 3.178 , -10.93 ± 5.465 , 38.25 ± 5.099 , 74.05 and 39.78, respectively (Table 4.1). In another study on Gemlik variety of black olive, L * values were 24.43 ± 1.57 ; a * values were $4.13 \pm$

1.28; b * values were 1.05 ± 0.83 ; Hue values were 14.49 ± 5.13 and the C values were found to be 4.28 ± 1.13 (Erdogan, 2014).

The total acidity level of Sarı Ulak olives determined as 0.2 g/Kg in terms of lactic acid by titration with 0.1N NaOH. López-López et al (2004) detected that the titratable acidity for ripe green olives was 0.64%. In olive samples of the Manzanilla types, some parameter values of titratable acidity were nearly below 0.5 g/100mL. Moreover, Marsilio et al (2000) found that the titratable acidity of the green table olive samples was about 0.1 to 1.44%. The amount of dry matter in Sarı Ulak olive variety used as raw material was 34.39%. Uylaşer et al (2009) reported that the dry matter of Gemlik variety olives grown in Gemlik, İzmit, Mudanya and Orhangazi regions was 49.68 %; 49.32%; 48.68%; 47.28% (w / w).

4.2. Chemical and Microbiological Analyses During the Fermentation Process of green cracked Sarı Ulak olives

4.2.1. The pH and the Total Acidity of the Brines of Sarı UlakOlives Variety during the fermentation

The changes in the pH and the total acidity in brines with different chloride salts mixtures during fermentation are given in Figures 4.1, 4.2, respectively.

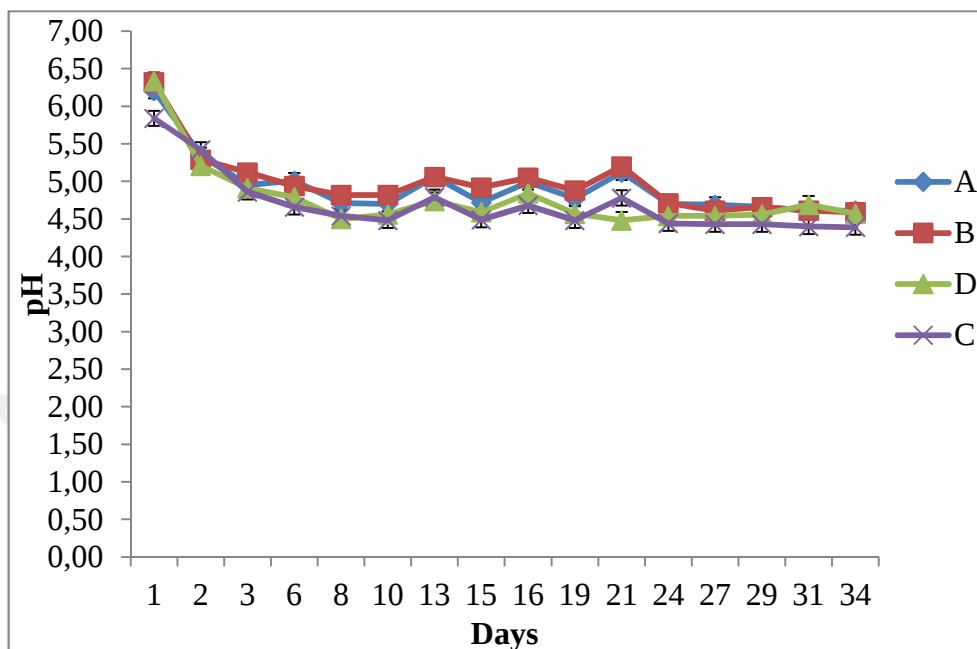


Figure 4.1. The pH of the brines during the fermentation (A: 8 % NaCl, B: 4 % NaCl+ 4 % KCl, C: 4 % NaCl+ 4 % CaCl₂, D: 4 % NaCl+ 4 % MgCl₂)

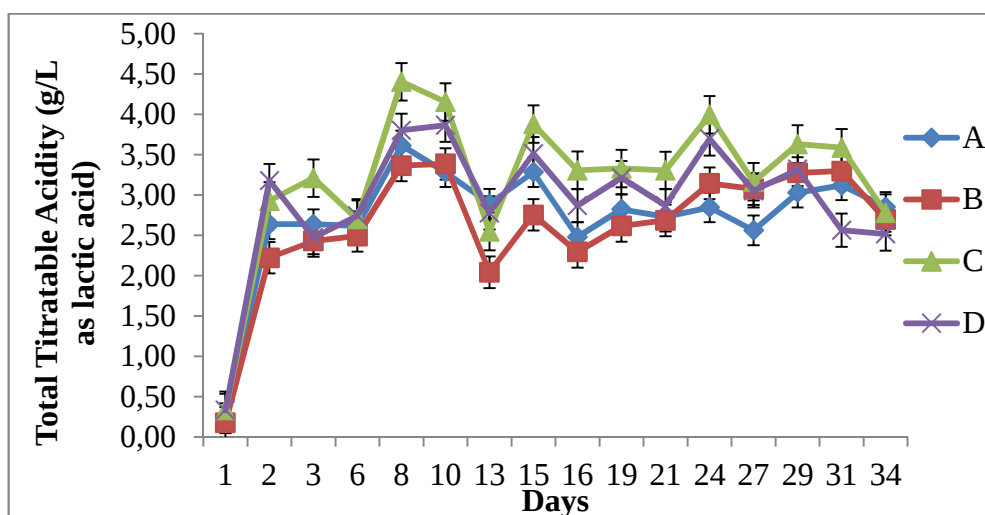


Figure 4.2. Changes in total acidity during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4%KCl, C: 4% NaCl+4 % CaCl₂, D: 4% NaCl+ 4% MgCl₂)

In the fermentation processes the most important parameters to follow the fermentation are pH and titratable acidity. The titratable acidity was always obtained regardless of the run of experiment. The pattern of titratable acidity value changes with time; following a sigmoidal curve, which comprises of a period of lag phase, followed by a variable increase and plateau phase. The minimum limit for this parameter in the IOOC standards is 0.4% (wt/vol) for green olives. It differs according to the types of olives. Low acidity value was noticed in ripe olives (López-López et al., 2004).

The initial pH values ranged from 5.84 to 6.34 as can be seen above in Figure 4.1. Bautista Gallego et al (2011b) detected the initial brine pH values for Spanish- style Gordal variety of green table olives ranged from 3.76 to 4.19. Mateus et al (2016) measured the pH in the initial brines of experiments between 6.24 to 6.53 in the fermentation of Maçanilha Algarvia variety of cracked green table olives. The initial risk of contamination by pathogenic organisms can be minimized by lowering the pH of the initial brine. This can be achieved by adding a certain amount of CaCl_2 (Bautista- Gallego et al., 2011). After the proceed of the fermentation, in all brines, pH values showed a remarkable drop within the first six days. Then, they came close to constant level. On 21th day the highest pH value was determined for B coded brine solution among the all treatments and it was the highest pH value during the fermentation. Also control brine showed very close to brine with code B during the fermentation process. This results were in agreement with the results reported by Panagou et al (2011) they observed that in the first 6 days of the fermentation of Conservolea variety of natural black table olives the pH value decreased to reach a stable level. At the end of the fermentation the pH value was ranged from 3.9 to 4.2. In this study, D coded brine had the lowest pH values during the all fermentation, but the brine with code C had close to it in pH values. Bautista-Gallego et al (2011) found that the pH at the beginning and the end of fermentation in the presence of CaCl_2 was lower and that was related to the delay of the diffusion of sugar in the brine.

In all the fermentation brines, the titratable acidity values remarkably increased in the first two days, then oscillated a little until the end of the fermentation. At the beginning of the olive fermentation, the total acidity values varied from 0.18- 0.33 g/L in terms of lactic acid. The highest acidity value was observed in 8th day for D coded brine and it was 4.40 g/L. The least acidity value during the fermentation was related to B brine. It was 2.04 g/L in 13th day of the process. The acidity values for A and B treatments were close to each other during the fermentation process. Panagou et al (2011) found the highest total acidity level in experiments which carried out with KCl and CaCl₂ salts in a study of table olive production with different chloride salts.

4.2.2. Microbiological Analyses During the Fermentation Process of Sarı Ulak Olives Variety

A research on the microbial features of the final product is essential in order to investigate any modification containing partial or total substitution of NaCl with other chlorides. This is an important action to perform, since fundamental principles and processing of food products declared whether reducing salts or replacing it with other salts have severe danger on safety and spoilage of the product (Mateus et al., 2016).

4.2.2.1. The Number of LAB

The media, MRS and M17 agar were used for the counting of rod and coccus LAB, respectively (Settanni et al., 2011). The change in the number of LAB grown on the MRS agar during the fermentation of Sarı Ulak variety of table olives is shown in Figure 4.3.

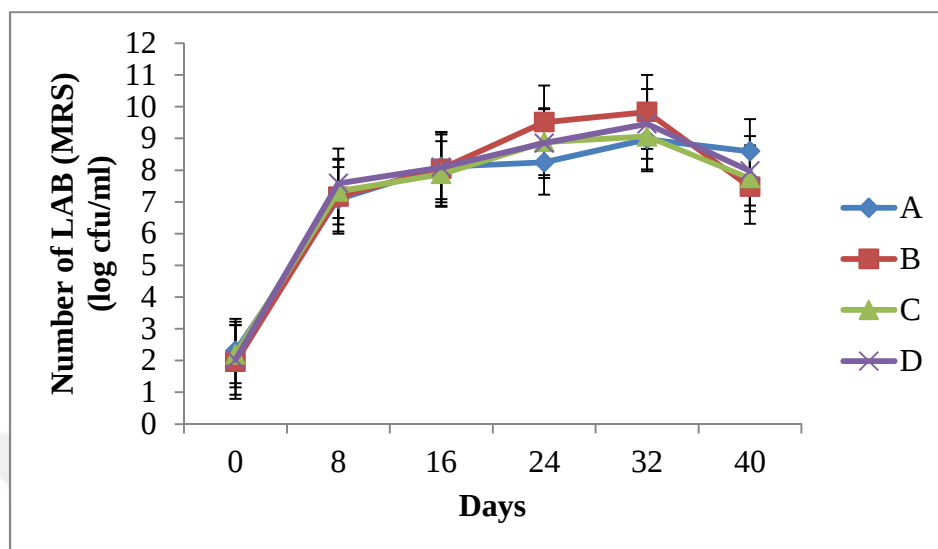


Figure 4.3. The changes of the number of LAB on MRS agar during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4% NaCl+ 4% CaCl₂, D: 4% NaCl+ 4% MgCl₂)

As can be seen from the Figure 4.3, at the beginning of the fermentation process (day 0) the number of LAB was in the range of 1.95 to 2.30 log cfu/mL.

The lowest number of LAB grown on MRS media was 1.95 log cfu/mL in B coded brine, whereas the highest number was 2.30 log cfu/mL in A coded control brine solution. Subsequently, during the fermentation process after two week) the highest number of LAB was 7.58 log cfu/mL in D coded brine, and the lowest number was 7.08 log cfu/mL in A coded control brine. Furthermore, after three week of fermentation the highest number of LAB dropped to 8.10 log cfu/mL in A coded control brine solution, and the lowest number of LAB was determined as 7.87 log cfu/mL in C coded brine. Meanwhile, there was a significant change in the bacterial count in the lag phase. As the fermentation process proceeds, the number of LAB began to increase. In the 24th day of the fermentation, the number of LAB was notably decreased especially B coded brine then their level increased reaching the maximum values on the 40th day of fermentation process. This result is in agreement with the result obtained by Bautista Gallego et al (2011) they noticed

that LAB did not present growth through runs of the brines and within the first 20 hours in the fermentation of Gordal olives variety of green Spanish- style table olives using different chloride salt brines. They noticed that when starter culture is added, this caused an intensely increase of its population to 6 log cfu/mL. In other studies on table olive production using different chloride salts, the number of LAB on the MRS agar ranged from 7.2 to 7.6 log cfu /mL (Panagou et al., 2011). The values obtained in this study are higher than those found by Panagou et al (2011). Erdogan (2014) observed that at the beginning of the fermentation of Gemlik black table olives the number of LAB ranged from 3.33 to 3.78 log cfu/mL, whereas at the end of the fermentation LAB number ranged from 6.51 to 7.50 log cfu/mL.

The changes of the number of LAB grown on M17 agar medium during the fermentation of Sari Ulak variety is shown in Figure 4.4. As can be seen from the following Figure, at the beginning of fermentation at day 0 lowest number of LAB was determined as 2.00 log cfu/mL and the highest LAB number was 3.80 log cfu/mL. On the 16th day of fermentation process, the highest number was 8.11 log cfu/mL for D coded brine, while the lowest number was 7.5 log cfu/mL for C coded brine among all the treatments. After three weeks of fermentation process on 24th day the highest number of LAB was counted as 8.5 log cfu/mL for A and B coded brines, while the lowest numbers were 7.83 and 7.85 log cfu/mL for both D and C coded brines, respectively. It was observed that the number of LAB decreased to 7.36 log cfu/mL which was belonging to the control brine and it was the lowest value among the others brines on 32th day of fermentation process. The number of LAB grown on MRS and M17 agar mediums was very close to each other. Erdogan (2014) detected that the number of LAB grown on M17 agar was more than those grown on MRS medium. The number of LAB at the end of the fermentation of Gemlik table olive variety was detected as 7.13 -8.17 log cfu/mL, while the initial number ranged from 2.61 to 4.78 log cfu/mL.

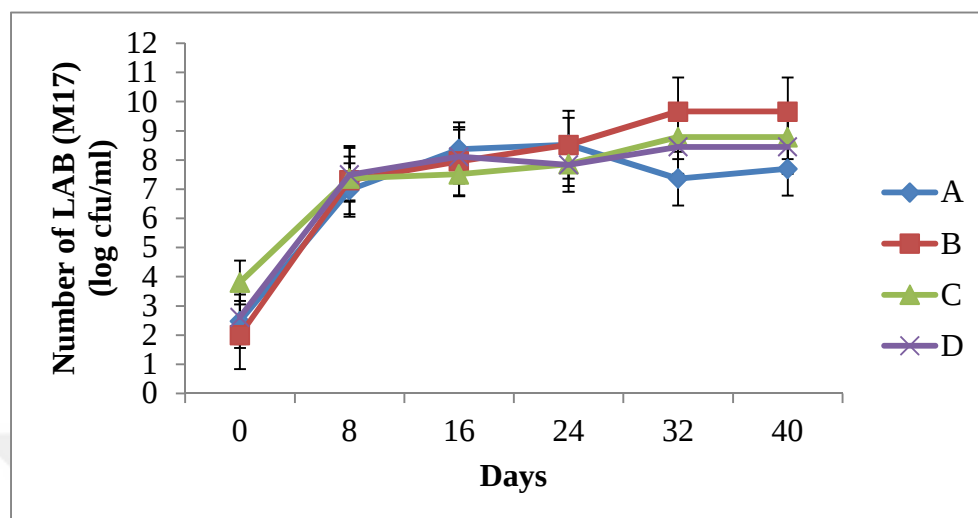


Figure 4.4. The changes of the number of LAB grown on M17 agar during the fermentation (A: 8% NaCl, B: 4%NaCl+ 4% KCl, C: 4% NaCl+4% CaCl₂, D: 4% NaCl+ 4% MgCl₂)

4.2.2.2. The Number of Total Mesophilic Aerobic Bacteria

The changes of the total number of mesophilic aerobic bacteria grown on PCA agar medium during the fermentation of Sarı Ulak variety of table olive is shown in Figure 4.5.

As can be seen from the following Figure, at the beginning of fermentation process on day 0th the number of mesophilic aerobic bacteria grown on plate count agar medium ranged from 3.56 log cfu/mL to 6.32 log cfu/mL for A and C experiments, respectively. On 16th day of fermentation process, the number of mesophilic bacteria increased as 7.84, 8.04, 8.06, 8.28 log cfu/mL for B, A, D and C coded brines respectively.

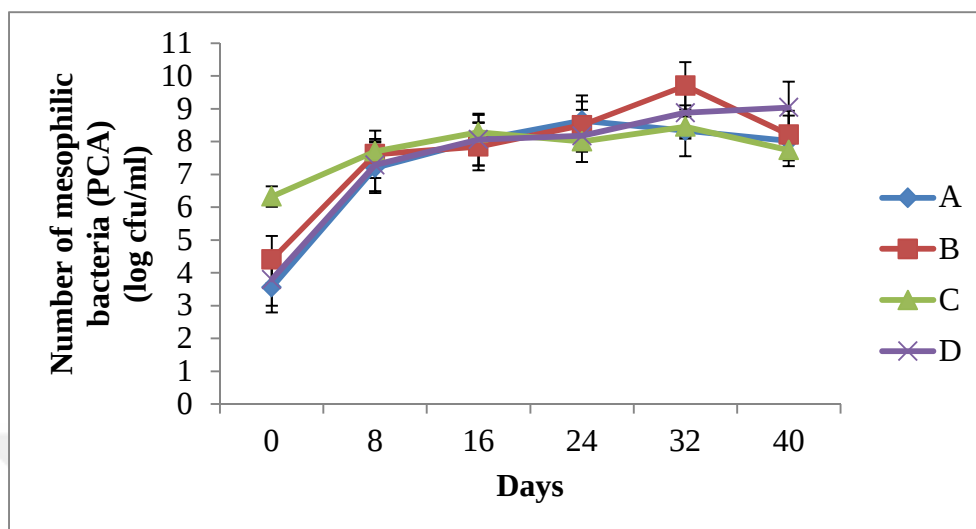


Figure 4.5. The changes of the number of mesophilic aerobic bacteria grown on PCA agar during the fermentation (A: 8% NaCl, B: 4% NaCl + 4% KCl, C: 4% NaCl+4 %CaCl₂, D: 4% NaCl +4 % MgCl₂)

The number was continue to increase on 32th day and the highest number was detected to be 9.70 log cfu/mL for B coded brine, while the lowest number was 8.33 log cfu/mL for A coded brine. On 40th day, the highest number of total mesophilic bacteria was determined as 9.04 log cfu/mL for D experiment.

Idoi et al (2009) reported that the number of total mesophilic aerobic bacteria in natural fermented Jijelian black olives varied between $2.40-9.76 \times 10^7$ cfu/g.

Erdogan (2014) counted the initial number of total mesophilic bacteria in the fermentation of Gemlik variety of black olives as 4.04 - 4.65 log cfu/mL and at the end of the fermentation the number was 7.25- 8.35 log cfu/mL.

4.2.2.3. The Number of Yeasts

The changes of the number of yeasts grown on PDA agar during the fermentation of Sarı Ulak variety is shown in Figure 4.6.

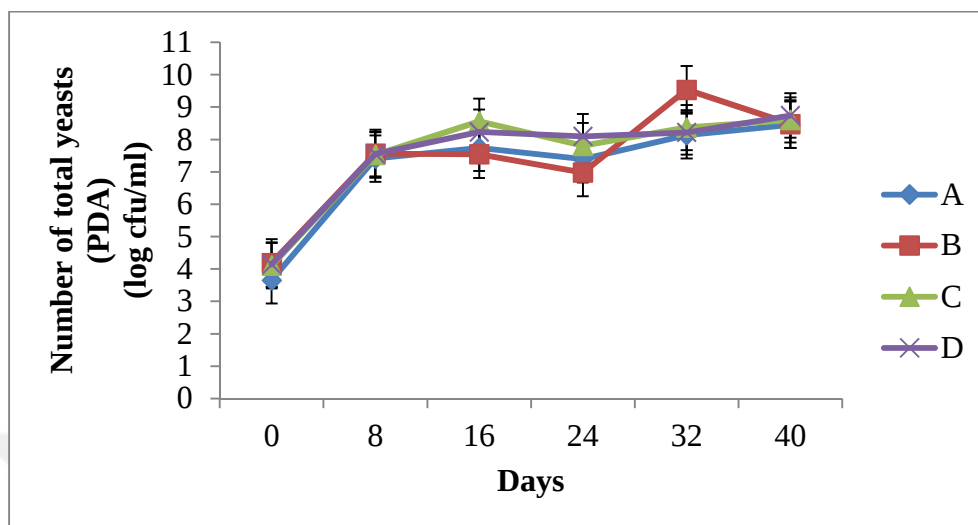


Figure 4.6. The changes of the number of yeasts grown on PDA agar during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4 % NaCl+ 4 % CaCl₂, D: 4 % NaCl+ 4 % MgCl₂)

The highest number of yeast was 9.53 log cfu/mL for B coded brine on 32th day of fermentation among the all treatments. Bautista-Gallego et al (2011) found that the highest yeast population was 6.7 log cfu/mL in the brine containing mixture of NaCl+ KCl+ CaCl₂ on 44th of the process, while the minimum yeast population was 2.73 log cfu/mL at the end of the fermentation phase.

Through the first 8 days, the whole yeast population displayed an adaptation period which was followed by exponential growth attaining the stationary phase. In another study at the beginning of the processes, counts of the yeast population risen from 4.9 to 5.0 log cfu/mL, to a maximum values of 6.0-6.5 log cfu/mL (Alves et al., 2012). The yeast counts in this study were higher than the values reported by Hernández et al (2007) who found the yeast level in the brine of seasoned green table olives were around 4.9 log cfu/mL. Arroyo-López et al (2008) reported yeasts to be present during the fermentation processes accomplishing populations that ranged from 4.0 to 6.0 log cfu/mL. Idoi et al (2009) reported that the number of yeast in the fruits of native fermented Jijelian black olives was 1.25-

8.00 log cfu/g. Leventdurur et al (2016) detected the total number of yeasts as 7.41-7.79 log cfu/mL in brines of fermentation of Gemlik black olives containing 10% NaCl at the end of the 180 days of fermentation, while in the brine containing 8% NaCl with glucose 0.5% the total yeast number was 8.33-8.84 log cfu/mL. Erdogan (2014) counted the number of yeast as 8.26-7.25 log cfu/mL at the end of the fermentation of Gemlik black table olives using different salt mixtures.

4.2.2.4. The Number of Non- *Saccharomyces* Yeasts

The changes of the number of non- *Saccharomyces* yeast grown on L-lysine agar medium during the fermentation of Sarı Ulak variety is shown in Figure 4.7.

As can be seen from the following Figure, the highest level of non-*Saccharomyces* yeast was determined from both A and D treatments as 2.23 log cfu/mL, while the lowest one was 2.54 log cfu/mL for C treatment at the beginning of the fermentation. After 24th days, the highest number was found in experiment A as 8.41 log cfu/mL. When the fermentation was processing, the number was increased and reached the maximum number 8.91 log cfu/mL for B coded brine after 40th days of fermentation process.

Erdogan (2014) reported that the number of non-*Saccharomyces* yeast at the beginning of the fermentation was between 2.77- 4.77 log cfu/mL, whereas at the end of the fermentation it was between 8.13- 6.00 log cfu/mL in the fermentation of Gemlik black table olive with different chloride salt brines.

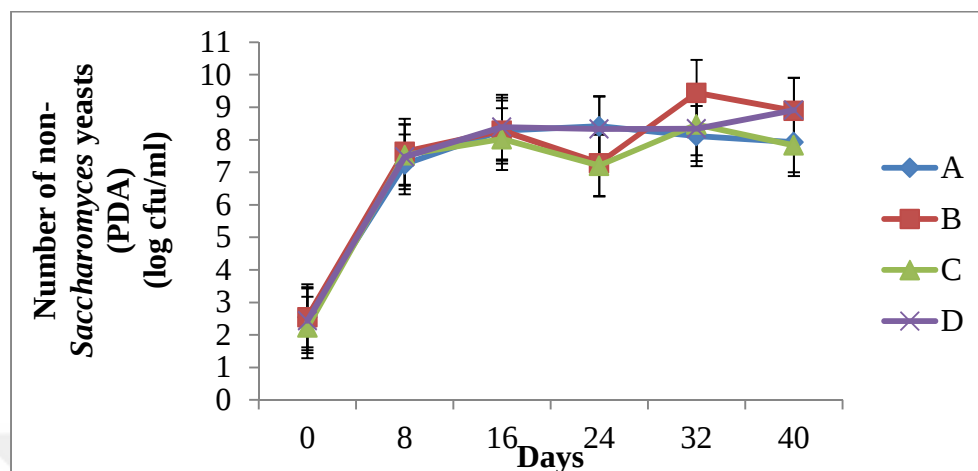


Figure 4.7. The changes of the number of non-*Saccharomyces* yeast grown on L-lysine agar during the fermentation (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4% NaCl+ 4% CaCl₂, D: 4% NaCl+ 4% MgCl₂)

4.2.2.5. The Number of Coliform Bacteria

The number of coliform bacteria was counted on violet red bile agar (VRBA). At the beginning of fermentation process on 0th day, the number of coliform bacteria was 1.47, 1.61, 0.84, 0 log cfu/mL for C, A, B and D coded brines respectively. After two weeks of fermentation process the numbers were reached zero for all experiments. Panagou et al (2011) found a wide group of Gram-negative bacteria (*Enterobacteriaceae*) was present from the starting of the fermentation of *Conservolea* natural black olives using different salt mixtures. Its number was between 4.0-4.50 log cfu/mL. After 10 day in control brine treatment, its number decreased, while in the other brines it took about 2 days to decline. This period coexisted nearly with the time where the population of LAB and yeasts has reached their highest values. The decline of coliform bacteria was in the opposite of the development of yeast and LAB, since they were invisible after 10 days in most treatments, and after 16 days, they completely disappeared. This period went along nearly, with the time LAB reached maximum population. There is restricted role of coliform bacteria through those processes.

During Greek olive fermentation, the growth of Gram- negative bacteria was inactivated rapidly specially in the treatment containing CaCl_2 , because of the calcium role in delaying the diffusion of sugar into the brine. The counts of Enteriobacteria ranged from in 0.35 log cfu/mL in treatment with NaCl and CaCl_2 to 6.0 log cfu/mL in control treatment (Bautista Gallego et al., 2011b).

4.3. Physiochemical Analyses of Proccessed Cracked Green Table Olives

The physiochemical analyses of fermented Sarı Ulak vriety are given in Table 4.2

Table 4.2 The physiochemical analyses of fermented Sarı Ulak vriety of cracked table olives at different salt brines (A: 8% NaCl, B : 4%NaCl+ 4% KCl, C: 4 % NaCl+ 4% CaCl_2 , D: 4% NaCl+ 4% MgCl_2)

	A (control)	B	C	D
Total Acidity(g/Kg)*	5.79±0,000 ^b	5.34±0,063 ^d	6.07±0,091 ^a	5.57±0,127 ^{bc}
pH	4.00±0,007 ^d	4.58±0,014 ^a	4.44±0,005 ^c	4.53±0,005 ^b
Total Ash (%)	6.42±0,671 ^a	4.89±0,113 ^b	5.27±0,007 ^b	3.86±0,127 ^c
Total Dry Matter (%)	30.31±0,353	31.28±0,890	32.85±0,770	30.40±3,917

*as lactic acid. Values with different superscripts indicate statistically significant differences at confidence level 95%.

From the table 4.2, the total dry matter ranged from 30.31- 32.85%. This result combatable with the result given by Özdemir et al (2011) the total dry matter of olives was varied from 31.11 to 37.27%. In present study, the total ash were found between 3.86- 6.42 %. The highest value was determined for fermented olives in A brine (control), while the lowest was for fermented olives in D brine. In another study, the total dry matter was between 43.19-43.88% for Gemlik black table olives fermented in different salt brines, while the total ash was 2.30-4.26% (Erdogan, 2014).

The final pH value was ranged from 4.00- 4.58. The highest value was for the olives fermented in B brine. Panagou et al (2011) found specific results in a study of natural black olives fermentation. They found that the final values pH ranged from 3.9- 4.2. Erdogan (2014) detected the pH value as 4.19-4.89 for fermented Gemlik olive.

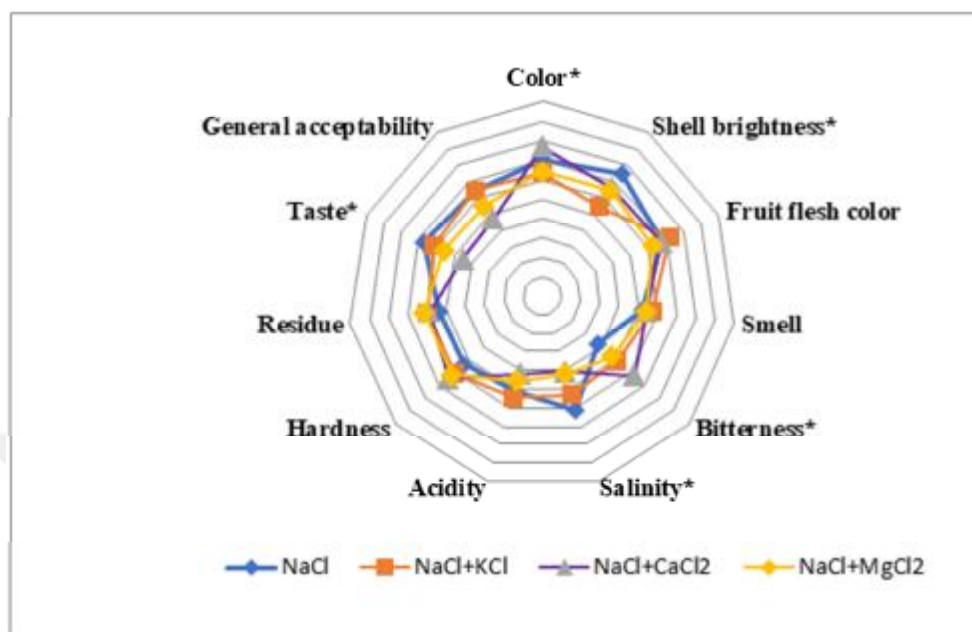
The total acidity expressed as lactic acid ranged from 5.34- 6.07 g/kg. The highest value was obtained for brine C, while the lowest one was in brine B. Panagou et al (2011), found that the higher values of acidity as 8.6 and 8.3g of lactic acid /L of fermenting brine, were measured in brines of 4% NaCl and 4% KCl and 4% NaCl and CaCl_2 . In another study the total acidity was in the range of 8.1- 9.7 g as lactic acid /L. It was stated that there was no relation between these values and the concentrations of the varied salts (Bautista-Gallego et al., 2010).

There are different factors that would explain the rise of total acidity noticed throughout the fermentation operations. These factors are: the organic acids founded in olives tissues (like citric acid), the organic acids (like lactic acid and produced during the lactic acid fermentation and the free fatty acids that are found in olives tissues (oleic acid) which resulted from the activity of esterases and lipases enzymes. Microorganisms mainly produce organic acids. The increased acidity values and the decreased pH values inhibit the growth of spoilage pathogens, thus maintain the safety of the final product (Mateus et al., 2016).

4.4. Sensory Analyses

After the fermentation process was completed, sensory analyses was performed. The sensory analyses results are given in the Figure 4.8. and the preference test results are given in Table 4.3.

For sensory analyses, the parameters used were: color, taste, smell, bitterness, flesh color, brightness, acidity, salinity, residue, hardness and general acceptability.



Properties indicated with superscript symbol () were significant statistically ($p < 0,05$).

Figure 4.8. The results of sensory analyses for fermented Sarı Ulak olives

Table 4.3. The results of preference test for fermented Sarı Ulak olives (A: 8% NaCl, B: 4% NaCl+ 4% KCl, C: 4% NaCl+ 4% CaCl₂, D: 4% NaCl+ 4% MgCl₂)

Experemints	Total points
NaCl	18
NaCl+KCl	25
NaCl+MgCl ₂	33
NaCl+CaCl ₂	35

The imagination of saltiness was concerning the former concentration of NaCl in the brines, since A control brine got the highest score by the panelists, it was 6.07 of 9. The scores for saltiness were incompatible with the initial concentration of NaCl in the brines and reduced constantly, as the level of NaCl declined. As anticipated, the lowest scores were in C brine (4% NaCl+ 4% CaCl₂),

it was 4.03. The employ of CaCl_2 had a remarkable impact on the recognition of bitterness of the final fermented product, and it was not favorable by the panelists.

The lowest scores in bitterness were given in brine A (8% NaCl) it was 3.75, and brine D (4% NaCl+ 4% MgCl_2) it was 4.75. The later handling was also be accepted by the panelists. Apparently, NaCl stables the bitterness caused by the existence of MgCl_2 , and thus a partial substitution of NaCl by MgCl_2 to reduce the amount of sodium in olives could be probable without affecting the acceptability of the end product. For another chloride salts, the concentration of CaCl_2 4% resulted in a gradually, raised perception of bitterness as concluded by the scores specified by the panelists. Regarding the perception of acidity, the highest value was received in the B coded brine containing (4% NaCl + 4% KCl) it was 5.44. Furthermore, the lowest value for C coded brine containing (4% NaCl+ 4% CaCl_2) it was 4.18. Chloride salts presence has an influence on olives hardness, especially CaCl_2 . Salt mixtures with CaCl_2 concentrations receipted the highest scores, whilst the same samples got also high scores for bitterness, and the taste panel did not finally accept them. Regarding to residue on the mouth, the highest score was received in the D coded brine containing (4% NaCl+ 4% MgCl_2) it was 6.17, immediately followed by B brine containing (4% NaCl+ 4% KCl) it was 5.99. With regard to taste, the highest score was received in the control A coded brine containing (8% NaCl) it was 6.70, while the lowest score was received in the C coded brine containing (4% NaCl+ 4% CaCl_2) it was 4.50. In related to shell brightness the most shell brightness was of control A coded brine containing (8% NaCl) it was 7.52, the next were for both C and D coded brine solutions (4% NaCl+ 4% CaCl_2) and (4% NaCl+ 4% MgCl_2) they were 6.47 and 6.65, while the least shell brightness was B coded brine solution containing (4% NaCl+ 4% KCl) it was 5.46.

The most accepted brine by panelists as was brine B (4% NaCl+ 4% KCl) it was 6.45 and A coded control brine it was 6.44, while the least accepted brine was that which contains calcium chloride salts C coded brine it was 4.75. In the

priority test as mentioned in Table 4.3, the most preferred brine in the first rank was A coded control brine containing (8% NaCl), followed by B coded brine containing (4% NaCl+ 4% KCl). The lowest preferred brine by the panelists was control C coded brine containing (4% NaCl+ 4% CaCl₂). In the middle was D coded brine containing (4% NaCl+ 4% MgCl₂) it was somewhat acceptable by the panelists.

These results were compatible with the result obtained by Bautista-Gallego et al (2011b), they detected that the mixture of NaCl and KCl had an important effect in increasing the general acceptability of the final product due to the role of this mixture in improving the quality and the nutritional values of the fermented olives.

5. CONCLUSION

This study was aimed to examine the influence of partial replacement of NaCl with different chloride salts (KCl, MgCl₂ and CaCl₂) on the physical, chemical, microbiological and sensory characteristics of cracked green table olives obtained from cv. Sarı Ulak of Tarsus District. The results can be summarized as follows:

- All chloride salt combinations did not lead to any abnormal fermentations.
- The pH values of fermented cracked Sarı Ulak olives varied from 4.00-4.58 and total acidity expressed as lactic acid in the range of 5.34-6.07 g/l.
- The numbers of lactic acid bacteria, yeasts, non-*Saccharomyces* yeasts and total mesophilic aerobic bacteria increased during the fermentations.
- Coliform bacteria did not survive throughout the fermentations and disappeared after two weeks.
- It was counted that yeasts were predominant during the fermentation of NaCl+ KCl mixture, however lactic acid bacteria dominated the fermentation of NaCl+ CaCl₂ combination.
- According to the sensory analysis, table olives obtained from NaCl containing brine was preferred the most and followed by NaCl+ KCl containing brine in the second rank, while brine containing NaCl+MgCl₂ was in the third rank.
- The NaCl+ CaCl₂ containing brine was not accepted by most of the panelists due to the better taste and hardness.
- Further studies need to clear the partial replacement of NaCl with different chloride salts for better knowledge of cracked table olive processing.



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CURRICULUM VITAE

She was born in Gaza on 01/10/1989. She completed his primary, secondary and high school education in Gaza. She graduated from Islamic University of Gaza, Faculty of science, Department of Biotechnology in 2012 and in the same year she worked as an assistant teacher at the Islamic University. In the same year, she worked at the ministry of agriculture as trainee. In the year of 2014 she was accepted in the Turkish scholarship programme to peruse her studies in Çukurova University in the department of biotechnology to have her master degree in biotechnology in the year 2014. She is married and has two children.