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Original Research Article

Microbiological Monitoring of Olive Fermentation Process of Gemlik Type Dry-Salted Black Olives and Investigation of the Effect of Pasteurization on the Product's Shelf Life

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ABSTRACT

In the present research, the microbiological changes that occurred during the fermentation process of traditionally produced Gemlik style dry-salted olives, locally known as "sele", were monitored. Following the fermentation process, microbiological, chemical and organoleptic changes were investigated for the products which were stored at room temperature for a period of 6 months after various pasteurization processes (non-brined, oiled and non-oiled). At the initial stages of fermentation, microbial flora consisted of molds and lactic acid bacteria, and in the following stages no presence of lactic acid bacteria could be detected. None of Gemlik style dry-salted (sele) olive samples contained coliform bacteria. During the storage period, the highest total bacteria count ($1,2x10^5$ cfu/g) was detected in the control-oiled group and the lowest total count (10 cfu/g) was observed in the pasteurized non-oiled group. The samples within the control group exhibited mold growth, whereas the pasteurized samples rarely exhibited yeast/mold growth. Throughout the storage period, dry matter, pH, total acidity, protein, oil content and oleuropein (absorbance) values of samples were monitored, and no significant change was observed among the sample groups. In general, all samples were relished according to the organoleptic evaluation results conducted after fermentation and throughout the storage period. However, with their attractive appearance, oiled group Gemlik style dry-salted (sele) olives were preferred at higher rates compared to non-oiled samples with dull appearance. Additionally, the slightly rancid taste of oiled group samples towards the end of the storage period, received adverse opinions of some of the panelists. Saltiness values, on the other hand, were found to be at normal levels.

Keywords: Olive, Dry curing, Pasteurization, Shelf life

INTRODUCTION

Olive tree (*Olea europea*, L.) is among the world's most ancient fruit trees. As widely stated in the literature, the presence of olive tree dates back to twelve thousand years ago and olive trees has been grown in Anatolia for six thousand years (Blazquez 1997). Olive cultivation is carried out in 38 countries, 30 in northern and 8 in southern hemisphere (Uylaşer *et al.* 2008, Toker and Aksoy 2013). 95% of all olive trees remain within the countries along the coast of Mediterranean Sea, including Turkey, and with its byproducts, olive constitutes one of the main ingredients of Mediterranean diet (Garcia *et al.* 2005, Uylaşer and Yıldız 2014). Due to its characteristic bitter taste arising from its oleuropein content, olive cannot be directly consumed before being processed as table olive or olive oil. According to the standards of International Olive Oil Council (IOOC 2017), world table olive production was recorded as 2 650 000 tons for 2015-2016 period. As the leading producer, 601 800 tons of this production belonged to Spain, and Turkey took the third place with a production of 397 000 tons. In the world olive production, EU countries hold 32,5%, and Turkey holds 15% market share.

All olive cultivars can be used in black table olive production, while Gemlik cultivar olive is distinguished with its higher quality due to its high flesh-to-pit ratio and thin peel (Şahin *et al.* 2000, Tuna and Bayizit 2009). Gemlik cultivar olive is widely grown in Gemlik, İznik and Orhangazi regions of Bursa, beside various other provinces with suitable climatic conditions. With its Protected Geographical Indication (PGI) certificate, granted by Turkish Patent Institute, Gemlik olive presents high flesh and oil efficiency and other specific characteristics as a result of the geographical, climatic and cultivation conditions under which it is grown (Uylaşer 2015). Gemlik cultivar olive is picked when it comes in violet-purple color and generally processed as natural fermented olive.

Oleuropein is a phenolic compound found in the flesh of olive fruit, and responsible for olive's bitter and harsh taste (Cemeroğlu *et al.* 2001, Savaş and Uylaşer 2013). It is widely known that phenolic compounds protect plants against pathogens, and with its anti-microbial characteristic, oleuropein retards and inhibits growth of various microorganisms including lactic acid bacteria which are effective in the fermentation process of olive

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(Ruiz-Barba *et al.* 1994, Uccella 2001, Romeo and Poiana 2007, Sanchez *et al.* 2007). Oleuropein is reported to be at highest levels (14% in dry matter) during the initial development stages of fruits and the types harvested in green maturity stage are reported to have high oleuropein content, even though it is lower than 14% (Bianchi 2003). In the black maturity period, the oleuropein content is reduced (Kailis and Harris 2007), and for some olive types it even drops down to zero level when it is fully black (Bianchi 2003). The bitter flavour given by oleuropein prevents direct consumption of olive (Uylaşer *et al.* 2008). Owing to its solubility in water, oleuropein can be removed from olive through classical pickling method. It can also be removed via alkali treatment, enzymatic applications as well as hydrolyzing with microorganisms (Soler-Rivas *et al.* 2000).

Table olive processing techniques, based on the principle of de-bittering, are generally applied to put olive in an edible form. Two main processes are applied for de-bittering of green, green-black and black olives: (1) natural method and (2) caustic (NaOH) treatment. Green olive includes Spanish, filled, kırma or çizme style olives; and black olive production includes Californian style ripe, confit (Moroccan), Gemlik (natural), sele, kalamata and teneke type black table olives (Savaş and Uylaşer 2013). In the production of Spanish, Californian and Moroccan style olives, de-bittering is made via lye (NaOH) treatment, and in Gemlik olive production it is made via natural fermentation in brine, putting the olives in their final edible form (Tokuşoğlu 2010). All olive cultivar can be processed as table olive, whereas the techniques used for the end-product vary due to the varying quality (Tunalıoğlu 2002). One of these techniques is dry-salted as a traditional black olive treatment method, which is also referred to as **"Gemlik dry-salted style"** or **"sele style"** (Kılıç 1994). In Turkish Food Codex Notification for Table Olive (Notification no: 2014/33), dry salted style (sele) olive is defined as: "black olive with creased outer surface cultivated in full maturity and put in edible form via laying olive in alternating layers of salt without the use of lye" (Anonymous 2014).

Despite the protection provided during storage with their low water and high salt content, sele style table olive is prone to formation of degrading microorganisms, particularly to mold growth and mycotoxin formation. Yeast/mold growth, induced by mycelium formation on the surface, has adverse effects on the product quality and nutritive value of olive, reduces its shelf life and poses various health issues (Tantaoui-Elaraki et al. 1990, Panagou et al. 2002, Panagou 2006). High salt concentration arising in sele style, results in a reduced nutritive value and a creased outer surface, due to the excessive juice loss in the fruit. Gemlik (sele) and Greek dry-salted style olive production involves the use of 10-20% and 40% coarse grained rock salt (Panagou 2006, Cardoso *et al.* 2008). In this study, salt concentration was kept at low levels as a means to eliminate its adverse effects.

The amount of salt used in olive fermentation is also effective on the development of microorganisms. Low salt concentrations result in the growth of proteolytic microorganisms giving off malodor, whereas high salt concentrations inhibit the development of degrading bacteria such as *Clostridium* and *Propionibacterium* which have lower salt tolerance as compared to lactic acid bacteria (Garrido-Fernandez *et al.* 1997). In sele style (dry salted) olives, high salt concentration becomes effective on microbial population and inhibits the growth of some of the microorganisms. In the case of Greek dry-salted style olive, prepared using 40g salt/100g olive proportion, lactic acid bacteria, *Pseudomonas* and *Enterobacteria* are reported to survive only until the 20th day of fermentation. In the same study, the weight loss for olives at the 80th day was 21% (Panagou 2006). As though the presence of other microorganisms was reported based on the process parameters in olive fermentation, generally lactic acid bacteria and yeasts coexist during fermentation and storage (Romeo 2012). Some yeast species synthesize the vitamins, amino acids and purines, necessary for the optimal development of *Lactobacillus* species (Viljoen 2006). As the final pH of the product reaches 3,8- 4,0 yeasts become predominant within the fermentation environment which is attributed to the reduced pH resulting from lactic acid production, and inhibition of toxic phenolic compounds present in brine (Bautista-Gallego *et al.* 2011, Mucilli *et al.* 2011). Yeasts developing towards the end of fermentation produce alcohol, ethyl acetate, acetaldehyde and organic acids using the glucose remaining after completion of lactic acid fermentation and contribute to the obtainment of the desired flavor for the end-product (Alves *et al.* 2012). Also, the predominance of yeasts during fermentation is reported to result in increased pH values, which in turn leads to formation of malodor, gas pockets, as well as softening and spoilage (Arroyo-López *et al.* 2008). Molds are also responsible for deteriorations in olive quality during the storage of sele style olives. Özdemir *et al.* (2013) reported that the majority of molds developing in olive fermentation brine mostly

consist of *Penicillium* molds; and *Aspergillus*, *Clodosporium*, *Alternaria*, *Eurotium*, *Paecilomyces*, *Rhizopus*, *Phoma* and *Thalaromyces* species are also observed. The compounds produced by *Penicillium* and *Aspergillus* species are reported to result in softening of the product (Hutkins 2006).

Table olives undergo various changes during their treatment and these changes have significant effects on physical, chemical and organoleptic characteristics of the end product. Salt concentration, pH and the applied temperature, polyphenol content in addition to reducing sugar content and microbial load of fruits constitute the main factors which are effective on olive quality (Duran Quintana *et al.* 1999, Uylaşer *et al.* 2000, Rejano *et al.* 2010).

Few studies are available on increasing the shelf life of Gemlik dry-salted (sele) style olives which have short shelf lives. New attempts to increase the storability of Gemlik dry-salted (sele) style olives will thus enable healthy and safe consumption of this product. In the present research, increasing the endurance of traditionally produced sele style Gemlik cultivar black olives was aimed through pasteurization with and without oil. This way, numerous chemical applications used in table olive production will be avoided and provision of a healthy end-product will be ensured by means of natural implementation of fermentation. The results derived from this study will be useful in the design of new processes that can be applied in the olive production industry.

MATERIALS and METHODS

Material

The olive cv. 'Gemlik' (*Olea europaea* L.), grown in Turkey with an average weight of 240 fruits/kg, were used for the experiments. The olive fruits which were in fully maturity and the colour of the skin of the olive fruits was black or purple-black were harvested in Gemlik district in Bursa, Turkey. The olives flavored by Gemlik style (sele) dry-salting method in 10 kg capacity bins were pasteurized as oiled (olive oil used) and non-oiled. Control groups were used to determine the effectiveness of pasteurization process and the effect of application of olive oil on the microbiological changes during the storage period.

Method

Gemlik Dry-Salted Style (Sele) Olive Production

Gemlik dry-salted style (sele) olive production method is summarized in Figure 1. During the research, olives were repetitively (3 times) laid in 10 kg bins and the fermentation process took place at 18-22 ^{o}C (ambient temperature). Microbiological analyses of olives were performed on the 0., 2., 5., 10., 15., 20., 31. days of fermentation. After the completion of fermentation process, olives were put in 370 mL jars and pasteurized oiled and non-oiled for 15 min at 80 ^{o}C. Applications were also performed with and without oil on the (non-pasteurized) control group. During the research 15 jars of samples were taken from each repetition and these jars were stored at 18-22 ^{o}C for six months. Microbiological, chemical and organoleptic analyses of the products were conducted on a monthly basis.

Physical and chemical analyses

Olives in raw and processed forms reserved for the chemical analyses were homogenized with a mixer after being pitted and afterwards they were placed in nylon bags and preserved in -18°C until the analysis.

The titratable acidity, pH, moisture, and salt content of the olive fruits were determined according to the methods described by Uylaser and Basoğlu (2011). The amount of reducing sugar was determined with Luff method (Cemeroğlu, 1992). For this purpose, the sample was then clarified using Carrez I and Carrez II solutions and filtered. 25 mL Luff solution was added into 25 mL erlenmeyer flask with socket along with 25 mL filtrate and then erlenmeyer flask was connected to a condenser. Hot plate temperature was adjusted so as to reach the boiling point of the solution within 2 min and the solution was boiled precisely for 10 min 10 mL 20% KI, 25 mL 25% H_2SO_4 and 2 mL 1% starch solution was added on the sample after rapid cooling under tap water, and the solution was titrated upon addition of 0,1 N $Na_2S_2O_3$ solution until the colour turned to cream yellow. The results

were obtained using the formula. The nitrogen content was determined using Kjeldahl method for crude protein determination (Uylaşer and Başoğlu 2011). Spectrophotometric determinations of oleuropein and fruit color were performed according to the method described by Tzika *et al.* (2004) and Mastorakis *et al.* (2004); a Schimadzu UV-1208 spectrophotometer was used. The oil content of the olive fruits was determined by Soxhelet extraction using n-hexane (AOCS Official Procedure Am 5-04: 2009). The analyses were carried out three times for each application.

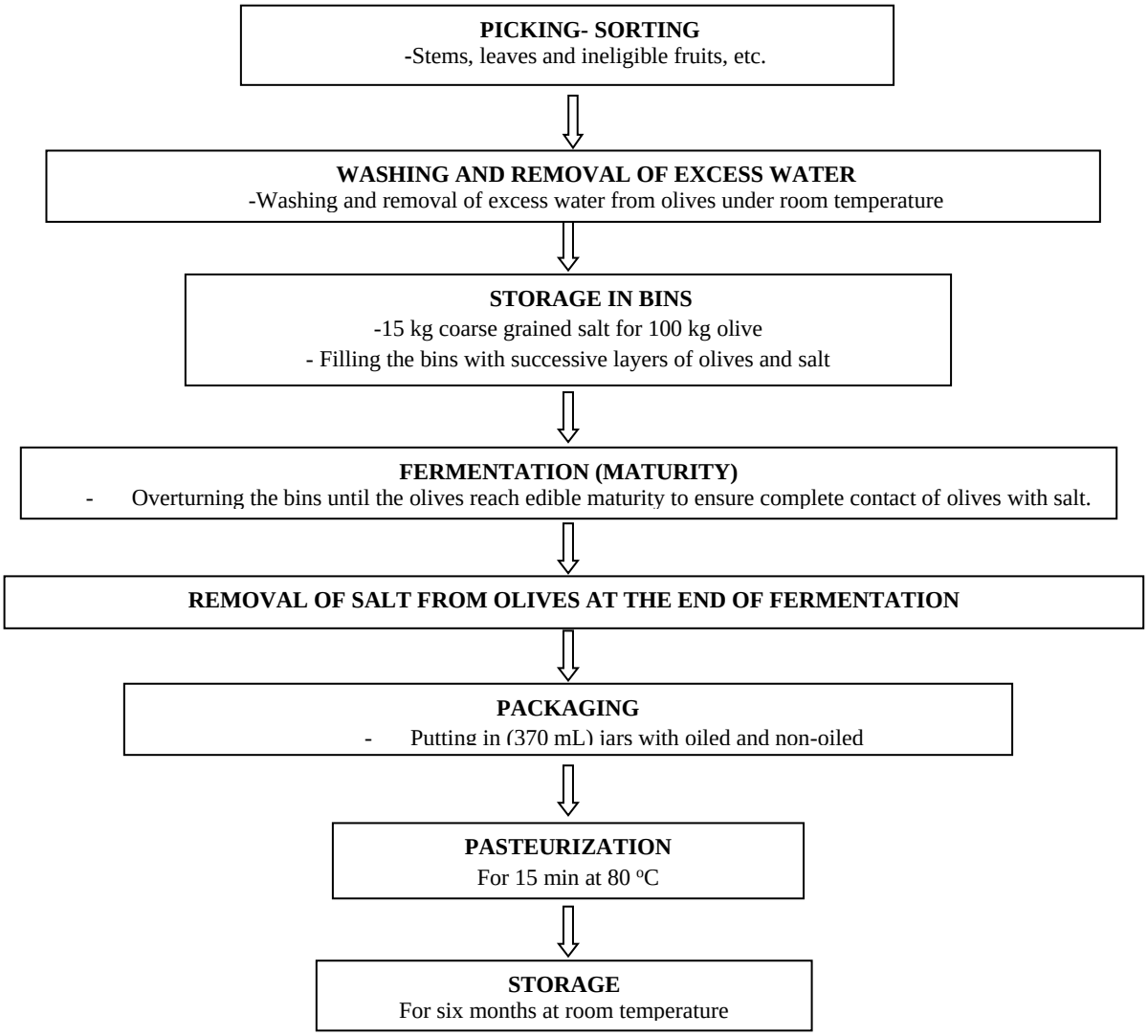

Figure 1. Production stages of Gemlik dry-salted style (sele) olive.

Microbiological analyses

10g olive sample taken under sterile conditions and solutions diluted at 1:9 ratio in 90 mL 0,85% sterile physiological salty water were used for total mesophilic aerobic bacteria (Anonymous 2005a), yeast-mold, lactic acid bacteria (Panagou 2006) and coliform bacteria (Anonymous 2005b) counts.

Organoleptic evaluation

The organoleptic evaluation was performed by ten panelists using a ten-point scale in which 5 indicated “extreme likeness” and 1 indicated “extreme dislike” (Panagou et al. 2002). The panelists who participated in the organoleptic analyses were academic personnel from the Uludag University, Agriculture Faculty, Food

Engineering Department and they had previously been trained for Gemlik dry-salted style (sele) olive evaluation criteria.

Statistical analysis

The significance tests on the differences between the stored Gemlik style sele olive groups were performed using Least Significant Difference (LSD) multiple comparison test within α=0,95 confidence interval. JMP Statistical Discovery Software 7.0 (SAS Institute Inc., Cary, NC, ABD) software package was for calculations.

RESULTS and DISCUSSION

Fruits characteristics

The results of the physical and chemical analyses of 'Gemlik' cultivar olives used as raw material are shown in Table 1. Also the results of the microbiological analyses of 'Gemlik' cultivar olives used as raw material with salt are shown in Table 2.

Physical and chemical analyses results of raw olive samples are in compliance with the specified characteristics of Gemlik cultivar olives. With exception of some negligible differences, the obtained results are in agreement with several other research results (Tanılgan *et al.* 2007, Uylaşer *et al.* 2008, Gür *et al.* 2011, Özdemir *et al.* 2013, Toker and Aksoy 2013, Idoui and Bouchefra 2014). However, 'Gemlik' cultivar olives studied in the present research had higher oil and reducing sugar content as compared to those reported in other studies. The differences can be ascribed to annual climatic changes, harvest maturity and farming applications.

Reportedly, the flora of Gemlik cultivar fresh olive mainly consists of gram-negative bacteria, coliform bacteria and yeasts with no sign of lactic acid bacteria (Özay and Borcaklı 1996, Borcaklı *et al.* 2000). Coliform and total yeast/mold counts prior to washing were found to be similar with those reported in other studies. However, the results differed in terms of lactic acid bacteria presence. Korukluoğlu *et al.* (2002) reported the presence of lactic acid bacteria in the micro-flora of fresh olives.

Table 1. Physical and chemical properties of 'Gemlik' cultivar olives.

Number of olive fruits/kg	240 ± 3,83
Fruit flesh ratio (%)	83,90 ± 0,30
Fruit flesh/Stone ratio	5,26 ± 0,02
Moisture (g/100g)	52,79±1,63
pH	5,22±0,05
Acidity (as lactic acid, g/100g)	0,90±0,08
Oil (g/100g)	29,89±0,00
Protein (g/100g)	2,02±0,07
Oleuropein (ABS)	1,001±0,06

Table 2. Microbiological properties of 'Gemlik' cultivar olives and salt.

	Olives Pre-washing	Olives Post - washing	Salt
Total mesophilic aerobic bacteria count (cfu/g)	1×10^7	$3{,}3 \times 10^4$	17
Lactic acid bacteria count (cfu/g)	1×10^4	1×10^3	<1
Total yeast-mold count (cfu/g)	1×10^5	6×10^3	10
Coliform bacteria count (cfu/g)	1×10^4	10	<1

Microbiological changes during the fermentation process

The changes in total mesophilic aerobic bacteria, total yeast/mold, lactic acid bacteria and coliform bacteria counts are given in Table 3.

Total mesophilic aerobic bacteria count fluctuated throughout the fermentation process and it was found as $1,7x10^4$ cfu/g at the beginning and as 10^4 cfu/g at the end of fermentation. Yeasts survived and maintained their existence throughout fermentation. Lactic acid bacteria, on the other hand, could not be detected as from the 5th day of fermentation. As hygiene indicators in foods, coliform bacteria was not observed throughout the fermentation process. Panagou *et al.* (2002) reported that no sign of lactic acid bacteria and enterobacteria was detected in the olives produced with dry-salted (sele) style after the 20th day of fermentation. They also reported that no microbial group except yeasts was observed due to the low aqueous activity at the end of fermentation, and that, the change in yeast count was negligible with 6 log cfu/g after fermentation.

Table 3. The microbial changes that occurred during the fermentation process.

	0th day	**2nd day**	**5th day**	**10th day**	**20th day**	**31th day**
Total mesophilic aerobic bacteria count (cfu/g)	$1,7x10^4$	$3x10^3$	$2,6x10^5$	$3x10^4$	$1x10^3$	$1x10^3$
Total yeast-mold count (cfu/g)	$1x10^4$	$2x10^3$	$3x10^3$	$1x10^4$	$1x10^3$	$2x10^3$
Lactic acid bacteria count (cfu/g)	$2,5x10^3$	<1	$1,3x10^3$	<1	<1	<1
Coliform bacteria count (cfu/g)	<1	<1	<1	<1	<1	<1

The obtained results indicate that the fermentation process in the present research followed a similar course with the ones reported in other researches, although with lower microorganism counts. High salt concentration, used in dry-salted (sele) method can be regarded as an adverse environment for various microorganisms. Initial microorganism load of the raw material and good hygiene conditions also account for low microorganism counts.

Microbiological changes in olives during storage period

The microbiological changes in Gemlik dry-salted style (sele) olives during the storage are shown in Table 4. Total mesophilic aerobic bacteria count for control group Gemlik dry-salted style (sele) olive samples were found as $1,6x10^4$ cfu/g after fermentation and as 10^2 cfu/g in the sixth month of storage. Total yeast/mold count was found as 10^4 cfu/g at the beginning and as 60 cfu/g at the end of the storage period. A slight increase was observed in lactic acid bacteria count as of the fourth month of storage, and afterwards it was found as 40 cfu/g in the 6th month. As a hygiene indicator for foods, no sign of coliform bacteria was observed throughout the storage period of Gemlik dry-salted style (sele) olives.

As seen in Table 4, total bacteria count in the control group (oiled) at the beginning of storage of Gemlik dry-salted style (sele) olives was found as $2,8x10^3$ cfu/g, and it increased to $1,2x10^5$ cfu/g on the 2nd month. Afterwards a consecutive drop and rise were observed in total mesophilic aerobic bacteria count and the count results were found as $3,0x10^4$ cfu/g on the last month. A significant decrease was observed in total yeast/mold count on the 1st month of storage, and yeast/mold amount reached its highest level with $2,7x10^4$ cfu/g as of the sixth month. In lactic acid bacteria count, the highest value was detected on the 2nd month with 10^5 cfu/g. A change of $9,8x10^3$ cfu/g was observed in lactic acid bacteria count throughout the storage period.

After pasteurization, total mesophilic aerobic bacteria count was found as 10 cfu/g for non-oiled olives, and the total count of yeast/mold, lactic acid and coliform bacteria was undetectable, which verifies the effectiveness of pasteurization process on the mentioned microorganism groups. At the later stages of storage, however, a general increase was observed in microorganism counts (Table 4).

Total mesophilic aerobic bacteria count in pasteurized and oiled Gemlik dry-salted style (sele) olives were found as $2,1x10^2$ cfu/g at the beginning of storage period, and reached its highest level with $2,5x10^4$ cfu/g on the 2nd month. In later months, a slight drop was observed. An abrupt increase was observed in yeast/mold count in the 2nd month of storage, and no sign of these microorganisms was observed in the 4th month of storage. Total yeast/mold count was found as $1,8x10^3$ cfu/g in the last month of storage. Lactic acid bacteria could maintain their

presence until the 5th month of storage and reached their highest level in this month. No sign of lactic acid bacteria was encountered in the last month of storage (Table 4). In the first month of storage, total microorganism load in oiled control group olives were found to be lower than those of non-oiled Gemlik dry-salted style (sele) olives (Table 4). Olive oil can be regarded as a protective material against the development of microorganisms as it covers the olive surface and exhibits microbial effect (Keçeli and Gordon 2001). A significant drop in the microbial load of non-oiled Gemlik dry-salted style (sele) olives was observed in the 6th month of storage. This is probably due to the presence of free fatty acids released by lipolytic microorganisms and/or lipase enzyme, and lactic acid bacteria (Asehraou *et al.* 1992). On the other hand, significant fluctuations were observed in Gemlik dry-salted style (sele) olives on a monthly basis. This can be ascribed to the changes in ambient conditions in accordance with microorganism requirements. After pasteurization, the microbial load in the non-oiled group was found to be lower than the oiled group. This is attributed to the heat insulating characteristic of oil which may have reduced the effectiveness of the applied heat treatment.

In their research, Ayhan and Ergen (2006), and Özer *et al*. (2003) also obtained similar results. According to the results stated by Asehraou *et al.* (1992) total bacteria count was $2{,}3x10^6$ cfu/g, total fecal coliform count was $9{,}1x10^3$ cfu/g, and total mold/yeast count was $2{,}1x10^6$ cfu/g in Moroccan style olive produced with dry-salting (sele) method. Panagou (2006) stated that, the microbial flora of dry-salted Greek olives packaged with different applications consisted mainly of yeasts during storage period; and due to the low aqueous activity and high salt content, no sign of lactic acid bacteria, enterobacteria, pseudomonas and *Staphylococcus aureus* development was observed.

Table 4. The changes of the microbiological in Gemlik style sele olives during the storage.

			0th day	**1st month**	**2nd month**	**3th month**	**4th month**	**5th month**	**6th month**
Total mesophilic aerobic bacteria count (cfu/g)	Control	Oiled	$2,8x10^3$	$3,6x10^2$	$1,2x10^5$	$9,8x10^3$	$2,5x10^3$	$4,7x10^4$	$3x10^4$
		Non-oiled	$1,6x10^4$	$9,7x10^3$	$2,7x10^3$	$6,5x10^3$	$1,6x10^4$	$1,3x10^4$	10^2
	Pasteurized	Oiled	$2,1x10^2$	$2,4x10^3$	$2,5x10^4$	$1,3x10^2$	$2,5x10^2$	$3,8x10^3$	$2x10^3$
		Non-oiled	10	30	$2x10^4$	$5,1x10^3$	$3,9x10^2$	$1,2x10^4$	$2,6x10^3$
Total yeast-mold count (cfu/g)	Control	Oiled	$1,1x10^3$	70	$2,3x10^4$	$3,6x10^3$	<1	$1,6x10^4$	$2,7x10^4$
		Non-oiled	10^4	$9,4x10^3$	$5,2x10^2$	$3,8x10^3$	$3,5x10^3$	$2,7x10^3$	60
	Pasteurized	Oiled	$1,8x10^2$	$2,4x10^2$	$3,5x10^3$	20	<1	$1,9x10^3$	$1,8x10^3$
		Non-oiled	<1	50	$3x10^3$	$3,1x10^3$	10^2	$5,9x10^3$	$2,2x10^3$
Lactic acid bacteria count (cfu/g)	Control	Oiled	$1,2x10^3$	$1,2x10^2$	$1x10^5$	$8,6x10^3$	$1,7x10^3$	$7,3x10^3$	$1,1x10^4$
		Non-oiled	$2,3x10^3$	$4,5x10^3$	$1,5x10^3$	$5,7x10^3$	$1,1x10^4$	$1,6x10^3$	40
	Pasteurized	Oiled	10^2	$1,2x10^2$	$5,4x10^3$	70	$2,1x10^2$	$2,2x10^3$	<1
		Non-oiled	<1	10	$2x10^4$	$1,6x10^3$	$3,4x10^2$	$3,2x10^3$	<1
Coliform bacteria count (cfu/g)	Control	Oiled	<1	<1	<1	<1	<1	<1	<1
		Non-oiled	<1	<1	<1	<1	<1	<1	<1
	Pasteurized	Oiled	<1	<1	<1	<1	<1	<1	<1
		Non-oiled	<1	<1	<1	<1	<1	<1	<1

The microbiological results obtained in the present study differ from those found by Panagou (2006). High salt concentration (40%) used in Greek dry-salted style olive production process an adverse environment for various microorganisms including lactic acid bacteria. The use of low salt concentration (15%) in the present research allowed survival of lactic acid bacteria beside other microorganisms.

Chemical changes in olives during storage period

The changes in dry matter, acidity (as lactic acid), pH, salt, reducing sugar, oil, protein and oleuropein (as ABS) in Gemlik dry-salted style (sele) olives during the storage are shown in Table 5. During processing, olives lose their water and water-soluble content, which results in a decrease in their net weight as compared to fresh olive. Dry matter amount used in this study was found as 52,70% in the fresh olive samples (Table 1), and as 76,71% at the end of fermentation (Table5). The lowest dry matter amount was found as 70,57 % in the 2nd month of storage in pasteurized-oiled Gemlik dry-salted style (sele) olive, and the highest value was found as 76,99% in the 1st month in oiled control group Gemlik dry-salted style (sele) olive. Time dependent water loss did not occur with a homogeneous distribution among olives, which may have resulted in a fluctuation in dry matter values, although they were not at significant levels. Panagou *et al.* (2002) reported that throughout the storage period the water content of dry-salted (sele) olives decreased to 21.7% from 23,6%, most of which was observed in olives stored at 20 ^{o}C. In their research Cardoso *et al.* (2008) used dry-salting method (40 g salt/100 g olive) to produce Greek Thassos style olive, and they found the dry matter ratio as 54,6% for raw olive and 73,9% after fermentation. In this research, raw olive dry matter ratio was slightly lower and group average was higher than those found by Cardoso *et al.* (2008). This is ascribed to the differences in a variety of applications used during these studies. As stated by Panagou *et al.* (2002) dry-salted olives underwent water loss throughout the storage period; however varying values were observed instead of a continuous increase in dry matter values.

In the research data received following pasteurization, the lowest acidity value belonged to pasteurized non-oiled Gemlik dry-salted style (sele) olives sample as 0,787%. No significant difference ($p<0,05$) was observed between acidity values among the groups in the 1st, 3th, 5th and 6th months of storage. At the end of the storage period acidity varied within the range of 0,655-0,750 % and no significant difference ($p<0,05$) was reported among all olive groups.

In the first four months of storage pH values of all samples were statistically similar ($p<0,05$). The highest values (5.19-5.26) were observed within the first month (Table 5). In the 5th month, no statistical difference was observed between the oiled and non-oiled control groups, whereas the average value for pasteurized non-oiled Gemlik dry-salted style (sele) olives differed from others. Balatsouras (2004) found the pH of fermented table olive as 4,5-5,5. Panagou (2006) reported the change in pH to be within 4,9-5,1 throughout the fermentation of cultivar olive, whereas Panagou and Katsaboxakis (2006) reported an average pH value of 4,31 for table olive samples. In Moroccan style olives pH values varied between 4,90-6,33 (Asehraou *et al.* 1992). In the present study, the changes in pH value throughout the storage period showed similarities with those reported by Panagou *et al.* (2002), Panagou (2006) and Panagou and Katsaboxakis (2006).

Table 5. The changes of in dry matter, titratable acidity (as lactic acid), pH, salt, reducing sugar, oil, protein and oleuropein (as ABS) in Gemlik dry-salted style (sele) olives during the storage.

			0th day	1st month	2nd month	3th month	4th month	5th month	6th month
% Dry matter	Control	Non-oiled	76,71 a ±0,59	75,86 a ±0,72	76,93 a ±0,96	75,50 a ±0,66	77,07 a ±0,25	73,54 ab ±0,17	75,04 ab ±0,12
		Oiled	76,42 a ±0,11	76,99 a ±0,28	76,58 a ±1,19	75,77 a ±0,21	75,90 a ±0,53	76,32 a ±0,14	75,24 a ±0,18
	Pasteurized	Non-oiled	72,65 b ±2,84	72,37 b ±2,54	70,57 b ±2,96	73,46 a ±2,78	71,22 b ±2,68	72,82 b ±2,28	72,23 b ±2,45
		Oiled	76,88 a ±1,26	74,88 ab ±1,54	72,53 b ±1,23	73,77 a ±1,36	75,27 a ±1,69	74,97 ab ±2,20	74,28 ab ±2,04
% Acidity	Control	Non-oiled	0,831 b ±0,00	0,652 a ±0,06	0,703 b ±0,00	0,658 a ±0,00	0,748 ab ±0,00	0,651 a ±0,00	0,655 a ±0,00
		Oiled	0,827 b ±0,00	0,621 a ±0,00	0,788 a ±0,04	0,684 a ±0,05	0,744 ab ±0,02	0,655 a ±0,00	0,750 a ±0,00
	Pasteurized	Non-oiled	0,787 c ±0,00	0,544 a ±0,12	0,710 b ±0,05	0,684 a ±0,05	0,712 b ±0,05	0,714 a ±0,06	0,685 a ±0,11
		Oiled	0,865 a ±0,01	0,542 a ±0,01	0,655 b ±0,00	0,655 a ±0,00	0,808 a ±0,05	0,685 a ±0,05	0,745 a ±0,01
% pH	Control	Non-oiled	5,03 a ±0,01	5,19 a ±0,00	5,07 a ±0,01	5,03 a ±0,00	5,02 a ±0,01	4,88 b ±0,06	4,82 b ±0,04
		Oiled	5,02 a ±0,01	5,23 a ±0,01	5,04 a ±0,00	4,95 a ±0,01	5,02 a ±0,01	4,88 b ±0,01	4,86 ab ±0,01
	Pasteurized	Non-oiled	5,03 a ±0,14	5,26 a ±0,08	5,05 a ±0,08	4,96 a ±0,14	4,96 a ±0,05	4,99 a ±0,06	4,97 a ±0,06
		Oiled	4,99 a ±0,12	5,22 a ±0,05	5,06 a ±0,05	4,96 a ±0,07	5,00 a ±0,10	4,94 ab ±0,07	4,86 ab ±0,11
% Salt	Control	Non-oiled	5,84 a ±0,00	5,99 a ±0,08	6,00 a ±0,08	5,44 ab ±0,00	5,75 a ±0,00	6,13 a ±0,07	6,67 a ±0,00
		Oiled	5,65 b ±0,00	5,24 b ±0,00	5,76 a ±0,00	4,40 b ±0,07	4,35 b ±0,07	4,37 b ±0,00	4,84 c ±0,00
	Pasteurized	Non-oiled	5,27 c ±0,13	4,40 c ±0,44	4,86 b ±0,35	4,47 b ±1,01	4,45 b ±0,51	4,46 b ±0,88	4,24 d ±0,35
		Oiled	5,32 c ±0,05	4,43 c ±0,52	5,68 a ±0,23	5,75 a ±0,55	5,23 a ±0,64	4,98 b ±0,76	5,65 b ±0,51
% Reducing	Control	Non-oiled	1,75 a ±0,01	1,51 ab ±0,05	1,72 a ±0,01	1,68 a ±0,01	1,63 ab ±0,01	1,49 a ±0,00	1,63 ab ±0,00
		Oiled	1,63 a ±0,01	1,70 a ±0,01	1,67 a ±0,02	1,57 a ±0,01	1,52 b ±0,00	1,77 a ±0,00	1,44 b ±0,00

	Pasteurized	Non-oiled	1,43 [a] ±0,34	1,30 [b] ±0,31	1,50 [a] ±0,69	1,69 [a] ±0,47	1,59 [ab] ±0,36	1,78 [a] ±0,48	1,70 [ab] ±0,32
		Oiled	1,74 [a] ±0,12	1,37 [b] ±0,15	1,71 [a] ±0,11	1,80 [a] ±0,22	1,91 [a] ±0,12	1,90 [a] ±0,20	1,88 [a] ±0,27
% Oil	**Control**	Non-oiled	35,54 [a] ±2,15	35,84 [a] ±3,53	38,80 [a] ±2,14	35,61 [a] ±2,81	37,06 [a] ±0,62	35,70 [a] ±3,25	37,49 [ab] ±4,30
		Oiled	35,54 [a] ±2,06	35,79 [a] ±1,39	37,39 [a] ±2,46	37,15 [a] ±1,38	34,86 [a] ±1,26	34,58 [a] ±0,69	34,55 [b] ±3,30
	Pasteurized	Non-oiled	37,68 [a] ±2,50	39,03 [a] ±7,58	35,41 [a] ±3,87	34,46 [a] ±2,24	37,57 [a] ±1,08	38,07 [a] ±3,9	40,01 [a] ±1,48
		Oiled	39,63 [a] ±3,48	36,47 [a] ±1,36	37,33 [a] ±1,72	36,01 [a] ±3,02	35,29 [a] ±5,42	36,91 [a] ±1,74	37,08 [ab] ±0,45
% Protein	**Control**	Non-oiled	2,33 [a] ±0,06	2,54 [a] ±0,04	2,32 [a] ±0,01	2,63 [a] ±0,02	2,28 [a] ±0,05	2,31 [ab] ±0,05	2,28 [b] ±0,02
		Oiled	2,51 [a] ±0,03	2,53 [a] ±0,06	2,37 [a] ±0,02	2,31 [b] ±0,07	2,50 [a] ±0,02	2,40 [ab] ±0,03	2,63 [a] ±0,02
	Pasteurized	Non-oiled	2,41 [a] ±0,14	2,49 [a] ±0,20	2,26 [a] ±0,06	2,38 [b] ±0,07	2,33 [a] ±0,22	2,46 [a] ±0,16	2,25 [b] ±0,10
		Oiled	2,43 [a] ±0,13	2,47 [a] ±0,09	2,38 [a] ±0,13	2,59 [a] ±0,04	2,44 [a] ±0,19	2,29 [b] ±0,04	2,58 [a] ±0,23
Oleuropein (ABS)	**Control**	Non-oiled	0,613 [a] ±0,028	0,591 [a] ±0,010	0,611 [a] ±0,042	0,559 [a] ±0,038	0,558 [a] ±0,035	0,533 [a] ±0,038	0,522 [a] ±0,033
		Oiled	0,585 [a] ±0,021	0,582 [a] ±0,018	0,565 [ab] ±0,037	0,552 [a] ±0,029	0,548 [a] ±0,030	0,547 [a] ±0,038	0,507 [a] ±0,032
	Pasteurized	Non-oiled	0,567 [a] ±0,069	0,487 [b] ±0,037	0,516 [bc] ±0,021	0,483 [b] ±0,018	0,503 [a] ±0,023	0,505 [a] ±0,021	0,478 [a] ±0,026
		Oiled	0,560 [a] ±0,101	0,484 [b] ±0,035	0,482 [c] ±0,045	0,486 [b] ±0,026	0,494 [a] ±0,019	0,515 [a] ±0,024	0,484 [a] ±0,026

Control group non-oiled Gemlik dry-salted style (sele) olives exhibited the highest salt content during the storage period, with exception of the third month. The monthly changes observed in the salt content can be attributed to the non-homogeneous distribution of coarse grained rock salt used in the production of Gemlik dry-salted style (sele) olives. Also, the water loss that occurred at varying levels throughout the storage period of Gemlik style olive may have resulted in a change in the salt content of fruit. Salt content in table olives were found as 7,4% by Panagou (2006), as 8,0% by Panagou and Katsaboxakis (2006) and 4-10% by Balatsouras (2004). Panagou *et al.* (2002) stated that, the salt content stored at 4 ºC varied between 7,2-7,6 % on a monthly basis, and an increase from 7,4% to 8,2% was detected at 20ºC. As stipulated in Turkish Food Codex Notification for Table Olive (Notification no:2014/33), the highest allowable salt content for olives produced without heat treatment is 8%, and the highest allowable salt content for pasteurized olives is 6% (Anonymous 2014). Accordingly, the results obtained in the present research are in compliance with the standards of Turkish Food Codex.

The average reducing sugar content was found as 2,17% (Table 5) in olive flesh prior to fermentation, and as 1,75% after fermentation. Such decrease in reducing sugar content is ascribed to the reducing sugar consumption by microorganisms, particularly by lactic acid bacteria and fermentative yeasts throughout the

fermentation process. No significant difference ($p<0,05$) was observed among the samples in the 2nd, 3th and 5th months of fermentation. Reducing sugar values varied between 1,30-1,70% within the 1st month of storage and the highest values were observed in the control group. In the last month of storage, the lowest value was observed in the oiled control group (1,44%) and the highest value was observed in the pasteurized oiled group. The lowest reducing sugar content was found as 1,30% in the 1st month in pasteurized non-oiled Gemlik dry-salted style (sele) olives, and the highest value was found as 1,91% in Gemlik dry-salted style (sele)olives in the 4th month. Reducing sugar content constantly varied on a monthly basis (Table 5). Panagou (2006) investigated the fermentation of black table olive and reported an increase from 2,0% to 2,2% in reducing sugar content. In the same research this change was attributed to the water loss within the fruit flesh throughout the process. In another study, reducing sugar content was reported to be within 2-2,25% interval for fermented black table olives (Balatsouras 2004). In the present research, however, reducing sugar content in black table olives were reported to be lower at the end of 6 months storage period. This is mainly attributable to the variety and differences among the used applications.

Fat content was found as 29,89% in fresh olive, and as 35,54% after fermentation. The water loss incurred throughout the fermentation process resulted in a proportional increase in the fat content. Fat content values varied between 34,46-40,01% throughout the storage period. No significant difference was observed between the pasteurized samples and the control groups with the exception of the last month of storage ($p<0,05$). A statistical difference was observed between oiled Gemlik dry-salted style (sele) olives and pasteurized non-oiled Gemlik dry-salted style (sele) olives (Table 5). The differences observed in the fat content throughout the storage process are ascribed to the changes in the dry matter content of olive samples. Fat content was reported 35-39% after the physico-chemical analyses conducted by Balatsouras (2004) on dry-salted black table olive. These values are in good agreement with the fat content values given in the present research.

2,02% average protein content in the raw material (Table 1) became 2,33% after fermentation (Table 5). Protein content within the fruit flesh is transferred to brine during fermentation, thus the nitrogen requirement of microorganisms is fulfilled (Balatsouras 1966). Accordingly, the increase in the protein content of fruit flesh is ascribed to the increase in the dry matter content which resulted from the water loss of olive during dry salt-curing process. In the first 3 months of storage, no significant difference was found between the samples ($p<0,05$), in the 4th month similarities were observed among the obtained values and the highest value belonged to control-oiled group with 2,63%. The lowest value during storage was observed as 2,25% in pasteurized non-oiled Gemlik dry-salted style (sele) olives in the 6th month. In the last month, statistical similarities were observed between non-oiled (control and pasteurized) groups and oiled (control and pasteurized) groups.

Average oleuropein absorbance was found as 1,001 in the raw material (Table 1) and as 0,613 absorbance after fermentation (Table 5). Oleuropein is hydrolyzed during the fermentation of olive. Accordingly, the absorbance value for oleuropein was found to be low for the fermented olive samples. No statistical difference was observed among the values from the 1st, 4th, 5th and 6th months ($p<0,05$). In the sixth month of storage, the highest value was found in control non-oiled group (0,522) and the lowest value belonged to pasteurized non-oiled group (0,478). Although fluctuating values were received throughout the storage period, a general decrease was observed in oleuropein absorbance. This is chiefly attributable to a certain amount of water loss by olive in addition to the hydrolysis of oleuropein.

The organoleptic evaluation results of the olives during storage

The organoleptic evaluation results of Gemlik dry-salted style (sele)olives are shown in Table 6. According to the findings of the first organoleptic evaluation performed immediately after pasteurization and at the end of storage, olive samples were quite relished by the panelists. The samples with the most favored fruit color belonged to non-oiled control group, and the least favored fruit color belonged to non-oiled pasteurized group. No sign of separation was observed on the samples. Panelists reported an easy flesh/pit separation for the studied samples. Saltiness values were found to be at normal levels, and non-oiled Gemlik dry-salted style (sele) olives were reported to be less salty than others. According to the received bitterness scores, all samples were “slightly bitter”. In general, the most favored sample was pasteurized oiled Gemlik dry-salted style (sele) olive, and the least favored sample

was pasteurized non-oiled olive, due to its dull appearance. Drying surface of fruits gives them a dull appearance, whereas oiled samples look blacker and brighter than their actual color with a more appealing appearance.

Softening was observed on some of the samples during the storage period. As from the 3th month, mold development was observed particularly on non-pasteurized samples. In general, skin separation was not observed on olive samples. The salt content used in the production of Gemlik dry-salted style (sele) olives tightened the fruit skin, which in turn prevented negative organoleptic experiences such as separation of the skin. Fruit pit was in general easily separable from the flesh, and fruit samples preserved this attribute throughout the storage period. This specific characteristic of Gemlik cultivar olive evidently increases the organoleptic quality of Gemlik dry-salted style (sele) olives. According to the panelists, olive samples tasted slightly bitter in the first months of storage. This situation was observed within the 2nd month of storage for non-oiled pasteurized Gemlik dry-salted style (sele) olives. In the 4th month, non-oiled Gemlik dry-salted style (sele) olive control group received the highest score with the least bitter samples. In the following periods of storage, the water loss in fruits in addition to de-bittering factors may have been effective on the change of score. Also, storage temperature is reportedly effective on bitterness, which arises from high fat content of olives (Balatsouras 1990). The sense of unpleasant odor or taste, also known as rancidity or bitterness, arises from production of free fatty acids as a result of hydrolysis of fats by lipase enzyme. Lipase is produced by *Debaryomyces hansenii* which is isolated from olive (Papagora *et al.* 2013). During organoleptic analysis, dry-salted (sele) olives received favorable opinions from the panelists in general. In the present research, application of pasteurization was effective in preserving the organoleptic quality of olives and extending their shelf life. Also, preservation of Gemlik dry-salted style (sele) olive in olive oil is suggested as a healthy alternative for consumption.

CONCLUSIONS

The following results are obtained from the present research carried out with various applications to extend the preservation period of Gemlik dry-salted style (sele) olives which is not commercially preferred as a result of its high wastage rate and short shelf life.

a. Owing to its thin peel and high flesh to pit ratio, production of Gemlik cultivar olive as dry-salted (sele) table olive is found suitable from organoleptic point of view.

b. The salt content (ratio) used in Gemlik cultivar olive has a highly important role in the fermentation stage and in terms of product quality. 15% salt was used in traditionally produced “sele” style Gemlik cultivar olive to keep the salt content at an acceptable level.

c. With 15% salt concentration, predominant flora consisted of yeasts, and development of lactic acid bacteria, which is effective on fermentation, was also observed at a certain rate.

d. The microbial load in the non-oiled group was found to be lower than the oiled group after pasteurization. This can be ascribed to the heat-insulating characteristic of oil which may have inhibited the effectiveness of heat treatment.

e. Towards the end of storage period, mold development was observed on some of the samples in the pasteurized non-oiled group. Accordingly, pasteurization can be regarded as an effective yet insufficient method in extension of shelf life.

f. In organoleptic analysis, all sample groups were relished and favored, although, with their appealing bright appearance, oiled groups were preferred more than non-oiled groups with dull appearance. However, the slightly rancid taste of oiled samples towards the end of storage period was found to direct consumers to non-oiled alternatives.

g. The low salt content of Gemlik dry-salted style (sele) olives in addition to preservation in olive oil and the attempts to extend the shelf life with pasteurization will enable a healthier consumption of this product.

Table 6. The changes of the organoleptic in Gemlik dry-salted style (sele) olives during the storage.

		0th day	1th month	2th month	3th month	4th month	5th month	6th month
Control (Non-oiled)	Fruit color	4,8±0,63	3,8±1,03	4,6±0,84	3,2±0,63	3,8±1,40	3,2±1,14	3,0±1,33
	Skin separation	1,8±0,42	2,0±0,00	1,8±0,42	1,8±0,42	2,2±0,32	1,9±0,32	1,9±0,32
	Flesh/pit separation	5,0±0,00	5,0±0,00	4,8±0,63	4,4±0,97	4,4±0,97	5,0±0,00	4,6±0,84
	Saltiness	3,2±1,99	4,2±1,69	5,0±0,00	5,0±0,00	3,8±1,93	3,8±1,93	3,4±2,07
	Bitterness	4,0±1,41	4,2±1,03	4,0±1,05	3,8±1,03	5,0±0,00	4,6±0,84	4,6±0,84
Control (Oiled)	Fruit color	4,2±1,69	5,0±0,00	5,0±0,00	5,0±0,00	5,0±0,00	5,0±0,00	4,6±1,26
	Skin separation	1,8±0,42	1,9±0,32	1,7±0,48	1,8±0,42	2,2±0,32	2,0±0,00	1,9±032
	Flesh/pit separation	4,6±0,84	4,8±0,63	5,0±0,00	4,8±0,63	5,0±0,00	4,8±0,63	4,6±084
	Saltiness	3,4±1,84	3,0±2,11	3,8±1,93	4,6±1,26	4,6±1,26	4,8±0,63	4,6±1,26
	Bitterness	3,8±1,69	3,8±1,40	4,4±0,97	4,2±1,03	4,4±0,97	4,4±0,97	4,6±0,84
Pasteurized (Non-oiled)	Fruit color	3,8±1,03	3,2±0,63	3,8±1,03	2,8±0,63	4,0±1,41	3,8±1,03	2,8±0,63
	Skin separation	1,9±0,32	2,0±0,00	1,7±0,48	1,9±0,32	2,1±0,42	1,8±042	1,9±0,32
	Flesh/pit separation	5,0±0,00	4,8±0,63	4,8±0,63	4,6±1,26	4,8±0,63	4,6±0,84	5,0±0,00
	Saltiness	4,0±1,70	4,4±1,35	4,4±1,35	4,8±0,63	5,0±0,00	4,4±1,35	4,0±1,70
	Bitterness	3,8±1,03	4,0±1,41	3,4±1,26	4,0±1,05	4,4±0,97	3,4±1,58	4,6±0,84
Pasteurized (Oiled)	Fruit color	4,6±1,26	5,0±0,00	4,8±0,63	5,0±0,00	4,8±0,63	4,2±1,69	4,2±1,40
	Skin separation	1,7±0,48	1,8±042	1,8±0,42	1,9±0,32	2,1±0,42	1,9±0,32	1,9±0,32
	Flesh/pit separation	4,8±0,63	4,8±0,63	5,0±0,00	5,0±0,00	5,0±0,00	5,0±0,00	4,6±0,84
	Saltiness	3,6±1,90	3,4±2,07	3,8±1,93	4,0±1,70	5,0±0,00	4,4±1,35	4,2±1,69
	Bitterness	4,0±1,05	4,4±0,97	4,4±0,97	4,2±1,03	4,4±0,97	4,6±0,84	4,4±0,97

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