

## Effect of Pretreatments on Microbial Growth and Sensory Properties of Dry-Salted Olives

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### ABSTRACT

The effect of various washing solutions (acetic acid, lactic acid, and chlorine dioxide) and NaCl concentrations (2.5, 5.0, and 10.0%) on the stability of dry-salted olives (cultivars Gemlik and Edincik) during storage was studied. Vacuum-packed olives were stored at 4°C for 7 months and monitored for microbiological changes that occurred in the dry-salted olives during the dry-salting process and for their stability during storage. Microbial populations were enumerated using pour plating (for aerobic plate counts) and spread plating (for counts of lactic acid bacteria and yeasts and molds). Aerobic plate counts were <2.5 log CFU/g for olive samples washed in chlorine dioxide at all NaCl concentrations. At 4°C, the population of yeasts and molds increased steadily during the shelf life in Gemlik olive samples washed with all of the solutions, except chlorine dioxide, whereas yeast and mold counts in Edincik olives decreased depending on the increase in salt concentration. Therefore, different combinations of organic acids, NaCl, and vacuum packaging can be successfully used to control the growth of yeasts and molds in these olives. The combination of vacuum sealing (with a 10-ppm chlorine dioxide wash) and storage at 4°C was the most effective approach for controlling the growth of lactic acid bacteria and yeasts and molds. Members of the sensory panel considered saltiness to be appropriate at 2.5 and 5.0% NaCl. Softness and bitterness scores increased with reduced NaCl concentrations, but rancidity and hardness scores increased as NaCl concentration increased.

Table olives are an important fermented food category, with an estimated worldwide production of 2,345,000 tons during the 2010 to 2011 season (31). Various types of table olives are produced with industrial treatments, e.g., green olives treated with alkali (Spanish style), ripe olives treated with alkaline oxidation (California style), and untreated or directly brined olives (green, turning color or naturally black) (6, 16, 25, 38, 51). Table olives prepared by traditional processes (e.g., dry salted or cracked) can be found in local markets according to consumers' demands (16). The most common two Turkish olive varieties, Gemlik and Edincik (or Edincik-Su), constitute the majority of the olive production in Turkey and are best suited for producing natural black olives for use as table olives. One type of black olive is prepared as either fermented olives (Gemlik style) or dry-salted olives (Sele style). Dry salting is also used in the Mediterranean region (Greece and some North African countries) for the production of naturally preserved black olives (18, 19). Table olives may be processed in many other ways according to fermentation conditions of the dry-salting processes (5, 19, 25, 37, 38, 46). Modern food processing technologies often rely on nonthermal

processes to provide food products that are microbiologically safe and stable (43).

Sodium chloride (NaCl) has long been employed in the preservation of food commodities and as a flavoring agent to enhance the organoleptic properties of food (25). The preparation of fermented vegetables in particular relies on the use of common NaCl as the main ingredient of the brine to reduce the water activity, increase the ionic strength of the solution, reduce the solubility of oxygen in water, and inhibit spoilage and pathogenic microbiota, thus ensuring the microbiological safety of the final product during storage (2).

For the production of dry-salted olives (basket style), the olives are harvested in December when they are fully mature and completely black. This traditional processing method consists of placing the olives in a basket, bag, or plastic container with coarse NaCl (4 to 10%, wt/wt). The container is turned over every other day. Because of the high osmotic pressure exerted by the NaCl (curing or desiccating agent), the olives lose water and other solutes, including much of the bittering agent oleuropein, and become gradually debittered and wrinkled (16, 28, 37). The low water activity (0.75 to 0.85) and high NaCl content of the flesh (4 to 10 g/100 g) of the dry-salted olives can ensure their safety during storage, although some spoilage microorganisms, mainly fungi, can grow under these

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conditions. Spoilage may occur when olives are not dried enough because of insufficient salt (16, 38, 39, 49).

Organic acids can be naturally present in fruits and vegetables or synthesized by microorganisms during fermentation. These acids have antimicrobial activity and are generally recognized as safe (1, 52). The antimicrobial activity of organic acids is attributed to pH reduction, depression of the internal pH of the microbial cells by ionization of undissociated acid molecules, chain length, cell physiology and metabolism, and disruption of substrate transport by altering the permeability of the cell's membrane (12, 34) or reduction of the proton motive force (22). Weak organic acids are more inhibitory for microorganisms than are strong acids. However, this effect depends on the type of acid used, the pH of the medium, and the concentration and temperature of the acid solution; the cell interior becomes acidified by becoming lipophilic (1, 43). The type of food product and the initial microbial load also affect antimicrobial activity. Acetic and lactic acid seem to be the best organic acids for the decontamination of food products (43).

To provide microbial safety, fruit and vegetables are passed through some processing steps, such as cleaning, trimming, peeling, coring, slicing, shredding, washing, and sanitizing. The objective of the washing and sanitizing steps is to remove soil and pesticide residues, reduce the microbial load, and lower the temperature of the product (9, 47). Chlorine-based agents often are used to sanitize the surfaces of products and to reduce microbial populations (20). These procedures reduce the initial microbiological load, thus reducing the rate of subsequent spoilage due to the presence of microbes and minimizing the populations of potential pathogens (24, 52). Chlorine dioxide ( $\text{ClO}_2$ ) is a strong oxidizing and sanitizing agent that can be used in aqueous or gaseous form to sanitize foods.  $\text{ClO}_2$  is gaining importance for maintaining the shelf life of fresh produce. The U.S. Food and Drug Administration (55) has allowed the use of aqueous  $\text{ClO}_2$  during the washing of fruits and vegetables at a residual level of 3 ppm. After the washing step, the fruits and vegetables should be rinsed with potable water (52). A maximum  $\text{ClO}_2$  concentration of 200 ppm should be used for sanitizing processing equipment (23).

Vacuum packaging is a common packaging method in the food industry. The product is placed in a pack that has low oxygen permeability, the air is evacuated, and the package is sealed. Because it is not possible to evacuate all of the air (0.3 to 3% may remain after sealing), the gaseous atmosphere of the vacuum package is likely to change during storage (because of microbial and product metabolism and gas permeation), so the atmosphere in the package may be different from the original atmosphere (32).

The objective of this study was to evaluate the effect of different concentrations of NaCl, organic acids (acetic and lactic acid), and commercial disinfectant ( $\text{ClO}_2$ ) on the microbiological, physicochemical, and organoleptic profiles of Gemlik and Edincik natural black olives during a dry-salting process and the stability of these olives during storage at 4°C. The results could be useful for the table olive industry to modify the existing processing scheme to obtain

a final product that has lower sodium concentrations but maintains its traditional properties.

## MATERIALS AND METHODS

**Raw material and sample treatment.** In December 2012, black olives (cultivars Gemlik and Edincik) were harvested in Erdek, Turkey and transported to the laboratory within 24 h. On arrival, the fruit was hand selected and separated into six lots of equal weight (approximately 50 kg each). For five lots, each lot of olives was immersed separately for 10 min in 100 liters of water containing 1 or 2% lactic acid (100366.2500, Merck, Darmstadt, Germany), 1 or 2% acetic acid (100063.2511, Merck), or 10 ppm of commercial disinfectant (DK-DOX 0.3% liquid  $\text{ClO}_2$ , Link Chemie AG, Dürrwiesen, Germany). The sixth lot (control) was dipped thoroughly in only distilled water, and a separator was used to keep the olives fully immersed. All olive lots were then dipped thoroughly in distilled water (olive:distilled water, 1:2, wt/vol, for 10 min) and left to dry at room temperature for 30 min. Olive samples of approximately 18 kg (from each of the lots prewashed with dipping solutions and distilled water) were each packed in a 30-liter polyvinyl chloride container with uniformly dispersed coarse salt at different NaCl concentrations (2.5, 5.0, and 10.0% per 18 kg of olives). To prevent fungal growth, the surface of the fruit was covered with a 2- to 3-cm layer of salt. During the process, the containers were kept at ambient temperature ( $20 \pm 1^\circ\text{C}$ ). Water and other solutes were drained through a hole at the bottom of the drum and collected during the dry-salting process (every 20 days). At the end of the dry-salting process (60 days), the six lots were each dipped separately in distilled water for 10 min to remove the excess NaCl and left to dry at room temperature for 20 min before packaging (18).

**Packaging.** After the dry-salting process, olives were vacuum packaged (VC 999/K12NA packing machine, Verpackungssysteme AG, Herisau, Switzerland) in FMXBK polyamide-polyethylene film ( $\text{PO}_2 = 15 \text{ cm}^3/\text{m}^2/24 \text{ h}$  at  $23^\circ\text{C}$  and 75% relative humidity; Flexopack S.A. Plastics Industry, Koropi, Greece). All of the samples, which had been dipped in different washing solutions (Table 1) or distilled water (control), were packaged separately and stored at  $4 \pm 1^\circ\text{C}$  (refrigerator temperature) for 7 months.

**Physicochemical analysis.** All pH values were determined with a microprocessor pH meter (HI 221, Hanna Instruments, Woonsocket, RI). The moisture and salt contents of the olive flesh were determined according to the method of Panagou et al. (39) and by the titrimetric method of the International Olive Council (31), respectively. All physicochemical analyses were conducted in triplicate.

**Microbiological analysis.** The level of microorganisms was determined for fresh olives and each type of treated sample. For each analysis, 25 g of each sample was weighed aseptically, placed in 225 ml of a sterile maximum recovery dilution solution (1 g/liter peptone plus 8.5 g/liter saline), homogenized in a stomacher (Masticator, IUL Instruments, Barcelona, Spain) for 60 s at room temperature ( $20 \pm 1^\circ\text{C}$ ), and used for the microbiological analyses. Decimal dilutions in the same maximum recovery dilution solution were prepared, and duplicates of 1 or 0.1 ml of at least three appropriate dilutions were pour plated or spread plated on the following agar media: plate count agar (CMO325, Oxoid, Basingstoke, UK) for aerobic microorganisms (AM), incubated at  $25^\circ\text{C}$  for 48 h; de Man Rogosa Sharpe medium (CM0361 supplemented with SR0222C, Oxoid) for lactic acid



TABLE 1. Abbreviations used for dry-salted olive samples

Run	Olive variety	Abbreviation	Washing solution concn	Salt concn (%)
1	Gemlik	GCS1	Control	2.5
2	Edincik	ECS1	Control	2.5
3	Gemlik	GCS2	Control	5.0
4	Edincik	ECS2	Control	5.0
5	Gemlik	GCS3	Control	10.0
6	Edincik	ECS3	Control	10.0
7	Gemlik	GL1S1	1% lactic acid	2.5
8	Edincik	EL1S1	1% lactic acid	2.5
9	Gemlik	GL1S2	1% lactic acid	5.0
10	Edincik	EL1S2	1% lactic acid	5.0
11	Gemlik	GL1S3	1% lactic acid	10.0
12	Edincik	EL1S3	1% lactic acid	10.0
13	Gemlik	GL2S1	2% lactic acid	2.5
14	Edincik	EL2S1	2% lactic acid	2.5
15	Gemlik	GL2S2	2% lactic acid	5.0
16	Edincik	EL2S2	2% lactic acid	5.0
17	Gemlik	GL2S3	2% lactic acid	10.0
18	Edincik	EL2S3	2% lactic acid	10.0
19	Gemlik	GA1S1	1% acetic acid	2.5
20	Edincik	EA1S1	1% acetic acid	2.5
21	Gemlik	GA1S2	1% acetic acid	5.0
22	Edincik	EA1S2	1% acetic acid	5.0
23	Gemlik	GA1S3	1% acetic acid	10.0
24	Edincik	EA1S3	1% acetic acid	10.0
25	Gemlik	GA2S1	2% acetic acid	2.5
26	Edincik	EA2S1	2% acetic acid	2.5
27	Gemlik	GA2S2	2% acetic acid	5.0
28	Edincik	EA2S2	2% acetic acid	5.0
29	Gemlik	GA2S3	2% acetic acid	10.0
30	Edincik	EA2S3	2% acetic acid	10.0
31	Gemlik	GDS1	Chlorine dioxide	2.5
32	Edincik	EDS1	Chlorine dioxide	2.5
33	Gemlik	GDS2	Chlorine dioxide	5.0
34	Edincik	EDS2	Chlorine dioxide	5.0
35	Gemlik	GDS3	Chlorine dioxide	10.0
36	Edincik	EDS3	Chlorine dioxide	10.0

bacteria (LAB), overlaid with the same medium and incubated at 30°C for 48 h; rose Bengal chloramphenicol agar (CM 549 supplemented with SR78, Oxoid) for yeasts and molds (YM), incubated at 25°C for 72 h or 5 days; cetrinide-fucidin-cephalorodine medium (CM 559 supplemented with SR 103, Oxoid) for *Pseudomonas* counts, incubated at 25°C for 48 h; and violet red bile glucose agar (1.10275.0500, Merck) for *Enterobacteriaceae* counts, incubated at 37°C for 24 h. The results for viable populations of microorganisms were expressed as log CFU per gram of olive pulp in fresh and packaged samples (18, 19). Experiments were conducted twice on different occasions with different dry-salted olives. Analyses were run in triplicate for each replicate ( $N = 3 \times 2$ ). The limits of detection were calculated by the International Union of Pure and Applied Chemistry method (35) for each microbiological analysis and were approximately 10 CFU/g for the media used.

**Sensory evaluation.** Sensory evaluation was conducted with 36 untrained assessors (16 women and 20 men) 24 to 45 years of age. The assessors indicated their sensory evaluation for each attribute using a 9-point hedonic scale of 1 to 9 (where 1 is "dislike extremely," 5 is "neither like nor dislike," and 9 is "like extremely"). Samples were identified by random numbers and

presented in individual trays to the assessors. The main quality attributes (Table 2) were divided into four groups corresponding to (i) color (black, black-brown, and brown), (ii) taste (salty, bitter, rancid, and off-flavor), (iii) texture and flesh stone (softness, pit-flesh detachment), and (iv) overall eating quality. All samples were tested at room temperature under normal daylight conditions. To reduce the likelihood of carryover, 2- and 10-min intervals were allowed between each sample and between each set of three samples, respectively. Each assessor was provided with filtered water and unsalted crackers and asked to cleanse her or his palate between tastings. Sensory attributes and their definitions were adapted from those of Değirmencioglu et al. (19).

**Statistical analysis.** The data were analyzed with the SPSS statistical package (version 16.0, SPSS, IBM, Chicago, IL). These analyses were conducted in triplicate for each sample. Viable populations of microorganisms were expressed statistically. The general linear model (repeated measures) and multiple comparison analyses were performed to estimate the significance ( $P < 0.05$ ) of the effects of washing treatments and salt concentrations on the microbiological quality and the sensory data for each attribute. The least significant difference test was used to determine differences between olives treated with the various washing solutions and salt



TABLE 2. Scores assigned for the evaluation of olive sensory attributes

Attribute	Score descriptions	
	Minimum (1)	Maximum (9)
Flesh color	Brown	Black
Flesh saltiness	Salty	Suitable
Flesh bitterness	Bitter	Not bitter
Flesh rancidity	Rancid	Not rancid
Flesh off-flavor	Dislike	Like
Flesh hardness	Stiff	Hard
Pit-flesh detachment	Easy	Hard
Overall eating quality	Dislike extremely	Like extremely

concentrations. The mean values were submitted to a multiple comparison test using the least significant difference procedure that allows the attributes that differentiate the samples to be determined.

## RESULTS AND DISCUSSION

**Microbiological changes in dry-salted olives.** Olives are one of the major agricultural products of Turkey. The most common two Turkish olive varieties, Gemlik and Edincik (or Edincik-Su), constitute the majority of the olive production in the region of the Sea of Marmara (northwestern Turkey) and the Aegean and are best suited for processing natural black olives. Gemlik is the common variety used for table olives and olive oil because of its thin skin, small stone, low acidity, high protein and sugar content, high fruit and oil yield, early maturation, low periodicity, and organoleptic qualities highly valued by consumers (7, 13, 48). The Edincik variety has large spherical fruits, a very small stone, low oil content, high moisture and sugar content, and firm flesh. The high moisture content gives the variety its name. Particular care must be taken during harvesting and processing of this variety because of its soft texture and very delicate skin and pulp (13, 54). In this study, the initial populations of AM, LAB, and YM of the olive samples were 5.38, 4.41, and 2.85 log CFU/g for Gemlik olives and 5.41, 4.41, and 2.80 log CFU/g for Edincik olives, respectively. After being packed in NaCl for 60 days, the counts of AM, LAB, and YM of control samples decreased to 3.26, 3.14, and 2.76 log CFU/g for Gemlik olives all salt concentrations and 3.26, 2.40, and 2.38 log CFU/g for Edincik olives of all salt concentrations, respectively. The data from the samples of all three NaCl concentrations (2.5, 5.0, and 10.0%) tested revealed that washing with distilled water (for 10 min) did not control the increases in AM and LAB counts as well as did washing with organic acids or ClO<sub>2</sub> before the dry-salting process. In raw olives after harvest, AM, LAB, and YM counts were detected at low levels. This finding was expected because the olives had been harvested and selected by hand, resulting in minimal damage. Similar microbiological results were reported in a survey of Moroccan dry-salted olives, in which the most common microbiota were YM (8). In the present study, the increase in AM counts in the Gemlik samples was not controlled in all washing

solutions during the dry-salting process and was reduced by higher NaCl concentrations; however, in the Edincik samples, AM counts were controlled by washing with 2.0% acetic acid (2.11 to 2.26 log CFU/g) or ClO<sub>2</sub> (2.06 to 2.08 log CFU/g) solutions at the end of the day 6, compared with AM counts of 2.20 log CFU/g (2.0% acetic acid) and 2.11 log CFU/g (ClO<sub>2</sub> solutions) at the day 0 of dry salting.

In this study, LAB counts in control samples increased in Gemlik olives from 3.14 log CFU/g initially to 3.36 to 3.54 log CFU/g and in Edincik olives from 2.40 log CFU/g initially to 3.25 to 3.34 log CFU/g for all salt concentrations; increases in salt concentrations did not make a significant difference ( $P > 0.05$ ). The effect of environmental stress factors on developing resistance of microorganisms to antimicrobials is of great importance. Therefore, the significant increase ( $P < 0.05$ ) in LAB counts from  $<1$  to 1.00 log CFU/g to 2.53 to 3.08 log CFU/g and 2.88–3.14 log CFU/g in Gemlik olives and from 1.00 log CFU/g to 2.10 to 2.27 log CFU/g and 2.24 to 3.14 log CFU/g in Edincik olives washed in lactic and acetic acid solutions (with 2.5% salt), respectively, during the dry-salting process was affected by microbial, intrinsic, and extrinsic factors such as resistance of strains, growth rate and phase, interaction with other microorganisms, cellular composition and status (injury), water activity, and pH and created a hurdle effect combined with the use of antimicrobial agents. No increase in LAB counts were found in the Edincik samples at 5.0 and 10.0% NaCl, with the exception of the control samples. All washing solutions were more effective than distilled water in Edincik olives than in Gemlik olives for restricting the growth of LAB, depending on the salt concentration.

Several studies have focused on the detection of yeasts that adhere to the surface of olives. Arroyo-López et al. (5) reported the presence of yeasts among the microbiota found on the surface of fresh mature olives, and the yeast species found depended on the maturation degree of the olives. However, yeast counts on the surface of fresh olives are generally low ( $<1$  log CFU/g), as was reported by Arroyo-López et al. (4) for Manzanilla-Aloreña olives during three consecutive seasons. The before and after washing with distilled water, YM counts were 2.85 and 2.76 log CFU/g (Gemlik olives) and 2.80 and 2.38 log CFU/g (Edincik olives), respectively. After 10 min of washing with lactic and acetic acid solutions, YM counts were 2.49 to 2.58 and 2.49 to 2.57 log CFU/g (Gemlik olives) and 1.04 to 1.23 and 1.11 to 1.23 log CFU/g (Edincik olives), respectively, whereas YM counts were  $<1$  log CFU/g in both olive cultivars washed with ClO<sub>2</sub>. During the dry-salting process, the YM counts of Gemlik olives increased slightly for the samples with 2.5% salt concentration that were washed in organic acid solutions. Washing with distilled water (control samples, Gemlik olives) was not effective for controlling the growth of YM at all three salt concentrations for 60 days. The various lactic and acetic acid solutions and higher NaCl concentrations were not effective for preventing the increase in YM counts in Gemlik olives ( $P > 0.05$ ), but washing in 1 and 2% acetic and lactic acid solutions for 10 min restricted the increase of YM counts at 5.0 and 10.0% NaCl



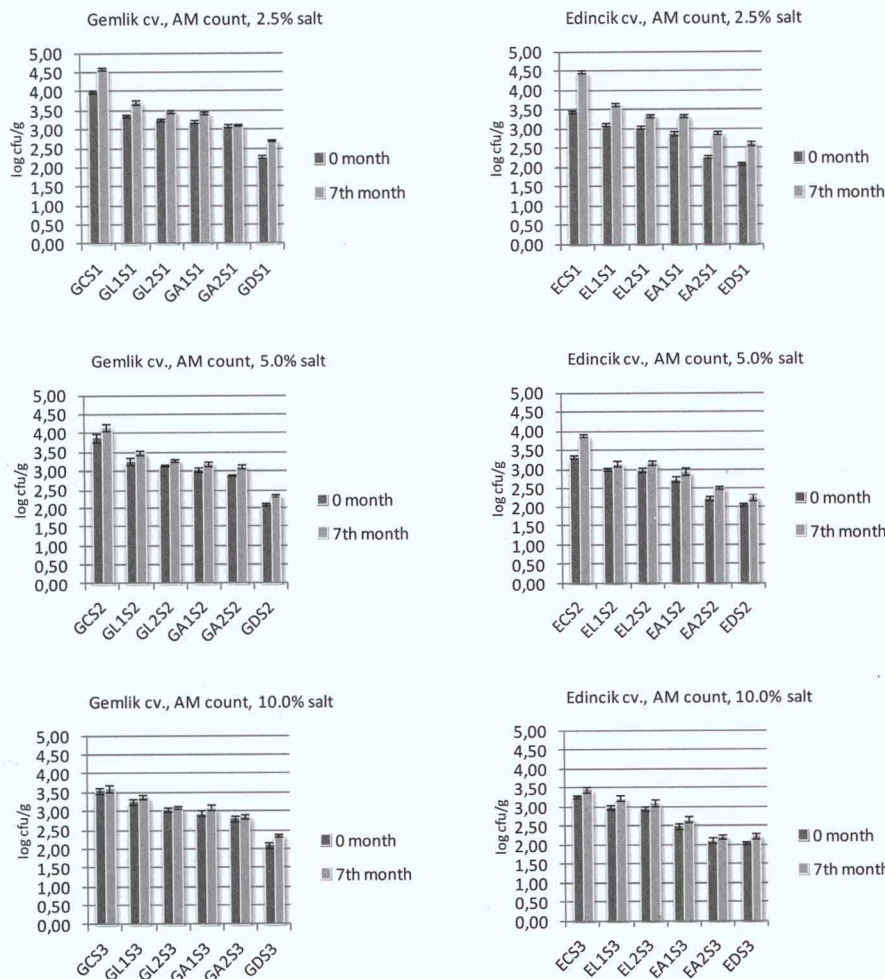


FIGURE 1. Aerobic microorganism (AM) counts in dry-salted olives before and after storage at 4°C. Data points are means of repeated measurements obtained from two replicates of dry-salted olive samples. The corresponding error bars represent the standard deviations. G, Gemlik olives; E, Edincik olives; C, control; L, lactic acid; A, acetic acid; D, disinfectant; S1, 2.5% NaCl concentration; S2, 5.0% NaCl concentration; S3, 10.0% NaCl concentration.

concentrations in Edincik olives. Organic acids may affect the integrity of the microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy metabolism, causing a bactericidal effect. An inhibition effect also can be created with a combination of organic acids and other preservation methods (42). Spoilage bacteria on fresh vegetables were inhibited by salt during production of sauerkraut, olives, and various pickles, whereas the fermenting LAB thrived (21). The preservative properties of NaCl are based on the direct toxicity of Cl<sup>-</sup>, removal of oxygen from the medium, sensitization of the organisms to CO<sub>2</sub>, and interference with the rapid action of proteolytic enzymes (50). In the present study, the NaCl concentrations in the olives determined which microorganisms would be dominant during the dry-salting process. The YM counts in Gemlik olives that were dry-salted after being washed in distilled water or organic acid solutions were not significantly different ( $P > 0.05$ ) regardless of the salt concentrations, and washing in ClO<sub>2</sub> solution (10 ppm) for 10 min was more effective than washing in the other solutions for both reducing initial LAB and YM counts and controlling the growth of LAB and YM during the debittering process during the 60-day study.

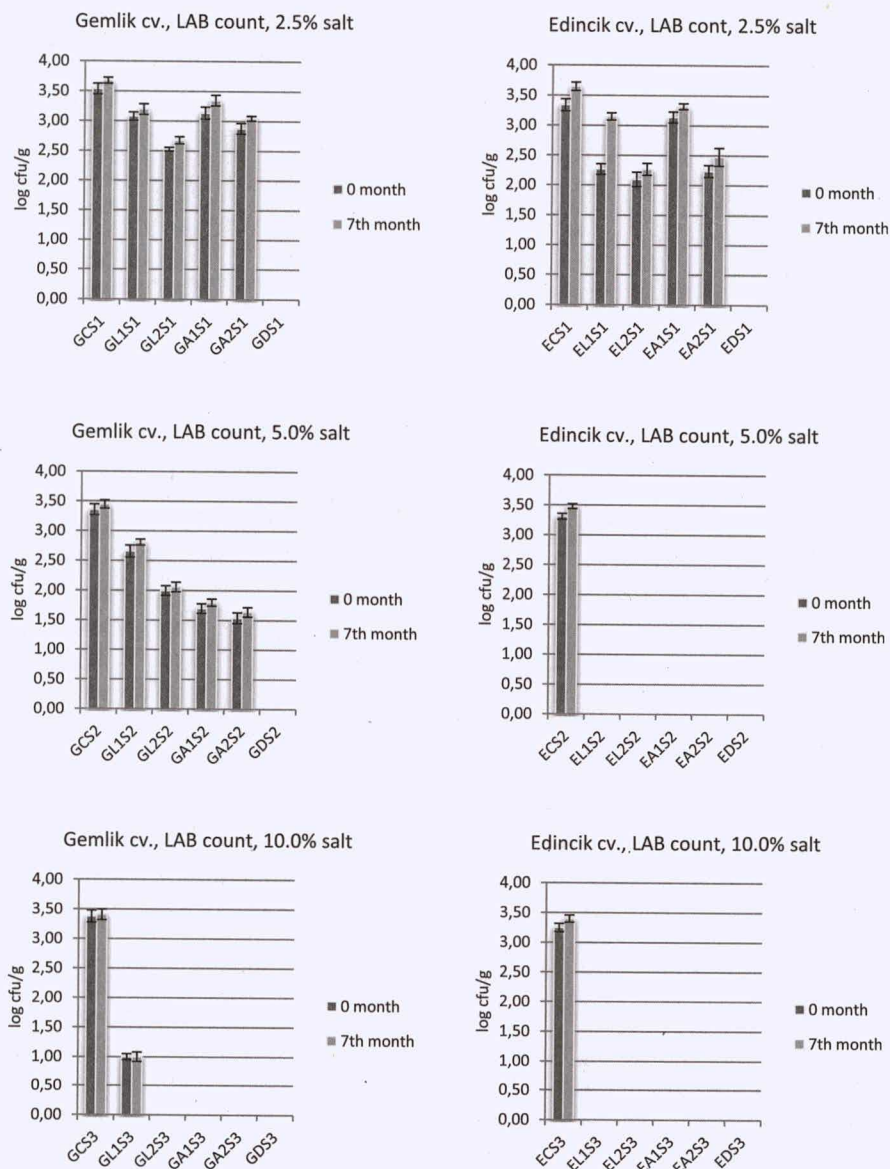
**Microbiological changes during storage of dry-salted olives.** The effects of NaCl concentration (2.5, 5.0, and 10.0%) on changes in AM, LAB, and YM counts of

dry-salted olives during storage are presented in Figures 1 through 3. The use of vacuum packaging for the shelf life of the fruit did not effectively control the increase in AM and LAB counts in the control samples that had not been washed in any chemical solutions (see Fig. 1) or dipped in the distilled water after the initial dry-salting process. The increases in AM and LAB counts were from 3.45 to 3.99 log CFU/g initially to 4.48 to 4.59 log CFU/g and from 3.34 to 3.54 log CFU/g initially to 3.65 to 3.68 log CFU/g, respectively, during the shelf life of 7 months at 4°C (Figs. 1 and 2). The AM and LAB counts were not affected ( $P > 0.05$ ) in the samples that had not been dipped in the ClO<sub>2</sub> solution. No significant differences in AM and LAB counts were found for either olive cultivar between the storage periods ( $P > 0.05$ ).

Panagou (36) found that the initial populations of AM and LAB changed only slightly and remained at about 7 log CFU/ml irrespective of packaging type (air, vacuum, or modified atmosphere) when a temperature of 20°C was maintained during the shelf life. Değirmencioğlu (18) found that the AM count remained stable until month 3 and then increased until the month 7 of shelf life and that vacuum treatment restricted the increase of LAB. In our study, the vacuum packaging did not significantly restrict the increase in LAB counts at low salt concentrations resulting from the differences of washing solutions, except for those olive washed in ClO<sub>2</sub>.



FIGURE 2. Lactic acid bacteria (LAB) counts in dry-salted olives before and after storage at 4°C. Data points are means of repeated measurements obtained from two replicates of dry-salted olive samples. The corresponding error bars represent the standard deviations. G, Gemlik olives; E, Edincik olives; C, control; L, lactic acid; A, acetic acid; D, disinfectant; S1, 2.5% NaCl concentration; S2, 5.0% NaCl concentration; S3, 10.0% NaCl concentration.



During storage, dry-salted olives are vacuum packed without brine in polyamide-polyethylene film. Yeasts and fungi are potential spoilage microorganisms because of their ability to grow in low water activity environments. Spoilage during storage also may occur when olives are not dry enough or not sufficiently salted. The growth of these organisms, mainly fungi, may negatively affect both the nutritional and the aesthetic value of the product. By the end of the shelf life studied, the total YM population increased from 3.02 to 3.25 log CFU/g initially to 3.60 to 3.65 log CFU/g in control samples at 2.5% salt concentration and 4°C. No significant differences were found throughout the storage period ( $P > 0.05$ ). Acetic acid solutions (1 and 2%) were more effective for controlling YM counts than were the other solutions, depending on the salt concentration, during the storage period (Fig. 3). The exception were the samples washed with  $\text{ClO}_2$ , which had YM counts of 2.85 and  $<1$  log CFU/g (Gemlik olives) and 2.80 and  $<1$  log CFU/g (Edincik olives) before and after washing, respectively. YM counts in these samples did not increase throughout the dry-salting and storage periods, in contrast

to samples that were dipped only in distilled water and stored at 4°C. Therefore, the  $\text{ClO}_2$  wash solution effectively inhibited or restricted YM growth in dry-salted samples at all NaCl concentrations. Although microorganisms entered the olives through cracks in the fruit, the  $\text{ClO}_2$  solution was able to sufficiently penetrate these areas. Dry-salted olives have low water activity and high NaCl content, which allows only salt-tolerant yeasts to grow (53). Panagou et al. (39) determined that yeast populations declined steadily throughout the storage period at 4°C. Panagou (36) also found that yeast populations in vacuum-packed olives started to decline when the experiments began, and the lowest count (3 log CFU/g) was reached after 150 days of storage at 20°C. Değirmencioğlu (18) established that chlorine treatment and modified atmosphere packaging (35%  $\text{CO}_2$  and 65%  $\text{N}_2$ ) to suppressed YM growth better than did vacuum packaging (1.22 log CFU/g, 6 months at 4°C). By comparison, in the present study, the YM count (Fig. 3) was lower at the end of the storage period than those reported by Değirmencioğlu (18), Panagou et al. (39), and Panagou (36).



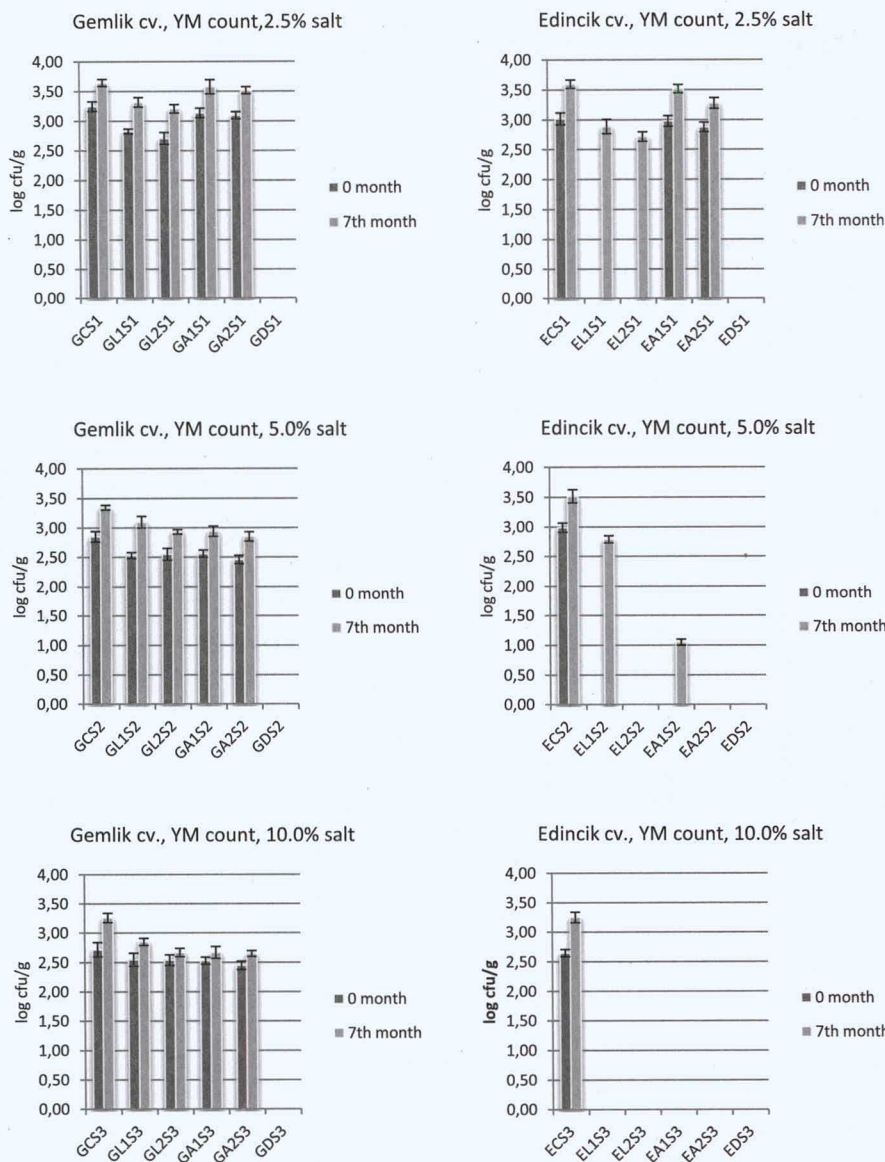


FIGURE 3. Yeast and mold (YM) counts in dry-salted olives before and after storage at 4°C. Data points are means of repeated measurements obtained from two replicates of dry-salted olive samples. The corresponding error bars represent the standard deviations. G, Gemlik olives; E, Edincik olives; C, control; L, lactic acid; A, acetic acid; D, disinfectant; S1, 2.5% NaCl concentration; S2, 5.0% NaCl concentration; S3, 10.0% NaCl concentration.

Panagou et al. (39) isolated some salt-tolerant yeast strains that grew in the presence of solutions of 15 g of NaCl per 100 g of water. The sample of dry-salted olives contained 2.1 to 9.7% NaCl. The results indicated that even when salt-tolerant yeast strains are present, the combination of ClO<sub>2</sub> (10 ppm) wash and vacuum packaging would effectively control YM growth on samples stored at 4°C. In the present study, we also evaluated the antimicrobial effectiveness of aqueous ClO<sub>2</sub> plus vacuum packaging, which is a more useful and less expensive olive packaging method for small companies compared with modified atmosphere packaging. The effect of vacuum packaging on microbial growth was impacted significantly by temperature and by ClO<sub>2</sub> washing.

Low temperature is an important factor for maintaining the microbial quality of food products. Low temperature during the shelf life of dry-salted olives is essential to control microbial growth and maintain sensory qualities. Betts et al. (11) suggested a synergistic effect between pH and NaCl at low temperatures that could be used for assessing the spoilage potential of new and existing product

formulations. Lowering the storage temperature and the use of washing solutions were more important factors than vacuum packaging for reducing microbial counts. Hurtado et al. (30) reported that higher oxygen concentrations more effectively retarded YM growth on the surface of control samples than did higher salinity. However, storage at a relatively low temperature is also essential for the successful processing of dry-salted olives. The conditions of low-temperature storage, vacuum packaging, and various salt concentrations in the present study restricted YM growth throughout the 7 months of shelf life. Consequently, the combination of treatment of olives with dry salting and a ClO<sub>2</sub> wash has the potential to increase the shelf life by slowing YM growth.

The microbiota of vegetables and fruits is made up largely of *Pseudomonas* spp., *Erwinia herbicola*, *Flavobacterium*, *Xanthomonas*, *Enterobacter agglomerans*, and various YM and LAB (15, 40, 41). *Pseudomonas* is not harmful to humans but is normally predominant and account for 50 to 90% of the microbial population on many vegetables (58). The levels of enteric bacteria on the surface

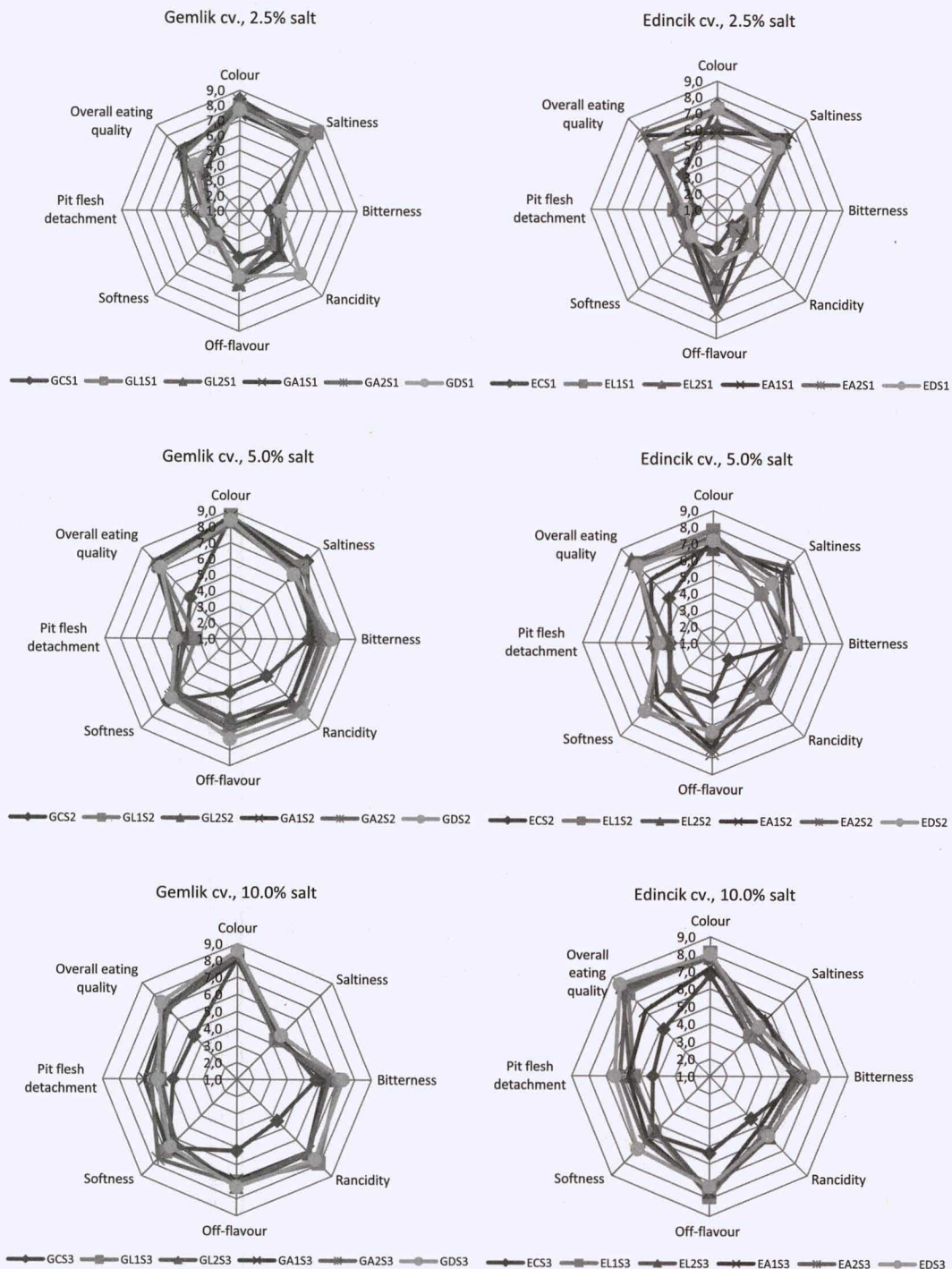


FIGURE 4. Radar plots of mean attributes values showing how the attributes differed among dry-salted olives. G, Gemlik olives; E, Edincik olives; C, control; L, lactic acid; A, acetic acid; D, disinfectant; S1, 2.5% NaCl concentration; S2, 5.0% NaCl concentration; S3, 10.0% NaCl concentration.

of olives could also be influenced by a higher proportion of damaged fruits in a sample, resulting in increased skin permeability in the ripe fruit (56). In the present study, no *Enterobacteriaceae* or *Pseudomonas* were detected probably because of the low water activity, high NaCl concentration, and hand harvesting (resulting in minimal damage to the fruits). Similar microbiological results were reported previously (8) in a survey of Moroccan dry-salted olives, on which the most prevalent microorganisms were *YM. Panagou et al.* (39), Panagou (37), and Değirmençioğlu (18) did not detect *Enterobacteriaceae* and *Pseudomonas* in

the Thassos and Gemlik varieties of dry-salted olives, possibly because of low water activity, high NaCl concentrations, and relatively low levels of spoilage bacteria.

**Physicochemical and sensory changes in vacuum-packed olives.** The various washing solutions had no effect on the physicochemical characteristics measured (pH, moisture, and NaCl concentration), indicating that the olives were stable throughout storage at 4°C. The same physicochemical characteristics were not affected by the



treatments, except for the NaCl concentration of the flesh, which increased as a result of moisture loss. Similar results were obtained in previous experiments reported by Değirmencioglu et al. (19), Jimenez et al. (33), Panagou et al. (39), and Panagou (36). In the present study, the NaCl concentrations in dry-salted olives were 2.05 to 9.72% at the end of the storage period at 4°C. The moisture content of the vacuum-packaged dry-salted olives decreased from an initial 18.1 to 22.8% at the beginning of storage to 16.2 to 21.6% at the end of storage. A corresponding increase in the NaCl concentration in the flesh occurred, resulting in slight shriveling of the olives.

Changes in the organoleptic characteristics of table olives are easier for the consumer to discern and identify than are the physicochemical and microbiological properties, and the changes also provide a description of the product, which in turn offers a starting point to evaluate product abnormalities, defects, and spoilage (19). The sensory evaluation in this study included color, taste, texture of flesh and stone, and overall eating quality. Figure 4 shows the radar plots of the sensory analysis of dry-salted olives that had been dipped in various solutions, vacuum sealed, and stored at 4°C for 7 months. According to the microbiological results, the effectiveness of vacuum packaging followed by a ClO<sub>2</sub> wash used in this study and storage at 4°C was greater than the other washing treatments for controlling or inhibiting LAB and YM, which impact sensory attributes.

Microorganisms play an important role in the production of table olives. Diverse microbial groups are involved throughout fermentation and determine the safety, quality, and flavor of the final product (25). Yeasts, which are present in fermented foods and beverages, can be beneficial (technological flora) or detrimental (spoilage flora) (17) and produce ethanol, glycerol, higher alcohols, esters, and other volatile compounds that play an important role in flavor generation and texture maintenance during fermentation and storage (5, 26). Most of these compounds are derived from the degradation of polyunsaturated fatty acids through lipoxygenase pathways (29, 44, 45). However, some polysaccharolytic strains of yeast can become dominant and negatively affect the product quality, causing deterioration of olives through gas pocket formation, softening, etc. (5, 38, 57). Various investigations have reaffirmed the negative effect of yeasts on the organoleptic characteristics of table olives (3, 5, 46).

Added NaCl, mainly as a flavoring agent, has a clearly established safety role and substantial effect on the growth or survival of pathogens (10). The exception to this effect is when the samples contain salt-tolerant yeast strains, and the use of a high concentration of NaCl during fermentation (>8% in the equilibrium) or the dry-salting process could allow the growth of these salt-tolerant yeasts (18, 39, 51). NaCl intake is closely related to water loss during dry salting. Therefore, the higher the water loss, the saltier the product becomes. However, NaCl intake cannot exceed certain limits; otherwise, olives would become organoleptically unacceptable (37). In our study, the flesh of dry-salted olives contained 2.05 to 9.72% NaCl, and no samples exceeded the specified limits for NaCl concentration. The NaCl concentration of dry-

salted olives creates the desired level of bitterness. The assessors on the sensory panel in the present study considered the saltiness to be appropriate at 2.5 and 5.0% and reported that softness and bitterness increased with reduced NaCl concentrations in both olive varieties. The high saltiness scores obtained with the higher NaCl concentrations was as a result of enhanced cell wall permeability associated with the soft texture and very delicate skin and pulp. An additional important finding was that the increase in salt diffusion, depending on the salt concentration, had a positive effect ( $P < 0.05$ ) on the softness of the olive flesh. No significant differences ( $P > 0.05$ ) in pit detachment were found between olives washed with various solutions and packaged with the same salt concentrations.

According to the sensory results for vacuum-packed dry-salted olives, Gemlik olives had higher color attribute scores ( $P < 0.05$ ) than did Edincik olives, possibly based on the fresh olive color (gray black). No significant differences ( $P > 0.05$ ) were found between trials of both olive cultivars for scores for rancidity, off-flavors, and overall eating quality (Fig. 4). Traditionally, fermented vegetables are processed using common NaCl solutions, resulting in Na as one of the major ingredients in the final product (25, 27). In vacuum-packaged samples, the lack of oxygen, the washing solutions used before dry salting, and the primary dry salting with high NaCl concentrations controlled YM growth; therefore, the assessors gave both rancidity and hardness attributes higher scores.

A combination of preservation treatments for olives is recommended to provide the required level of protection while retaining the organoleptic attributes of the product, such as color, flavor, texture, and nutritional value (14). Treatment with a ClO<sub>2</sub> wash (10 ppm) followed by dry salting with 10.0% salt, vacuum packaging, and storage at 4°C was more effective than use of other washing solutions for controlling LAB counts and suppressing YM growth. However, acetic and lactic acid solutions (2%) are useful washing solutions as an alternative to ClO<sub>2</sub> when salt concentrations are 5.0 and 10.0%.

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