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INFLUENCE OF TEMPERATURE AND MODIFIED ATMOSPHERE ON THE MICROBIAL PROFILE OF PACKED GEMLIK DRY-SALTED OLIVES

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ABSTRACT

The effect of a chlorine wash on microbial growth in packed (vacuum and MAP), dry-salted olives of the Gemlik variety stored at 4C and 20C was studied for 7 months. The study was based on microbiological changes occurring in dry-salted olive samples during their shelf life. The microbiota were comprised of total viable bacteria, LAB and yeasts, mold, Enterobacteria and Pseudomonads. At 4 and 20C, the population of yeasts increased steadily in control samples during the shelf-life period (with and without chlorine exposure). At 20C, neither of the packaging methods was effective in suppressing total viable and LAB growth. The count of TYM increased in the MAP samples after the third month at 20C; therefore, different combinations of chlorine and CO_2 and N_2 (or combinations of chlorine with only CO_2 or N_2) can be used to control yeast–mold growth. The combination of a 35% CO_2 and 65% N_2 (with a 10-ppm chlorine wash) at 4C was the most effective at controlling the growth of total viable, LAB and TYM. No Enterobacteria and Pseudomonads were detected since the high salt content is not favorable for their growth.

PRACTICAL APPLICATIONS

MAP has been used for several years for preserving fresh fruits and vegetables. The use of MAP for the storage of fruits and vegetables at a low temperature is more effective than vacuum packaging alone for preventing the growth of microorganisms due to its bacteriostatic effects. Better results can be obtained if MAP is supported with a low-concentration antimicrobial agent (chlorine). In future research, combinations of CO_2 and N_2 at various concentrations in the packaging should be evaluated to determine their ability to increase microbiological quality, especially to control yeast–mold growth in packaged olives.

INTRODUCTION

Since ancient times, olives (*Olea europaea* L.) have been prepared for human consumption (Cardoso *et al.* 2008). Table olives are one of the major agricultural commodities cultivated around the world and probably the most important and popular fermented food worldwide. Olives and olive oil are main constituents of the Mediterranean diet. Today, this popular product is primarily processed in one of three ways: (1) Spanish-style green olives that are alkali-treated and fermented; (2) black olives (Greek style) that are naturally fermented; and (3) black olives that are alkali-treated and oxidized (California style) (Tassou *et al.* 2002; Cardoso *et al.* 2008; Panagou *et al.*, 2008). In addition to these three methods, other traditionally processed table olives (e.g. dry-salted, cracked) can also be found in local market places according to consumers' demands (Cardoso *et al.* 2008).

Olives are an important agricultural product in Turkey. In 2008, 512,103 tons of table olives were produced (DIE 2009). Among the large number of olive varieties grown in Turkey, the Gemlik olive is the main variety used in the production of different types of table olives. One special type of naturally

black olives is prepared as either "naturally black fermented olives, Gemlik style, or naturally black dry-salted olives, Sele style" (Kılıç 1994). Dry salting is also used in the Mediterranean region (in Greece and some North African countries) for the production of naturally preserved black olives (Panagou 2006).

The industrial processing of table olives in Turkey, both green and black, is essentially an empirical process despite its economic importance. However, there are still some traditional preparations that have attracted little attention to date. For the production of dry-salted olives (basket style), the olives are harvested in December when fully mature and completely black in color. This traditional processing method consists of placing the olives in a basket, bag, or plastic container with coarse sodium chloride in a range of 4–10 parts of salt to 100 parts of olives (w/w). The container is turned over every other day. Due to the high osmotic pressure exerted by the salt (curing or desiccating agent), the olives lose water and other solutes, including much of the bitter agent, oleuropein, and become gradually debittered and wrinkled (dry-salt processing). Under the above conditions, practically no fermentation takes place since the fruits are not immersed in brine (Kılıç 1994; Harris 1998; Panagou 2006; Cardoso *et al.* 2008). This method is similar to the methodology applied in other countries (Panagou *et al.* 2002; Panagou 2006).

The low water/high salt content (water activity, A_w, 0.75–0.85; NaCl content of the flesh [4–10 g/100 g]) of the dry-salted olives can ensure their safety during storage, although potential spoilage microorganisms, mainly fungi, can grow in these conditions. Fungal growth negatively affects both the nutritive and aesthetic value of the product, often due to the presence of mycelium on the surface. This spoilage may occur when olives are not dry enough as a result of insufficient salt. There is increasing scientific evidence linking fungal growth and mycotoxin production to olives processed by dry-salting (Tantaoui-Elaraki *et al.* 1990; Panagou *et al.* 2002; Panagou 2006; Cardoso *et al.* 2008).

Since dry-salted olives are not packaged in brine, an alternative would be to use modified atmospheres, such as CO_2, that could irreversibly impact the survival of fungi (Panagou 2006). Packaging of olives under modified atmospheres effectively controls the growth of fungi, thus minimizing the potential production of mycotoxins (Panagou *et al.* 2002).

MAP techniques are now used for a wide range of fresh or chilled foods, including raw and cooked meats, poultry, fish, fresh pasta, fruits, vegetables and, more recently, coffee, tea and bakery products (Phillips 1996). Modification of the atmosphere surrounding the food provides an environment that restricts the growth of microorganisms. Another technique is storage at low temperatures (<4C). The combination of low temperatures and MAP generally results in a more effective and safer storage time as well as a longer shelf life (Leistner 1995).

Another common packaging method in the food industry is vacuum packaging. The product is placed in a pack with low oxygen permeability, air is evacuated and the package is sealed. Since it is not possible to evacuate all the air (0.3–3% may remain after sealing), the gaseous atmosphere of the vacuum package is likely to change during storage (owing to microbial and product metabolism and gas permeation), and therefore, the atmosphere in the package may be different from the original atmosphere (Irtwange 2006). Vacuum packaging may not be appropriate for fruit and vegetable products because it accelerates the deterioration of quality (Soylemez *et al.* 2001).

To provide microbial safety, fruit and vegetables are passed through some preprocessing steps, such as cleaning, trimming, peeling, coring, slicing, shredding, washing and sanitizing. The objective of the washing or sanitizing steps is to remove soil and pesticide residues, reduce microbial load and lower product temperature (Simons and Sanguansri 1997; Baur *et al.* 2005). To ensure the reduction of microbial contamination, chlorine has long been the most commonly used sanitizer at various stages (Adams *et al.* 1989; Nguyen-The and Carlin 1994; Beuchat *et al.* 1998; Beuchat 1999; Roberts and Reymond, 1994; Li *et al.* 2001). Appropriate washing systems must ensure the removal or destruction of harmful microbes in whole vegetables on a commercial scale. The United States Food and Drug Administration (USFDA) recommends 50–200 mg/L chlorine at pH 6.0–7.5 and contact times of 1–2 min for this purpose (Beuchat *et al.* 1998, U.S. Food and Drug Administration [USFDA] 1998; Parish *et al.* 2001). Minimum chlorine levels (between 2 and 7 mg/L) have been recommended to ensure the microbiological quality of water used for washing vegetables (Delaquis *et al.* 2004).

The aim of the present work was to determine the effect of a chlorine wash on the microbial characteristics of dry-salted olives of Gemlik cultivar packed in vacuum and modified atmosphere conditions and stored either at 4 or 20C.

MATERIALS AND METHODS

Dry-Salting Process

Black olives cv. Gemlik were harvested in Erdek, Turkey, in December 2008 and transported to the laboratory within 24 h. On arrival, the fruit was hand selected and separated into two equal lots. The first lot was immersed in water containing 10 ppm of chlorine (10 L) for 10 min. The olives were then washed thoroughly in distilled water and left to dry at room temperature for 30 min. A sample of approximately 50 kg of fresh olives was packed in a 100-L polyvinyl chloride (PVC) container with 5 kg of uniformly dispersed coarse salt. To avoid fungal growth on the surface, the fruit was covered with a top layer of 2–3 cm of salt, which was a part of the 5 kg total. During the process, the drum was kept at ambient tem-

perature (20 ± 1C). Water and other solutes were drained through a hole located at the bottom of the drum and collected during the dry-salting process (every 20 days). The second lot was washed thoroughly using only distilled water, dried at room temperature and then put into a PVC container and packed in a 100-L PVC container in the same manner as the first lot. At end of the dry-salting process (60 days), both lots were washed with distilled water to remove the excess salt and dried at room temperature. This process was adapted from Panagou (2006).

Packaging

Dry-salted olive samples were packaged in high-density polyethylene (HDPE) trays (density 1.26–1.28, thickness range 190–1000 μm, comparable thickness 750 μm, Neo Plastica/Klöckner Pentaplast, Germany). A film (85 μm thick) consisting of HDPE and having an oxygen transmission rate of 3.5 $cm^3/m^2/24$ h (23C, 0% RH) with antifog property was used to cover or seal the package (Neo Plastica/Klöckner Pentaplast, Germany; data from the manufacturer). Olive samples (250 g) were placed into these trays. The trays were vacuumed and flushed with vacuum, and an atmosphere of 35% CO_2-65% N_2 and air (control samples) was provided prior to their being sealed in a machine (Tiromat Compact M 380–3.11 packaging machine, Convenience Food Systems, Tiromat Kraemer + Grebe GmbH & Co. KG, Germany). Vacuum packaging was performed by using a VC 999/K12NA (Switzerland) packaging machine, and the dry-salted olive samples were packed in polyamid/polyethylene. The samples, some of which had been dipped in an aqueous solution of chlorine and the remainder had not, were packaged separately and stored at 4 ± 1C and 20 ± 1C for 7 months.

Microbiological Analysis

The determination of microorganisms was performed on fresh olives and each type of sample. For the olive pulp analyses, 25 g of each sample were weighed out aseptically, transferred into 225 mL of a sterile, Maximum Recovery Dilution solution (1 g/L peptone and 8.5 g/L saline) and homogenized in a stomacher (Masticator, IUL Instruments, Spain) for 60 s at room temperature (20 ± 1C). 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 mixed or spread on the following agar media: Plate Count Agar (PCA; Oxoid CMO325, Basingstoke, U.K.) for TVC, incubated at 25C for 48 h; de Man–Rogosa–Sharp medium (Oxoid CM0361) for LAB, overlaid with the same medium and incubated at 30C for 48 h; Cetrimide–Fucidin–Cephalorodine Medium (Oxoid CM 559 supplemented with SR 103) for *Pseudomonads* counts, incubated at 25C for 48 h; Rose Bengal Chloramphenicol agar (Oxoid CM 549 supplemented with SR 78) for yeasts and molds, incubated at 25C for 72 h; Violet Red Bile Glucose agar (Merck 1.10275.0500) for Enterobacteria counts, incubated at 37C for 24 h. The results of all microorganisms were expressed as $\log_{10}$ values of the colony forming units per gram (cfu/g) of pulp olive in fresh and packaged olive samples (Panagou *et al.* 2002; Panagou 2004). All analyses were conducted at least in triplicate for each type of sample.

Statistical Analysis

Data were analyzed with the SPSS statistical package (SPSS 15.0, Chicago, IL). All microbiological counts were evaluated statistically. General Linear Model (repeated measures) and multiple comparisons analysis were performed to estimate the significance ($P < 0.05$) of the effects of chlorine treatment on the microbiological quality, temperature during shelf life and packaging methods. The least significant difference (LSD) test was used to determine differences between the packaging methods.

RESULTS AND DISCUSSION

Effects of Dipping in Chlorine Solution on the TVC and LAB Counts

The use of different temperatures during the shelf life of the fruit did not effectively restrict the increase of TVC (Fig. 1) and LAB count (Fig. 2) ($P > 0.05$) in the control samples that had not been dipped in the chorine solution. Also, control samples that were packed in air after being dipped in the chlorine solution showed no statistical differences ($P > 0.05$) in TVC (Fig. 1) and LAB count (Fig. 2). Increases in the TVC and LAB count were observed (approximately 2 log cfu/g) during the shelf life of the samples at 4 and 20C, and the increases were affected by temperature. Also, shelf life at different temperatures did not limit the increase of TVC counts. On the contrary, it led to an increase in the number of bacteria found in vacuum-packed and MAP-packed samples (without the chlorine wash) at 20C. This is possibly due to the fact that total viable bacteria have entered a stationary phase of growth in vacuum-packed and MAP-packed samples after 2 months of shelf life. However, the increases of LAB counts in samples stored at 4C were less than those stored at 20C in vacuum-packaged samples. Panagou (2004) found that the initial population of TVC and LAB presented slight changes and remained around 7 log cfu/mL regardless of air, vacuum and MAP packaging when a temperature of 20C was maintained during the shelf life. In comparison, the TVC and LAB counts of our study did not reach levels as high as those observed in Panagou's research (Panagou 2004).

In vacuum-packaged samples that had been dipped in chlorine and held at 4C, the TVC count remained stable until

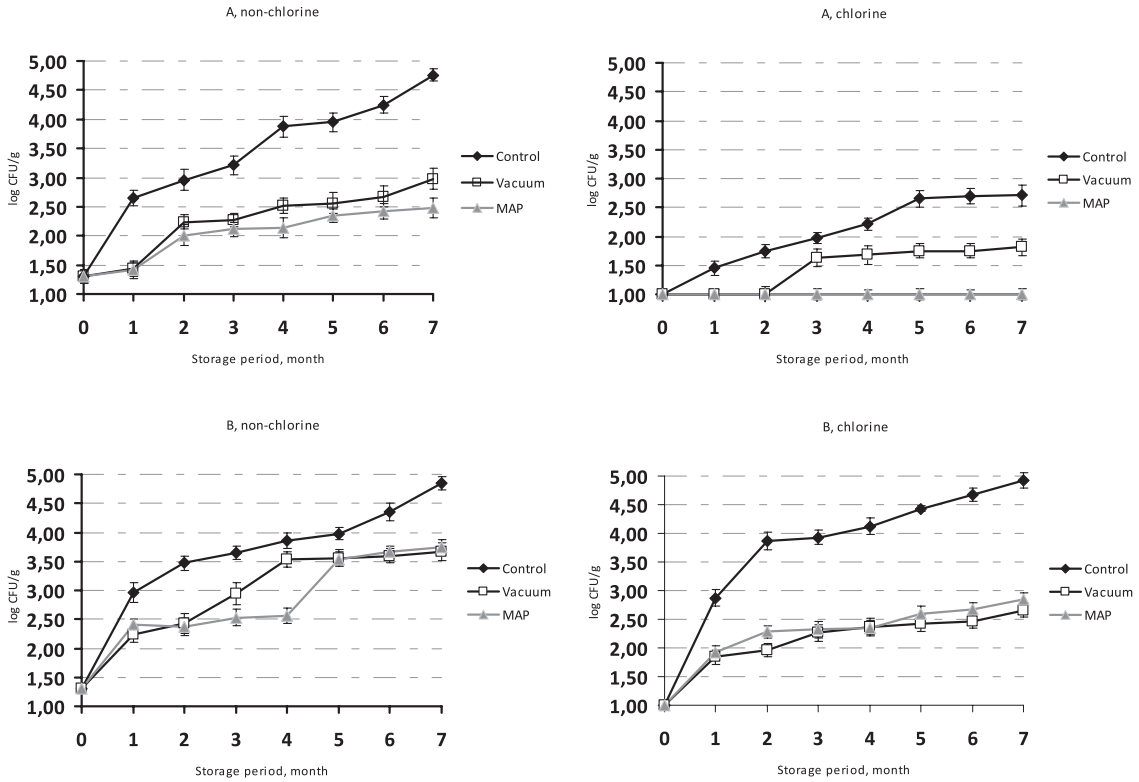

FIG. 1. CHANGES IN TOTAL VIABLE COUNT (log cfu/g) OF DRY-SALTED OLIVES STORED AT 4C (A) AND 20C (B) DURING SHELF-LIFE PERIODS UNDER DIFFERENT PACKAGING AND CHLORINE TREATMENT
MAP, modified atmosphere packaging.

the third month, and, after this period, the counts increased until the seventh month of shelf life. The LAB count determined at the end of shelf life (1.45 log cfu/g) was similar to vacuum samples (1.47 log cfu/g) that had no chlorine applied and were also stored at 4C. It can be said that the vacuum treatment restricted the increase of LAB. Similar to TVC count, the LAB counts remained stable in the vacuum packaging samples until the fifth month, after which, a steady increase was observed until the end of the shelf life in this study. It is possible that this situation was caused by the remaining small amount of oxygen available in the vacuum packaging.

Carbon dioxide, which is both fungistatic and bacteriostatic, prevents insect growth in packaged and stored food products. The effectiveness of carbon dioxide is influenced by the original and final concentrations of the gas, the temperature of storage and the original population of organisms. Nitrogen is an inert, tasteless gas that is often used as a filler gas. Because of its low solubility in water, the presence of nitrogen in MAP food can prevent package collapse that occurs when high concentrations of carbon dioxide are used (Phillips 1996). Nitrogen is also used to replace oxygen in MAP products to prevent rancidity and inhibit the growth of aerobic organisms (Farber 1991). Although previous studies used 100% carbon dioxide and various combinations of carbon dioxide and nitrogen as packaging atmospheres (Panagou *et al.* 2002; Panagou 2004, 2006), in this study, the same fungistatic and bacteriostatic effects were provided by a prewash with a solution containing chlorine, followed by the use of MAP containing a low concentration of CO_2.

Several studies have determined the efficacy of washing, sanitizing and MAP conditions in order to inhibit browning and spoilage in fresh-cut fruit and vegetables (Beltran *et al.* 2005). Agents that are chlorine based have been used often to sanitize the surfaces of products, as well as reduce microbial populations (Delaquis *et al.* 2004). These procedures reduce initial microbiological load, thus reducing the rate of subsequent microbial spoilage and minimizing the populations of potential pathogens. The washing agent can be water alone, but the efficacy of washing is improved by including antimicrobials, typically chlorine at concentrations that range from 100 to 200 ppm in the wash water (Wei *et al.* 1985; Francis *et al.* 1999). However, the use of chlorine is of concern due to the potential formation of harmful by-products (Richardson *et al.* 1998), and it can only achieve approximately 2 to 3 log reductions of native microbiota (Ukuku *et al.* 2001). The

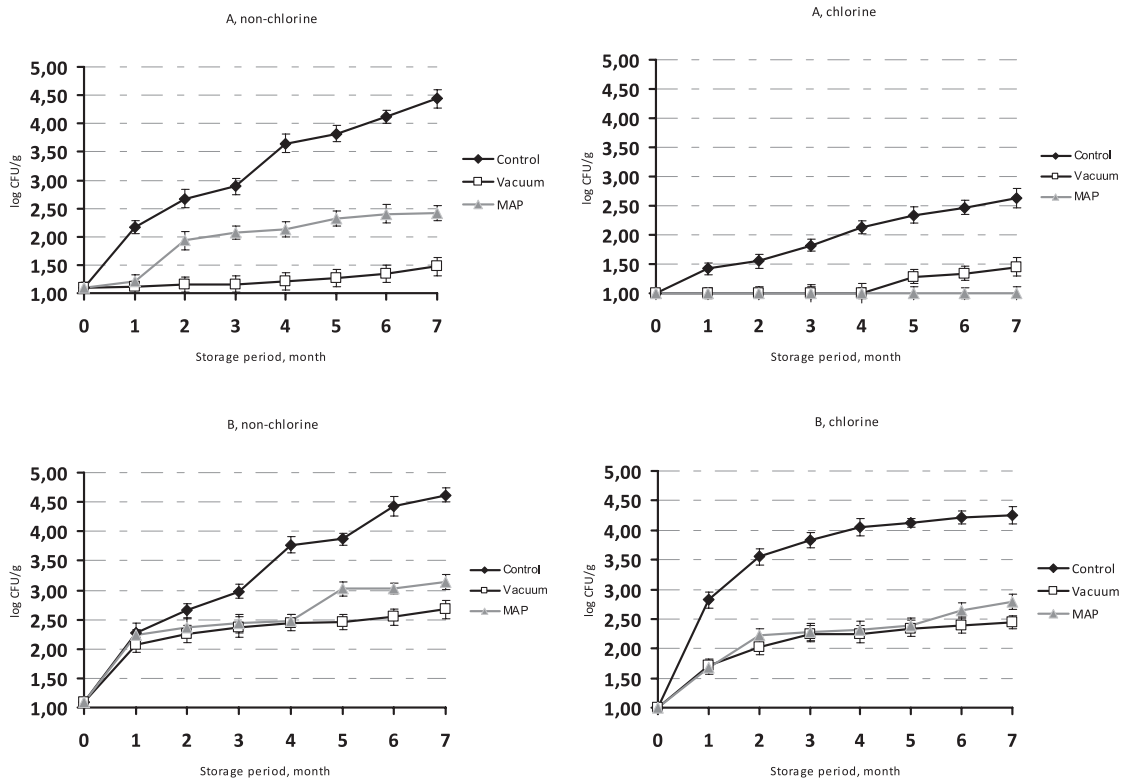

FIG. 2. CHANGES IN LACTIC ACID BACTERIA COUNT (log cfu/g) OF DRY-SALTED OLIVES STORED AT 4C (A) AND 20C (B) DURING SHELF-LIFE PERIODS UNDER DIFFERENT PACKAGING AND CHLORINE TREATMENT
MAP, modified atmosphere packaging.

TVC and LAB counts in the MAP samples of this study that were not dipped in chlorine solution and were stored at 4C (2.47 log cfu/g, 2.42 log cfu/g) as compared with samples that were dipped in chlorine solution and stored at 20C (2.85 log cfu/g, 2.79 log cfu/g), respectively, were determined to be close to each other. It was thought that the effectiveness of MAP treatment with chlorine for controlling the increase of TVC and LAB counts (Figs. 1 and 2) was higher statistically ($P < 0.05$) than the other packaging treatments at 4C.

Effects of Dipping Chlorine Solution on the TYM Counts

Several studies have focused on the detection of yeasts that adhere to the surface of olives. Arroyo-Lopez *et al.* (2008) reported the presence of yeasts among the microbiota found on the surface of fresh mature olives. It was observed that the species found depended on the maturation degree of the olive. However, yeast counts on the surface of fresh olives are generally low (<1 $\log_{10}$ cfu/g), as was reported by Arroyo-Lopez *et al.* (2009) for Manzanilla-Alorena olives during three consecutive seasons. The TYM count found in our study, pre-dipping and post-dipping in a chlorine solution, was <1 log cfu/g. Similar results were found for control treatments with and without chlorine, and it was determined that chlorine treatment was not effective for controlling the TYM growth at 4 and 20C ($P > 0.05$). The total population of yeast–mold was suppressed in vacuum (2.25 log cfu/g) and MAP (2.11 log cfu/g) treatments, with no chlorine wash, until the second and third months at 4C, respectively, after which, the yeast–mold count increased by the end of the shelf-life period studied (Fig. 3).

Although the vacuum-packaged samples were stored at 20C, the TYM growth started a month earlier (1.54 log cfu/g at the end of the first month). The MAP samples that were dipped in chlorine solution and packaged had a TYM growth of 1.96 log cfu/g at the end of the second month, as compared to the samples that were washed only in distilled water and stored at 4C. Therefore, it was found that the chlorine wash solution did not effectively limit the yeast growth in dry-salted samples stored in vacuum and MAP packaging conditions at 20C. Also, it can be said that the microorganisms entered the olives through cracks in the fruit, but the chlorine solution was not able to sufficiently penetrate these areas. Panagou *et al.* (2002) determined that the population of yeast declined steadily throughout the

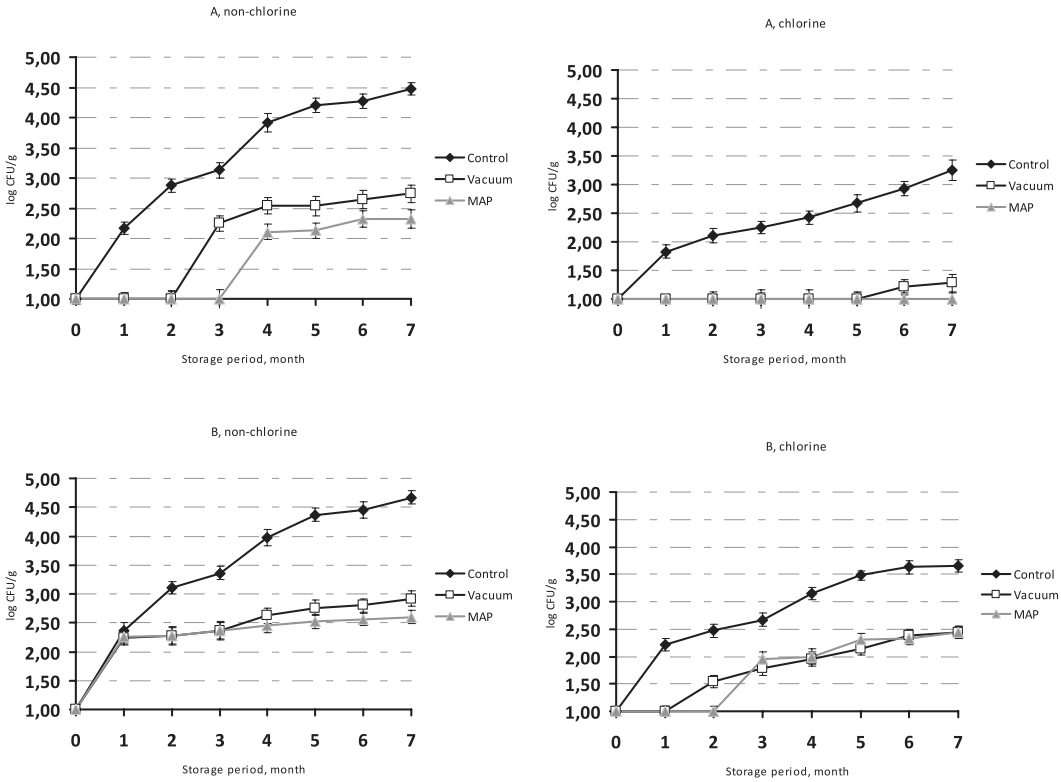

FIG. 3. CHANGES IN TOTAL YEAST AND MOLD COUNT (log cfu/g) OF DRY-SALTED OLIVES STORED AT 4C (A) AND 20C (B) DURING SHELF-LIFE PERIODS UNDER DIFFERENT PACKAGING AND CHLORINE TREATMENT
MAP, modified atmosphere packaging.

storage period at 4C. They also observed that the death rate of yeasts in samples stored in 100% CO_2 and 40% CO_2-30% O_2-30% N_2 was higher than in olive samples stored under aerobic conditions. Panagou (2004) found that the population of yeasts in vacuum-packed olives started to decline when the experiments began, and, after 150 days of storage at 20C, the lowest count (3 log cfu/g) was observed. Compared with these results, it was determined that the TYM count (2.44 log cfu/g) found in vacuum-packaged and MAP-packaged samples was lower than those reported by Panagou *et al.* (2002) and Panagou (2004) at the end of the storage period. However, in MAP-packed samples, no yeast–mold growth was detected at the end of the seventh month in this study. Therefore, it could be said that chlorine treatment and MAP (35% CO_2-65% N_2) were able to suppress the TYM growth better than vacuum packaging (1.22 log cfu/g, sixth month) at 4C. Panagou *et al.* (2002) and Panagou (2006) suggested that carbon dioxide was very effective in keeping the counts low. This was an expected observation, since dry-salted olives have lower water activity and high salt content in which only salt-tolerant yeasts can grow (Tokuoka 1993). Panagou *et al.* (2002) did isolate some salt-tolerant yeast strains that grew in the presence of aqueous solutions of salt containing 15 g of NaCl/100 g of water. In this research, the sample of dry salted olives contained 8.6–9.7% NaCl. According to the results, even if the samples contained salt-tolerant yeast strains, it is thought that the combination of a wash of chlorine (10 ppm) and a 35% CO_2-65% N_2 packaging condition would effectively control yeast–mold growth on dry-salted olive samples stored at 4C.

MAP costs twice as much as vacuum packaging because it requires special packaging material and gases (Murcia *et al.* 2003). However, with the elimination of O_2 from the packaging and the introduction of different concentrations of CO_2 and N_2, together with adequate refrigeration, spoilage due to psychotropic and aerobic microorganisms, proteolytic bacteria, yeasts and fungi can be delayed. It has also been reported that CO_2 gases are related to a reduction in intracellular and extracellular pH values and to the direct inhibition of enzymatic processes. This effect generally increases at lower temperature since solubility of the CO_2 is enhanced (López-Caballero *et al.* 2000; Murcia *et al.* 2003). Therefore, the microbial growth of all microorganisms in dry-salted olives stored under MAP conditions showed the lowest values during the seventh month. The effect of vacuum packaging

on microbial growth was overshadowed by the effect of temperature and the chlorine wash. Low temperature was the most important factor in maintaining microbial quality of food products. The use of low-temperature shelf life of dry-salted olives was essential for controlling microbial growth and maintaining quality attributes. When storage temperature was increased from 4 to 20C, the counts of TYM increased by approximately 2 log cfu/g in vacuum samples and by approximately 3 log cfu/g in MAP samples, and therefore, it is evident that lowering the storage temperature was a more critical factor than MAP in reducing microbial counts.

Variance analysis for TVC, LAB and TYM for dry-salted olive samples that had been dipped in chlorine solution, stored at different temperatures and packaged with different methods showed that the effects of chlorine dipping, packaging methods, temperature and their interactions were all significant ($P < 0.05$) during the shelf life of the product (Tables 1–3). According to LSD results after 7 months of shelf life, dipping in chlorine solution significantly ($P < 0.05$) controlled TVC, LAB and TYM counts compared to samples that were not dipped in the chlorine solution. MAP packaging also significantly ($P < 0.01$) controlled TVC, LAB and TYM counts compared to vacuum-packed and air-packed control samples (Tables 1–3).

Effects of Dipping in Chlorine Solution on the Enterobacteria and Pseudomonads Counts

The bacteria normally found on fresh-cut products are the same as those normally found on produce in the field. The microbiota of vegetables and fruits are made up largely of *Pseudomonas* spp., *Erwinia herbicola*, *Flavobacterium*, *Xanthomonas* and *Enterobacter agglomerans*, as well as various molds. LAB, such as *Leuconostoc mesenteroides* and *Lactobacillus* spp., are also commonly found, as are several species of yeasts (primarily on fruit). *Pseudomonas* is normally predominant and may make up 50–90% of the microbial population on many vegetables. Typically, these microorganisms are not harmful to humans (Zagory 1999). The enteric bacteria content on the surface of olives could possibly be influenced by a higher amount of damaged fruit, resulting in increased skin permeability in the ripe fruit (Vaughn *et al.* 1969).

In this study, no Enterobacteria or *Pseudomonads* was detected. This was expected, since the product had low water activity, high salt content and had been harvested by hand, resulting in minimal damage. Similar microbiological results were reported previously (Asehraou *et al.* 1992) in a survey of Moroccan dry-salted olives, according to which the prevailing microbiota were comprised of yeasts and molds. Also, Panagou *et al.* (2002) and Panagou (2006) did not detect Enterobacteria and *Pseudomonads* in the Thassos variety of

TABLE 1. VARIANCE ANALYSIS FOR TVC OF DRY-SALTED OLIVE SAMPLES DIPPING CHLORINE SOLUTION AND PACKED WITH THREE DIFFERENT GAS COMBINATIONS AT 4 AND 20C

	0		1		2		3		4		5		6		7	
Days	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F
Temperature (T)	0.000	1.000	7.022	28.092*	7.388	37.121*	7.263	7.645*	7.218	27.073*	8.604	301.059*	11.122	32.032*	12.145	34.164*
Chlorine (C)	0.828	49.692	2.300	9.201*	3.381	16.992*	3.355	3.532*	5.649	21.183*	7.526	263.322*	8.151	23.472*	10.552	29.682*
Packaging method (P)	0.000	1.000	5.413	10.834*	9.591	24.100*	9.283	4.886*	14.098	26.432*	12.557	219.684*	17.200	24.773*	22.577	31.752*
T × C	0.000	1.000	0.281	1.124*	2.665	13.382*	1.460	1,537*	1.613	6.048*	1.247	43.629*	1.085	3.125*	2.301	5.711*
C × P	0.000	1.000	0.089	178.678*	0.324	813.099*	0.073	38.398*	0.199	372.948*	1.090	19.072*	0.535	769.848*	0.056	78.875*
T × P	0.000	1.000	0.029	57.700*	0.827	2.077*	0.457	240.667*	0.015	28.135*	0.719	12.570*	0.630	970.416*	0.945	1.329*
T × C × P	0.000	1.000	0.650	1.300*	0.309	777.047*	0.954	502.082*	1.895	3.553*	1.216	21.820*	1.719	2.475*	1.597	2.246*

* Significant at 0.05 probability level.

TABLE 2. VARIANCE ANALYSIS FOR LAB OF DRY-SALTED OLIVE SAMPLES DIPPING CHLORINE SOLUTION AND PACKED WITH THREE DIFFERENT GAS COMBINATIONS AT 4 AND 20C

	0		1		2		3		4		5		6		7	
	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F
Temperature (T)	0.015	1.776*	5.945	18.512*	8.371	24.112*	9.631	43.894*	9.466	16.793*	9.425	23.400*	10.901	71.351*	10.123	3.307*
Chlorine (C)	0.178	21.340*	0.516	1.606*	0.706	2.032*	0.740	3.370*	2.171	3.850*	2.712	6.732*	3.604	23.592*	4.716	1.541*
Packaging method (P)	2.222.132	1.333	3.604	5.606*	6.385	9.194*	9.383	21.382*	19.610	17.392*	19.319	23.980*	24.567	80.042*	26.492	4.327*
T × C	0.015	1.776*	0.119	370.672*	1.841	5.301*	2.054	9.362*	1.941	3.443*	1.322	3.283	1.296	8.482*	1.517	495.573*
C × P	2.222.134	1.333	0.120	185.975*	0.299	430.872*	0.482	1.098*	0.361	320.399*	1.276	1.583*	1.387	4.540*	1.719	280.830*
T × P	2.222.129	1.333	0.016	24.745*	0.082	117.416*	0.249	567.696*	0.253	223.897*	0.068	84.352*	0.022	73.382*	0.118	19.024*
T × C × P	2.222.132	1.333	1.260	1.960*	1.680	2.419*	1.347	3.069*	1.212	1.075*	1.286	1.596*	1.016	3.324*	1.217	198.845*

* Significant at 0.05 probability level.
LAB, lactic acid bacteria.

TABLE 3. VARIANCE ANALYSIS FOR TYM OF DRY-SALTED OLIVE SAMPLES DIPPING CHLORINE SOLUTION AND PACKED WITH THREE DIFFERENT GAS COMBINATIONS AT 4 AND 20C

	0		1		2		3		4		5		6		7	
	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F
Temperature (T)	2.778.142	1.000	2.372	13.142	3.392	12.464*	3.744	17.282*	2.523	5.190*	4.033	14.822*	3.597	10.444*	3.367	7.721*
Chlorine (C)	2.778.142	1.000	2.230	12.352*	2.918	10.724*	3.654	16.872*	9.313	19.164*	8.831	32.440*	7.691	22.332*	7.981	18.300*
Packaging method (P)	5.555.236	1.000	5.600	15.510*	12.724	23.374*	10.747	24.810*	15.872	16.332*	21.330	39.184*	22.407	32.532*	26.066	29.883*
T × C	2.778.142	1.000	1.323	7.325*	0.893	3.281*	0.048	220.103*	1.214	2.497*	1.533	5.633*	1.673	4.856*	1.388	3.184*
C × P	5.555.236	1.000	0.284	785.831*	0.198	362.765*	0.860	1.985*	0.241	247.446*	0.465	854.704*	0.135	196.363*	0.209	240.076*
T × P	5.555.236	1.000	0.218	603.185*	0.564	1.036*	1.244	2.872*	0.119	122.817*	0.193	354.194*	0.181	262.992*	0.472	541.223*
T × C × P	5.555.236	1.000	1.037	2.871*	0.742	1.362*	0.441	1.019*	0.020	20.749*	0.036	66.194*	0.137	198.782*	0.377	432.408*

* Significant at 0.05 probability level.
TYM, total yeast–mold.

dry-salted olives. According to the authors, the results of their study are thought to be the result of low water activity, high salt content and relatively low numbers of bacteria that cause spoilage.

CONCLUSIONS

The effect of chlorination and different packaging conditions (vacuum and MAP) on microbial growth was studied for 7 months in packed Gemlik dry-salted olives that were stored at 4 and 20C. According to the results, the effectiveness of treatment with a chlorine wash followed by MAP (35% CO_2 and 65% N_2) and storage at 4C was more effective than the other packaging treatments in controlling the increase of TVC and LAB counts. In MAP-packed samples, there was no evidence of yeast–mould growth at the end of the seventh month of shelf life. Therefore, it can be concluded that chlorine treatment and MAP, along with storage at 4C, were able to suppress the TYM growth better than vacuum packaging.

NOMENCLATURE

LAB	lactic acid bacteria
MAP	modified atmosphere packaging
TVC	total viable count
TYM	total yeast–mold

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