Effect of absorbent pads containing oregano essential oil on the shelf life extension of overwrap packed chicken drumsticks stored at four degrees Celsius

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ABSTRACT The addition of sachets or pads containing volatile antimicrobial agents into packages has been the most successful commercial application of antimicrobials to packaging. In this study, the effect of oregano (*Origanum onites*) essential oil on the extension of shelf life of overwrap packed fresh chicken drumsticks was investigated. Meat exudate absorbent pads were sprayed with 5 mL of oregano essential oil at a concentration of 1.5% in distillate water. Sampling was carried out at 0, 3, 5, and 7 d of the refrigerated storage. Total viable count, psychrotrophs, pseudomonads, members of the family Enterobacteriaceae, yeasts, and lactic acid bacteria were enumerated. Physicochemical analysis and sensorial evaluation were also conducted. The shelf life of fresh chicken drumsticks was approximately 3 d. Oregano essential oil extended product shelf life by approximately 2 d. Thus, incorporation of essential oils to absorbent pads may have supplementary applications in food packaging.

Key words: oregano, essential oil, packaging, drumstick

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INTRODUCTION

Poultry meat is a very popular food commodity around the world and its consumption has increased over the last decades in many countries (Chouliara et al., 2007). However, raw meat can be easily contaminated by microorganisms and support the growth of pathogens, leading to serious foodborne illnesses. Refrigeration is the most common preservation method of raw meat (Solomakos et al., 2008). Considering the fact that poultry is a perishable food, the main concern of industry is the shelf life extension of these products (Chouliara et al., 2007). The shelf life of refrigerated meat can be extended by adding synthetic additives to poultry meat. Because the safety of synthetic additives has been questioned in recent years, consumers are demanding the use of natural products as alternative preservatives in food (Solomakos et al., 2008). Therefore, modern trends to achieve this goal include the use of natural food preservatives to ensure protection from both spoilage and pathogenic microorganisms (Chouliara et al., 2007).

Recent foodborne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness, and safety. One option is to use packaging to provide an increased margin of safety and quality. The next generation of food packaging may include materials with antimicrobial properties. Antimicrobial food packaging, which is a form of active packaging, acts to reduce, inhibit, or retard the growth of microorganisms that may be present in the packed food or packaging material itself. Addition of sachets or pads containing volatile antimicrobial agents into packages has been the most successful commercial application of antimicrobial packaging (Appendini and Hotchkiss, 2002). Absorbent pads (diapers) are used in trays for packaged retail meats and poultry to soak up meat exudates. Organic acids and surfactants have been incorporated into these pads to prevent microbial growth in the exudates, which are rich in nutrients (Hansen et al., 1989).

Much attention lately has been focused on extracts from herbs and spices that have been used traditionally to improve the sensory characteristics and extend the shelf life of foods (Botsoglou et al., 2003). Plant essential oils (**EO**) also exhibit antimicrobial activity

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by interfering and destabilizing the operation of the phospholipids bilayer of the cell membrane, enzyme systems, and genetic material of bacteria (Kim et al., 1995). Generally, the EO possessing strong antibacterial properties against foodborne pathogens contain phenolic compounds. These compounds exhibit a wide range of biological effects including antioxidant and antimicrobial properties. The mode of action of these compounds is the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, or coagulation of cell contents (Burt, 2004).

The antimicrobial properties of many EO have been mostly studied in in vitro experiments; however, little information exists, in general, on the in vivo antimicrobial efficacy of plant EO against microorganisms in meat (Burt and Reinders, 2003). Practical application of several EO in foods is limited due to the strong flavor they impart to foods and also to their interaction with some food ingredients (Burt, 2004). For these reasons the preservative effect of EO may be achieved by using lower concentrations of them in combination with other preservation technologies such as low temperature (Skandamis and Nychas, 2001).

Oregano is a characteristic spice of the Mediterranean cuisine. The 2 phenols, carvacrol and thymol, the major components of oregano EO, are mainly responsible for its antimicrobial activity (Adam et al., 1998). Considering the above, the aim of the present study was to investigate the effect of oregano (*Origanum onites*) EO, which was sprayed on meat exudate absorbent pads, on the shelf life extension of fresh chicken drumsticks, overwrap packed in trays and stored at 4°C.

MATERIALS AND METHODS

EO and Absorbent Pads

The oregano EO (*O. onites*) was provided by Türer Tarım Ltd. Şti. (Türer Tarım ve Orman Ürünleri İthalat İhracat Sanayii ve Ticaret Limited Şirketi, Kavaklıdere Köyü, Bornova, İzmir, Turkey). The meat exudate absorbent pads had 3 layers made of perforated polyethylene, cellulose, and polyethylene. The size of the pads was 90 × 170 mm. These materials were provided by MNM Hijyen Ped (Adapazarı-Ankara E-5 Karayolu üzeri 10. km Çaykışla Sapağı Adapazarı, Turkey).

Packaging Film and Foam Trays

The stretch food packaging film was supplied by Rotopak (Rotopak Matbaacılık Ambalaj Sanayii ve Ticaret A.Ş., Tepeören, İstanbul, Turkey). The film width, thickness, and length in roll were 500 mm, 20 μm, and 1,500 m, respectively. It was highly transparent and permeable for oxygen and carbon dioxide. The foam packaging trays were bought from a local market in Kars, Turkey. The size of the trays was 265 × 175 × 35 mm.

Drumsticks

The fresh drumsticks were obtained from a local poultry market (Kars, Turkey) within 6 h after slaughter. The cold chain was carefully maintained during transport of samples to the laboratory.

Determining the Composition of Oregano Oil

The gas chromatography-mass spectrometry (**GC-MS**) analysis was carried out with an Agilent 5975 GC-MSD system (Agilent Technologies Inc., Santa Clara, CA). An Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies Inc.) was used with helium as a carrier gas (0.8 mL/min.). Gas chromatography oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted 40:1. The injector temperature was at 250°C. Mass spectrometry was taken at 70 eV. Mass range was from *m/z* 35 to 450. The gas chromatography analysis was carried out using an Agilent 6890N GC system (Agilent Technologies Inc.). To obtain the same elution order with GC-MS, simultaneous injection was done by using same column and appropriate operational conditions. Flame ionization detector temperature was 300°C.

The components of EO were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC-MS Library, Adams Library, Mass Finder Library, and were confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Experimental Design

In this study, a total of 32 drumsticks were divided into 2 groups as control and treatment. Each group consisted of 4 packages for each analysis day and each package included 4 drumsticks. Experiments were replicated 2 times on different occasions with different chicken samples (n = 64).

In the control groups, absorbent pads placed onto foam food packaging trays were sprayed on both sides with 5 mL of physiological saline (**PS**; 0.85% NaCl), whereas the others were sprayed with 5 mL of 1.5% oregano EO concentrate in distillate water. For this purpose, spray bottles (270550250, Bürkle GmbH, Bad Bellingen, Germany) were used. Tween 80 at a concentration of 0.1% was also added to EO solution to obtain an emulsion between the distillate water and EO. The drumsticks were laid on top of the pad. The stretch food packaging film was then placed over the

package to seal the tray. The packages were kept at 4°C. Sampling was carried out at d 0, 3, 5, and 7 of the refrigerated storage.

Sensorial Evaluation

Sensory analysis was conducted according to Ruiz et al. (2001) with some modifications. Six assessors from the Department of Food Hygiene and Technology evaluated the sensorial characteristics of the samples. The same people were used for evaluations throughout the study. Panelists were asked to evaluate color and odor intensities of raw samples. Along with the test samples, the panelists were presented with a freshly thawed chicken sample, stored at −20°C throughout the experiment. This serving was used as the reference sample. The different attributes were quantified on a rating scale from 0 to 5. Eight descriptors were analyzed from raw chicken drumsticks (Table 1) and scores of samples for each descriptor were summed.

Microbiological Analysis

Two of the whole drumsticks from each of the packages were transferred aseptically into individual stomacher bags and weighed. One hundred milliliters of PS was also added into the bags. Then, the drumsticks were rinsed for 1 min. For each sample, serial dilutions were prepared in PS. An amount of 0.1 mL of each dilution was spread on the surface of dry media. Total viable counts (**TVC**) were determined using Plate Count Agar (Oxoid CM 0325, Basingstoke, UK), after incubation for 2 d at 30°C. Concentrated oregano oil was tested for TVC by plating 0.1 mL on Plate Count Agar as describe above. Psychrotrophs were counted using Plate Count Agar, after incubation for 10 d at 7°C. Pseudomonads were determined on Cetrimide Fucidin Cephaloridine Agar (Oxoid CM 559, supplemented with Oxoid SR 103) after incubation at 25°C for 2 d. An oxidase test (Oxidase Touch Sticks, Oxoid BR 0064) was also conducted to confirm the colonies as pseudomonads. For members of the family Enterobacteriaceae, 1 mL of sample was inoculated into 15 mL of molten (45°C) Violet Red Bile Glucose Agar (Oxoid CM 485). After setting, a 10-mL overlay of molten medium was added and incubation was carried out at 37°C for 24 h. The large colonies with purple haloes were counted. Yeasts were enumerated using Rose Bengal Chloramphenicol Agar (Oxoid CM 0549, supplemented with Oxoid SR 0078), after incubation at 25°C for 5 d in the dark. Lactic acid bacteria (**LAB**) were determined on Chalmers Agar (Vanos and Cox, 1986), after microaerophilic incubation (CampyGen Atmosphere Generation System, Oxoid CNO35A) at 25°C for 5 d. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium.

Physicochemical Analysis

The pH value was recorded using a pH meter (HI 8314, Hanna Instruments, Woonsocket, RI). Deboned chicken samples were thoroughly homogenized with 10 mL of distilled water and the homogenate was used. Ammonia and hydrogen sulfide were determined using Nessler's reagent (Svehla, 1979) and lead acetate paper (Gill and Newton, 1979) to qualitatively determine spoilage of samples.

Statistical Analysis

Microbiological data were transformed into logarithms of the number of colony-forming units (cfu/g) and data were expressed as means ± SD. The data were initially tested for normal distribution by 1-sample Kolmogorov-Smirnov test. After the confirmation of normal distribution ($P > 0.05$), differences for individual parameters between control and treated groups were tested by paired-sample *t*-test using SPSS (Version 9.05 for Windows, SPSS Inc., Chicago, IL). When the distribution was abnormal, Mann-Whitney U test was used. Differences were considered significant if the *P*-value was less than 0.05.

Table 1. Descriptive attributes and definitions used to evaluate raw chicken drumsticks

Attributes	Definition and procedure used for their evaulation[1]
Visual	
White/yellow skin color	Yellow color intensity in the skin of the drumstick (white = 0, yellow = 5)
White/pink skin color	Pink color intensity in the skin of the drumstick (white = 0, pink = 5)
White/green skin color	Green color intensity in the skin of the drumstick (white = 0, green = 5)
Lightness/darkness of meat	Intensity of meat darkness when raising the leg skin (white = 0, black = 5)
Odor	
Rancid	Intensity of rancid odor perception (fresh poultry meat odor = 0, rancid odor = 5)
Blood/liver/metallic	Quantification of metallic odor similar to that produced by fresh blood (fresh poultry meat odor = 0, metallic odor = 5)
Oregano oil	Intensity of EO[2] odor of drumstick when the pack was opened (fresh poultry meat odor = 0, EO odor = 5)
Oregano oil	Intensity of EO odor of drumstick within 1 h after opening the pack (fresh poultry meat odor = 0, EO odor = 5)

[1]A high score is indicative of a high intensity of the attribute.

[2]EO = group treated with oregano essential oil.

Table 2. The composition of *Origanum onites* oil

RRI[1]	Main compounds	%
1,280	*p*-Cymene	7.0
1,553	Linalool	3.6
1,611	Terpinen-4-ol	1.7
1,719	Borneol	1.2
1,741	β-Bisabolene	1.9
2,198	Thymol	7.4
2,239	Carvacrol	70.2
	Others	7

[1]RRI = relative retention indices.

RESULTS AND DISCUSSION

Composition of Oregano Oil

The GC-MS results indicated that the 2 phenols, carvacrol and thymol, were the major components of oregano EO (Table 2). Some researchers have reported that these components are mainly responsible for its antimicrobial activity (Adam et al., 1998; Burt, 2004).

Sensory Evaluation

On each sampling day, EO treatment resulted in color and odor and overall acceptability scores that were significantly different ($P < 0.05$) from controls. Samples treated with oregano EO were assessed with attribute scores between 2 and 10 throughout storage, whereas the controls were 0 and 16. Sensory properties of drumsticks are given in Table 3. The significant odor of oregano oil on treated drumsticks had decreased from the first to third day of storage. Based on sensory scores, it can be stated that 1.5% of oregano oil gave a characteristic desirable odor to chicken meat. Sensory data, with the exception of oregano oil odor, were in a good agreement with microbiological data. However, Chouliara et al. (2007) reported that they found different results than our findings. In that study, oregano oil was pipetted to the fresh chicken breast meat in chunks of approximate dimensions 2 × 2 × 2 cm so as to obtain final concentrations equal to 0.1% wt/wt and 1%. Due to the very strong odor of the oregano oil at the concentration of 1%, samples containing this concentration of oregano oil were not sensorially evaluated. Based on their results, the researchers reported that oregano oil gave a characteristic desirable odor and taste to chicken meat, very compatible to cooked chicken flavor, when its concentration was at 0.1%. They also found that, in contrast to our results, sensory data were not in very good agreement with microbiological data.

Microbiological Changes

The initial value of TVC (d 0) for each treated sample was approximately 5.94 log cfu/g. The TVC of PS samples reached the value of 9.01, 10.6, and 11.86 log cfu/g on the third, fifth, and seventh day, respectively, whereas the levels of that microorganisms for EO were different ($P < 0.05$) as 7.00, 7.71, and 9.43 log cfu/g on the same days. With respect to the use of oregano oil, the present results are in agreement with those of Tsigarida et al. (2000), who reported a reduction in initial microflora of beef meat fillets by 2 to 3 log cfu/g with the addition of 0.8% oregano EO. Skandamis and Nychas (2001) and Chouliara et al. (2007) also indicated a suppression of TVC in meat when oregano oil was added. In this present study, the growth pattern of psychrotrophs showed the same behavior as that of TVC (Table 4) and this finding is in agreement with those of Oral et al. (2008).

Pseudomonads (Table 4) are gram-negative bacteria comprising the main spoilage microorganisms in meat (Jay, 1986). In this study, the number of that microorganism was 8.77, 10.19, and 11.80 log cfu/g for PS samples on the third, fifth, and seventh day, respectively. On the other hand, the count of pseudomonads

Table 3. Effect of oregano essential oil on sensory properties of drumsticks stored at 4°C at d 0, 3, 5, and 7 of storage

	Scores[1]							
	0 d		3 d		5 d		7 d	
Attributes	PS	EO	PS	EO	PS	EO	PS	EO
White/yellow skin color	0.00 ± 0.00[2]	0.00 ± 0.00	2.00 ± 0.82[a]	0.00 ± 0.00[b]	2.00 ± 0.82	1.25 ± 0.50	3.00[a]	1.75 ± 0.50[b]
White/pink skin color	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.82	0.00 ± 0.00
White/green skin color	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00	1.75 ± 0.50[a]	0.00 ± 0.00[b]	3.50 ± 0.58[a]	1.75 ± 0.50[b]
Lightness/darkness of meat	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.50	0.00 ± 0.00	0.50 ± 0.58	0.25 ± 0.50	1.25 ± 0.96	0.25 ± 0.50
Rancid odor	0.00 ± 0.00	0.00 ± 0.00	1.75 ± 0.50[a]	0.00 ± 0.00[b]	3.25 ± 0.50[a]	0.00 ± 0.00[b]	5.00	4.00 ± 1.16
Blood/liver/metallic odor	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Oregano oil odor (when the pack was opened)	0.00 ± 0.00[a]	1.75 ± 0.50[b]	0.00 ± 0.00[a]	1.00 ± 0.00[b]	0.00 ± 0.00[a]	1.00 ± 0.00[b]	0.00 ± 0.00[a]	1.00 ± 0.00[b]
Oregano oil odor (within 1 h after opening the pack)	0.00 ± 0.00	0.50 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total	0.00 ± 0.00[a]	2.50 ± 0.58[b]	4.25 ± 0.50[a]	1.00[b]	7.50 ± 1.29[a]	2.50 ± 0.58[b]	13.75 ± 1.71[a]	8.50 ± 1.29[b]

[a,b]Means with different letters in the same row for each sampling day are significantly different ($P < 0.05$).

[1]PS = group treated with physiological saline (control); EO = group treated with oregano essential oil.

[2]Mean value ± SD.

was 7.04, 7.59, and 9.06 log cfu/g on the same days. Our findings showed that oregano oil resulted in approximately 2 log cfu/g reduction of pseudomonads. This is in agreement with Deans and Richie (1987), who showed that thyme oil, an EO containing similar components as oregano oil, was very effective against *Pseudomonas aeruginosa*. Some researchers reported low inhibition effects of oregano oil on pseudomonads (Elgayyar et al., 2000; Skandamis et al., 2002; Chouliara et al., 2007). However, in our study, a reduction in pseudomonads was seen.

With respect to Enterobacteriaceae (Table 4), considered a hygiene indicator, the initial counts were 2.89 and 2.90 log cfu/g for PS and EO groups. The number of that microorganism was 3.87, 4.89, and 5.48 log cfu/g for PS samples on the third, fifth, and seventh day, respectively. However, the count of Enterobacteriaceae was 3.10, 3.90, and 4.57 log cfu/g for EO groups on the same days. Similarly, Chouliara et al. (2007) reported that oregano oil at a 1% concentration level had a strong effect against Enterobacteriaceae counts of modified atmosphere packed chicken breast meat giving a reduction of more than 6 log cfu/g. Oral et al. (2008) also found that the hydrosol of wild thyme, a plant containing similar main components as oregano, was very effective against Enterobacteriaceae. In another study, it was found that oregano oil had bactericidal effects against *Escherichia coli* O157:H7 (Shekarforoush et al., 2007). Some of the other investigators who examined *Origanum vulgare* and *Thymus vulgaris* oils also reported similar findings (Dorman and Deans, 2000; Nedorostova et al., 2009). In a study conducted by Lim and Mustapha (2007), the effects of cetylpyridinium chloride and acidified sodium chlorite sprayed on the beef surfaces and tray absorbent pads against *Escherichia coli* O157:H7 were evaluated. Based on their results, the researchers reported that the application of cetylpyridinium chloride can extend the shelf life of the product. They also indicated that the use of an antimicrobial containing a tray absorbent pad that is in direct contact with the underside of a food, combined with a treated film overwrap that has contact with the top surface of the food, may help to increase product shelf life and reduce the potential for foodborne illness. Natrajan and Sheldon (2000) reported parallel results using protein- and polysaccharide-based films containing bacteriocin formulations for inhibiting salmonellae on fresh broiler skin.

In the present study, the initial yeast population of each treated sample was approximately 2.89 log cfu/g, whereas on d 3, 5, and 7 of storage, a count of 4.27, 4.55, and 5.16 and 3.43, 3.81, and 4.06 log cfu/g was recorded for PS and EO groups, respectively. The level of yeasts of treatment and control groups was different ($P < 0.05$) on d 3 and 7 (Table 4). These results are in agreement with those of Conner and Beuchat (1984) and Chouliara et al. (2007), who reported the inhibitory action of oregano oil on growth of yeasts. Brr and Mahmoud (2005) also showed that thyme extract, consisting of carvacrol and thymol as major components like oregano, had an inhibitory effect on the growth of *Candida albicans*, *Debaryomyces hansenii*, and *Saccharomyces cerevisiae*.

Finally, the initial LAB counts of both groups (Table 4) were approximately 5.46 log cfu/g and increased during the storage period. Treating with oregano EO significantly ($P < 0.05$) inhibited the growth of LAB in comparison to control groups. Other researchers also found that oregano oil had an inhibitory effect against LAB (Ouattara et al., 1997; Seydim and Sarikus, 2006; Chouliara et al., 2007). In a study conducted by Horošová et al. (2006), it was found that oregano oil had strong antibacterial activity against lactobacilli.

Physicochemical Changes

A small increase in the pH values was recorded throughout the 7 d of storage of chicken meat. The level

Table 4. Effect of oregano essential oil on microbial flora of drumsticks stored at 4°C at d 0, 3, 5, and 7 of storage

Microorganisms[1]	Groups[2]	0 d	3 d	5 d	7 d
TVC	PS	5.94 ± 0.29[3]	9.01 ± 0.37[a]	10.6 ± 0.32[a]	11.86 ± 0.37[a]
	EO	5.95 ± 0.32	7.00 ± 0.73[b]	7.71 ± 0.91[b]	9.43 ± 0.45[b]
PC	PS	5.74 ± 0.36	8.93 ± 0.38[a]	10.42 ± 0.44[a]	11.79 ± 0.42[a]
	EO	5.71 ± 0.38	6.80 ± 0.77[b]	7.66 ± 0.91[b]	9.12 ± 0.40[b]
Pseudomonads	PS	5.43 ± 0.60	8.77 ± 0.39[a]	10.19 ± 0.56[a]	11.80 ± 0.40[a]
	EO	5.39 ± 0.59	7.04 ± 0.85[b]	7.59 ± 0.92[b]	9.06 ± 0.72[b]
Enterobacteriaceae	PS	2.89 ± 0.06	3.87 ± 1.00	4.89 ± 0.03[a]	5.48 ± 0.07[a]
	EO	2.90 ± 0.06	3.10 ± 0.16	3.90 ± 0.36[b]	4.57 ± 0.26[b]
Yeasts	PS	2.89 ± 0.07	4.27 ± 0.09[a]	4.55 ± 0.17	5.16 ± 0.08[a]
	EO	2.87 ± 0.06	3.43 ± 0.34[b]	3.81 ± 0.66	4.06 ± 0.40[b]
LAB	PS	5.46 ± 0.64	8.83 ± 0.36[a]	10.35 ± 0.52[a]	11.54 ± 0.64[a]
	EO	5.44 ± 0.64	6.20 ± 0.84[b]	7.65 ± 0.93[b]	9.21 ± 0.64[b]

[a,b]Means with different letters in the same column for each microorganism group are significantly different ($P < 0.05$).

[1]TVC = total viable counts; PC = psychrotrophs; LAB = lactic acid bacteria.

[2]PS = group treated with physiological saline (control); EO = group treated with oregano essential oil.

[3]Mean value (cfu/g) ± SD.

Table 5. Effect of oregano essential oil on pH of drumsticks stored at 4°C at d 0, 3, 5, and 7 of storage

Groups[1]	0 d	3 d	5 d	7 d
PS	6.44 ± 0.11[2]	6.77 ± 0.12[a]	7.00 ± 0.00[a]	7.43 ± 0.15[a]
EO	6.44 ± 0.11	6.51 ± 0.12[b]	6.62 ± 0.10[b]	6.99 ± 0.27[b]

[a,b]Means with different letters in the same column for each sampling day are significantly different ($P < 0.05$).
[1]PS = group treated with physiological saline (control); EO = group treated with oregano essential oil.
[2]Mean value (cfu/g) ± SD.

of pH of treatment and control groups was significantly different ($P < 0.05$) on d 3 and 7 (Table 5). Silva and Glória (2002) also observed that pH increased gradually with refrigerated storage time for chicken thigh. In similar research, Mano et al. (1993) found that the increase in pH could be related to the formation and accumulation of amines and ammonia. In this study, ammonia and hydrogen sulfide evaluation was detected qualitatively on d 3 for the control group and on d 5 for the treatment group.

As a conclusion, based primarily on sensory data, the shelf life of overwrap packed fresh chicken drumsticks was approximately 3 d. Oregano EO extended product shelf life by approximately 2 d. Direct addition of EO to food will result in immediate reduction of bacterial population, and may alter the sensory characteristics of added food. Thus, incorporation of EO to absorbent pads may have supplementary applications in food packaging.

REFERENCES

Adam, K., A. Sivropoulou, S. Kokkini, T. Lanaras, and M. Arsenakis. 1998. Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. J. Agric. Food Chem. 46:1739–1745.

Appendini, A., and J. H. Hotchkiss. 2002. Review of antimicrobial food packaging. Innov. Food Sci. Emerg. Technol. 3:113–126.

Botsoglou, N. A., S. M. Grigoropoulou, E. Botsoglou, A. Govaris, and A. Papageorgiou. 2003. The effects of dietary oregano essential oil and α-tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. Meat Sci. 65:1193–1200.

Brr, A.-A. H., and Y. A.-G. Mahmoud. 2005. Anti-yeast effects of some plant extracts on yeasts contaminating processed poultry products in Egypt. Czech. J. Food Sci. 23:12–19.

Burt, S. 2004. Essential oils: Their antibacterial properties and potential applications in foods – A review. Int. J. Food Microbiol. 94:223–253.

Burt, S., and R. D. Reinders. 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett. Appl. Microbiol. 36:162–167.

Chouliara, E., A. Karatapanis, I. N. Savvaidis, and M. G. Kontominas. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf life extension of fresh chicken breast meat, stored at 4°C. Food Microbiol. 24:607–617.

Conner, D. E., and L. R. Beuchat. 1984. Effects of essential oils from plants on food spoilage yeasts. J. Food Sci. 49:429–434.

Deans, S. G., and G. Richie. 1987. Antimicrobial properties of plant essential oils. Int. J. Food Microbiol. 5:165–180.

Dorman, H. J. D., and S. G. Deans. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88:308–316.

Elgayyar, M., F. A. Draughon, D. A. Golden, and J. R. Mount. 2000. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot. 64:1019–1024.

Gill, C., and K. G. Newton. 1979. Spoilage of vacuum-packaged dark, firm, dry meat at chill temperatures. Appl. Environ. Microbiol. 37:362–364.

Hansen, R., C. Rippl, D. Midkiff, and J. Neuwirth. 1989. Antimicrobial absorbent food pad. US Patent 4 865 855. Kimberly-Clark Corporation, assignee.

Horošová, K., D. Bujňáková, and V. Kmeť. 2006. Effect of oregano essential oil on chicken lactobacilli and *E. coli*. Folia Microbiol. (Praha) 51:278–280.

Jay, J. M. 1986. Microbial spoilage indicators and metabolites. Pages 213–240 in Foodborne Microorganisms and Their Toxins. Developing Methodology. M. D. Pierson and A. Stern, ed. Marcel Dekker Inc., Basel, Switzerland.

Kim, J., M. R. Marshall, and C. I. Wei. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. J. Agric. Food Chem. 43:2839–2845.

Lim, K., and A. Mustapha. 2007. Inhibition of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* on sliced roast beef by cetylpyridinium chloride and acidified sodium chlorite. Food Microbiol. 24:89–94.

Mano, S. B., H. S. Pardi, M. F. Queiroz, and A. C. C. Sardinha. 1993. Avaliação comparative de métodos físico-químicos utilizados no exame de carne de aves (*Gallus domesticus*) resfriadas. Hig. Alim. 7:23–25.

Natrajan, N., and B. W. Sheldon. 2000. Inhibition of *Salmonella* on poultry skin using protein- and polysaccharide-based films containing a nisin formulation. J. Food Prot. 63:1268–1272.

Nedorostova, L., P. Kloucek, L. Kokoska, M. Stolcova, and J. Pulkrabek. 2009. Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. Food Contr. 20:157–160.

Oral, N., M. Gülmez, L. Vatansever, and A. Güven. 2008. Application of antimicrobial ice for extending shelf life of fish. J. Food Prot. 71:218–222.

Ouattara, B., R. E. Simard, R. A. Holley, and G. J.-P. Piette. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 37:155–162.

Ruiz, J. A., L. Guerreo, J. Arnau, M. D. Guardia, and E. Esteve-Garcia. 2001. Descriptive sensory analysis of meat from broilers fed diets containing vitamin E or β-carotene as antioxidants and different supplemental fats. Poult. Sci. 80:976–982.

Seydim, A. C., and G. Sarikus. 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food Res. Int. 39:639–644.

Shekarforoush, S. S., A. H. K. Nazer, R. Firouzi, and M. Rostami. 2007. Effects of storage temperatures and essential oils of oregano and nutmeg on the growth and survival of *Escherichia coli* O157:H7 in barbecued chicken used in Iran. Food Contr. 18:1428–1433.

Silva, C. M. G., and M. B. A. Glória. 2002. Bioactive amines in chicken breast and thigh after slaughter and during storage at 4±1°C and in chicken-based meat products. Food Chem. 78:241–248.

Skandamis, P., and G.-J. E. Nychas. 2001. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. J. Appl. Microbiol. 91:1011–1022.

Skandamis, P., E. Tsigarida, and G.-J. E. Nychas. 2002. The effect of oregano essential oil on survival/death of *Salmonella* Typhimurium in meat stored at 5°C under aerobic, VP/MAP conditions. Food Microbiol. 19:97–103.

Solomakos, N., A. Govaris, P. Koidis, and N. Botsoglou. 2008. The antimicrobial effect of thyme essential oil, nisin, and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage. Food Microbiol. 25:120–127.

Svehla, G. 1979. Vogel's Textbook of Macro and Semimicro Qualitative Inorganic Analysis. Longman, London, UK.

Tsigarida, E., P. Skandamis, and G.-J. E. Nychas. 2000. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5°C. J. Appl. Microbiol. 89:901–909.

Vanos, V., and L. Cox. 1986. Rapid routine method for detection of lactic acid bacteria among competitive flora. Food Microbiol. 3:223–234.