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Original Scientific Article

THE EFFECT OF THYME OIL ON THE SHELF LIFE OF CHICKEN BALLS DURING STORAGE PERIOD

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Summary: The effects of thyme essential oil on the shelf life of chicken balls during refrigerated storage for 12 days were examined. Treatments examined in the present study were as follows: A (control samples, untreated), B (final concentration of thyme essential oil; 0.4% w/w, added to ball), C (final concentration of thyme essential oil; 0.4% w/w, on surface of ball). The shelf-lives of samples were determined by means of microbiological, chemical, sensory, colour and texture analyses. Microbial populations in B group samples were determined to be reduced at the end of the storage period. Lactic acid bacteria (LAB) and Enterobacteriaceae were the most sensitive groups among the microorganisms examined. B group treatment samples resulted in lower microbiological counts in comparison to the control (A) and C group samples, but LAB was found to be lower in group C samples. Salmonella spp. and Staphylococcus spp. were not detected in all samples. TBA values for C group samples remained lower than 1mg MDA/kg throughout the 12 day storage period. The pH values varied between 6.4 (day 0) and 5.9 (day 12). The C group samples were found to be desirable (organoleptically acceptable) following sensory analysis. Based primarily on sensory data (taste attribute) B and C groups samples extended the product's shelf-life by ca. 4 and 6 days, respectively, as compared to the control sample. The results of the analyses of chicken balls showed no significant differences ($p>0.05$) in colour and texture among all samples.

Key words: thyme oil; chicken ball; microbiology; chemical and sensory quality; colour and texture analysis

Introduction

Poultry meat is very popular in the world and nowadays it is consumed increasingly in many countries. Chicken meat has a low cost of production, low fat content and high nutritional value. However, poultry products are pathogenic and spoilage microorganisms (*Salmonella*, *Yersinia enterocolitica*, such as) and remains a significant concern for consumers and public health officials worldwide. High consumption of poultry products leads to concerns in the industry about shelf-life extension. *Escherichia coli* and *Staphylococcus aureus* have been used in poultry products to assess microbiological safety, sanitation conditions during processing and the retention of the quality of the product (1, 2). Natural food preservatives are used to ensure protection from both spoilage and pathogenic microorganisms (3). Recently, herb and spice extracts have been a particular focus. These extracts have been used to improve sensory characteristics and to extend the shelf-life of foods (4). There has been a great interest within the food industry during the last decade regarding the antimicrobial substances used in foods, such as thyme oil (5). Thyme (Thymus vulgaris L.) is used both as a spice and a condiment. The essential oil of thyme (Thymus vulgaris L.) has a significant rate of fungal and antibacterial activity with

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strongly inhibited lipid peroxidation and high off radical scavenging (6, 7).

This study was carried out to investigate the effects of thyme oil (on the surface of and as an additive to chicken balls) on the quality of chicken balls during storage.

Material and methods

Preparation of chicken balls

Chickens were purchased from a local market. After washing, the skin and the apparent fat and bone tissues were removed. The meat was treated using a kitchen food processor with a pore size of 5 mm. The chicken ball included 85% chicken mince, 2% salt, 0.5% cumin, 0.5% red pepper, 10% onion and 2% garlic. The ingredients were homogenized with a kitchen blender. The spices were purchased from Bağdat Company (Istanbul, Turkey). The chicken meat batter was shaped into balls using stainless steel equipment (approximately 20 g).

Preparation of experimental groups

The following lots of samples were prepared: The first lot of samples comprised the controls (aerobic packaging, group A). Lot two consisted of samples with thyme oil (Sigma, Germany) 0.4% (B and C group). The B group which thyme oil %0.4 added batter chicken meatball (considering amount of batter meat ball). The preparation of C group, thyme oil was surface balls (final concentr ations equal to 0.4% w/w). Thyme oil was added undiluted using a micropipette. The thyme oil was massaged onto the product, so as to get even distribution of the oil using gloved fingers (to avoid cross-contamination of samples and also transmission of food poisoning organisms). After three groups of chicken balls were produced, they were refrigerated at 4 ± 1 °C in straphor trays covered with aluminium foil for testing microbiological, chemical, sensory, colour measurement and texture analysis on 0, 3, 6, 9 and 12 days of the storage.

Microbiological analysis

Approximately 25 g of the chicken meat balls were sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag, containing 225 ml of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a Lab Blender 400 Stomacher at room temperature. Microbiological analyses were conducted using standard microbiological methods (8). The amount of 0.1 ml of these serial dilutions of chicken homogenates was spread on the surface of dry media. Total viable counts (TVC) were determined using Plate Count Agar (PCA, Merck code 1.05463), after incubation for three days at 30 °C. *Pseudomonads* were determined on cetrimide fusidin cephaloridine agar (Oxoid code CM 559, supplemented with SR 103) after incubation at 25 °C for 2 days (9, 10). For members of the family *Enterobacteriaceae*, 1.0 ml sample was inoculated into 5 ml of molten (45 °C) Violet Red Bile Glucose Agar (Oxoid code 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. Lactic acid bacteria (LAB) were determined on de Man Rogosa Sharpe Medium (Oxoid code CM 361) after incubation at 25 °C for 5 days. Yeasts and moulds were enumerated using Rose Bengal Chloroamphenicol Agar (RBC, Merck code 1.00467) after incubation at 25 °C for 5 days in the dark. Isolation of *Salmonella* spp. was carried out in four stages. After incubation at 35–37 °C for 16–20 h for the pre-enrichment step, 0.1 and 1 ml of the homogenate was transferred to RV (Rappaport Vassiliadis, Merck) and Selenite Cystein Broth (Merck) for selective enrichment with an incubation period of 42 and 35 °C for 24 h, respectively. After incubation, a loopful from each tube was streaked on Brillant Green Phenol Red Lactose Agar (Merck) and Bismuth Sulfite Agar (Merck). These plates were incubated for 20–24 h at 35 °C and checked for typical colonies. Five colonies were selected for biochemical tests and were grown in Nutrient Agar (Oxoid) at 35 °C for 18–24 h (11).

Baird Parker Agar (Merck) was used for the estimation of *Staphylococcus* spp. counts. After incubation at 35 °C for 45–48 h, typical colonies were tested for the detection of coagulase production. (12). Duplicate plates were spread from each dilution of 0.1 ml. All plates were examined for typical colony types and morphology characteristics associated with each growth medium.

Chemical analysis

The pH value was recorded using a pH meter. Chicken samples were thoroughly homogenized

with 10 ml of distilled water and the homogenate used for pH determination (13). Thiobarbituric acid (TBA) was determined according to the method proposed by Pearson (14).

Sensory analysis

The door and taste of cooked chicken balls were evaluated by a panel of seven judges, experienced in chicken ball evaluation on each day of sampling. Chicken ball samples were cooked individually in an oven, for 5 min and were then immediately presented to the panellists. The scale points were: excellent, 5; very good, 4; good, 3; acceptable, 2; poor (first off-odour, off-taste development), 1; a score of acceptability (15).

Colour measurement

Colorimetric measurements of cooked chicken balls were determined in triplicate using a Colorimeter (Minolta spectrophotometer CM 3500d, Japan). The colour reading includes lightness (L), redness (a) and yellowness (b). The equipment was standardized with a white colour Standard. Five replicate measurements were taken for each sample, following the guidelines for colour measurements from American Meat Science Association (16).

Texture profile analysis (TPA)

Texture measurement of cooked chicken balls were used a Texture Analyzer (TA.XTPlus Stable Micro Systems, UK). Texture Profile Analysis (TPA) was used to determine hardness, cohesiveness, chewiness and springiness (17). This test was carried out by using compression plate with a diameter of 75 mm. The mean of five measurements was taken for each hardness, cohesiveness, chewiness and springiness.

Statistical analysis

Analysis of the data was conducted using a Statistical Analysis System (SAS) packages software. Values between groups and within groups-between days were compared. Data were subjected to variance analysis in accordance with 3 x 5 x 3 x 2 factorial design and in terms of fix effects and inter-variable interactions so that "repetition number x sampling time x test groups x number of samples examined at one instance from each test group". According to the General Linear Models (GLM) procedure, the Fisher's smallest squares average (LSD) test was used. Standard deviation figures of all averages were calculated (18). The alpha value was determined as 0.05.

Result

Microbiological changes are given in Fig.1. The thyme oil treated groups had a lower count TVC. The control samples reached a TVC count of upper 7 log cfu/g storage day. Listeria spp. and *Salmonella* spp was not detected in any of the groups. Whereas on the 3rd day of storage, the number of *Pseudomonads* in group B was 1 log cfu/g, no reduction was determined in the *Pseudomonads* values of the C group ($P > 0.05$). On day 9 of storage, group B was 3.11 log cfu/g ($P<0.05$), while groups C and A were determined as 5.11, 7.37 log cfu/g. Initial populations of LAB were ca. 3.42 and increased to reach counts of 7.13 log cfu/g on day 9 of storage, group A samples ($P<0.05$). However, significantly lower LAB counts ($P < 0.05$) were recorded for C samples during the entire storage period under refrigeration. The *Enterobacteriaceae* counts were determined by 7.56 log cfu/g, 2.13 log cfu/g and 2.59 log cfu/g group A, B and C on day 9, respectively. The yeast and mould counts were initially (0 day) determined as 2.78 log cfu/g all samples. In the A group of samples, the yeast and mould count was shown to have increased steadily during storage time (2.78, 4.26, 4.6 and 5.13 log cfu/g, 0, 3, 6 and 9 day, respectively). B and C group samples were found to contain a yeast and mould count of <10 log cfu/g, on the third and sixth days of storage. B group samples were not observed on day 9 while the yeast and mould counts were found to be 2.56 log cfu/g, at the end of storage. C group samples were found to be 2.19, 2.43 log cfu/g, on days 9 and 12, respectively.

Changes of pH values are shown in Fig. 2a. Values of pH initial were recorded for groups A, B and C as 6.17, 6.21 and 6.19, respectively. The TBA values for all chicken ball treatments are given in Fig. 2b. The TBA values for the control group varied between 0.3 and 1.8 mg MDA/kg. A very low lipid oxidation value of between 0.3 and 0.9 mg MDA / kg was determined for the treatment group.

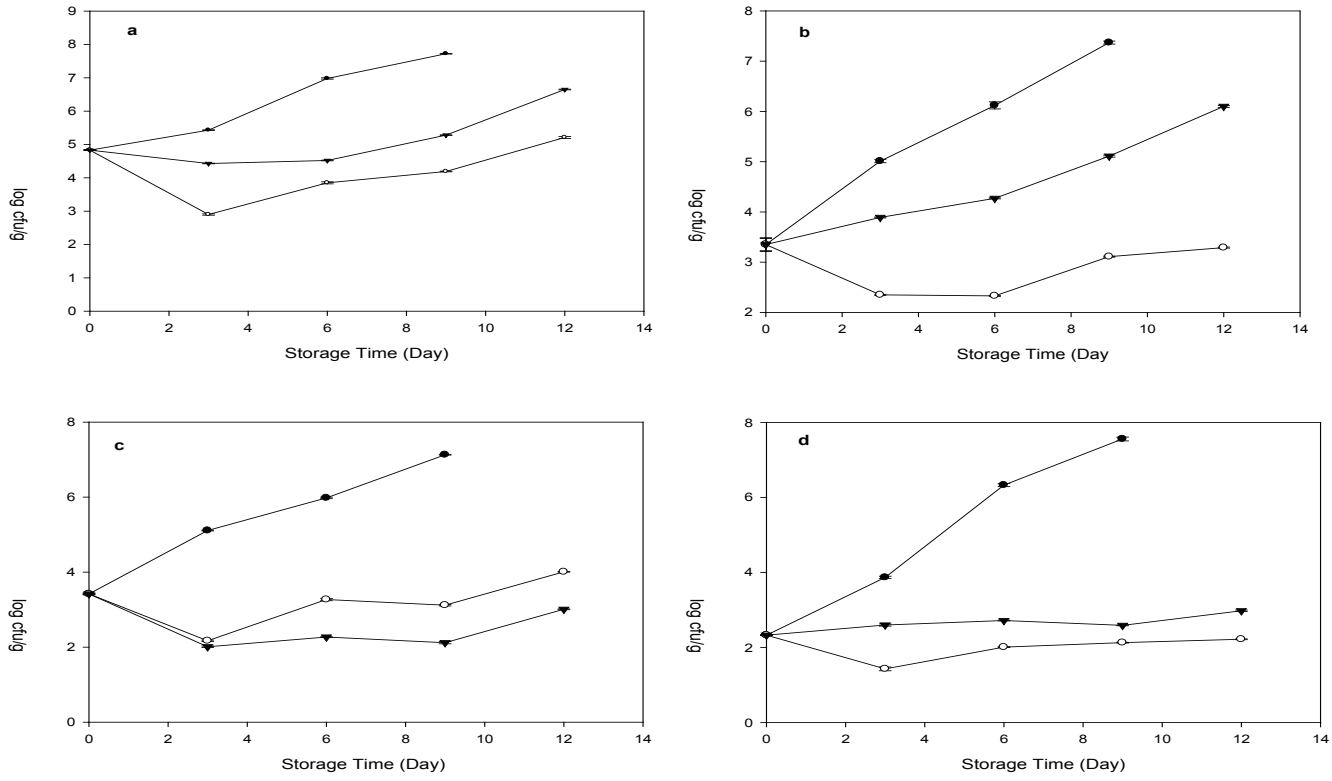

Figure 1: Changes (log cfu/g) in (a) Total Viable Counts (TVC); (b) *Pseudomonas* spp.; (c) LAB and (d) *Enterobacteriaceae*, control (A), batter with added thyme EO 0.4% (B) and ball surface thyme EO 0.4% (C). Each point is the mean of three samples taken from two replicate experiments (n: 3 x 2: 6). Error bars show SD. —●— A —○— B —▼— C

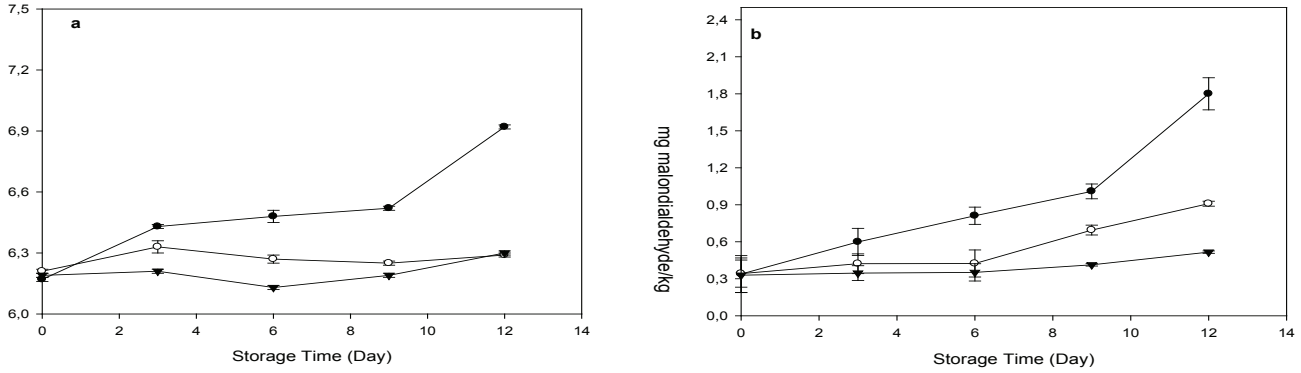

Figure 2: Changes of pH (a) and TBA (b) values of chicken balls, control (A), batter with added thyme EO 0.4% (B) and ball surface thyme EO 0.4% (C). Each point is the mean of three samples taken from two replicate experiments (n: 3 x 2: 6). Error bars show SD. —●— A —○— B —▼— C

The sensory properties (odour and taste) of the cooked chicken balls are given in Fig. 3a-b. Sensory analysis was carried out until the 6th day of storage for control group samples and until the 12th day of storage for treatment group samples. Due to the microbiological quality of the control group samples, exceeded limit values were not subject to sensorial evaluation. Taste attribute has usually been a more sensitive parameter than odour; therefore, taste attribute was used for the sensory evaluation of the thyme products and the determination of sensorial shelf-life in the present study. On day 0 of storage, the cooked chicken balls had a pleasant taste and odour. The presence of thyme oil (on ball surface) in the samples produced a very pleasant taste ($P<0.05$).

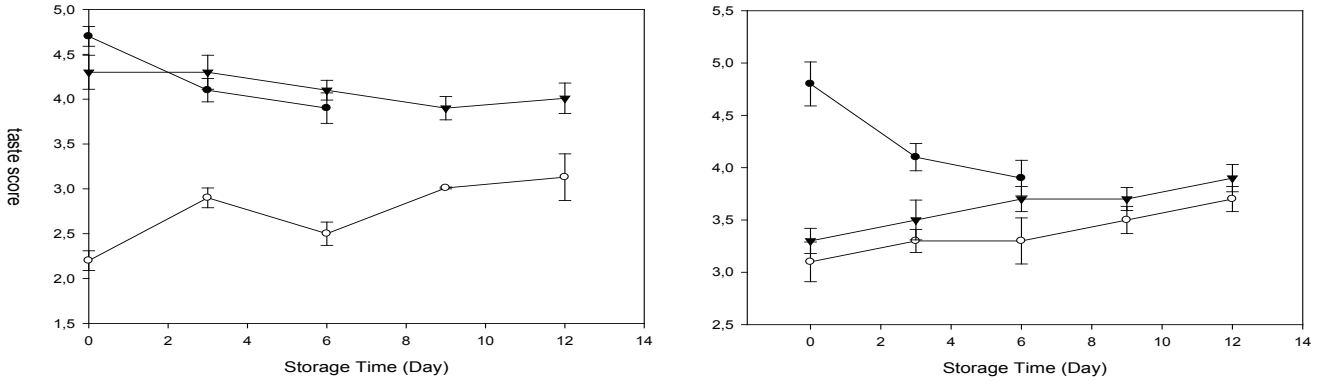

Figure 3: Changes in the taste (a) and odour (b) of chicken balls, control (A), batter with added thyme EO 0.4% (B) and ball surface thyme EO 0.4% (C). Each point is the mean of three samples taken from two replicate experiments (n: 3 x 2: 6). Error bars show SD. —●— A —○— B —▼— C

Table 1 shows the colour measurement results of chicken balls. All chicken balls varied insignificant in their L, a and b values ($p>0.05$).

Table 1: Colour properties of experimental chicken balls

Group	Storage time (day)					
	Analysis	0	3	6	9	12
A	L*	75.42±0.46 ^{a,z}	75.42±0.33 ^{a,z}	75.33±0.56 ^{a,z}	75.56±0.41 ^{a,z}	75.33±0.39 ^{a,z}
	a*	0.90±0.09 ^{a,z}	0.90±0.06 ^{a,z}	0.91±0.4 ^{a,z}	0.93±0.21 ^{a,z}	0.90±0.36 ^{a,z}
	b*	16.77±0.25 ^{a,z}	16.77±0.21 ^{a,z}	16.81±0.56 ^{a,z}	16.85±0.43 ^{a,z}	16.77±0.21 ^{a,z}
B	L*	75.33±0.19 ^{a,z}	76.16±0.26 ^{a,z}	76.01±0.31 ^{a,z}	75.19±0.18 ^{a,z}	75.96±0.16 ^{a,z}
	a*	0.91±0.04 ^{a,z}	0.90±0.02 ^{a,z}	0.96±0.05 ^{a,z}	0.91±0.04 ^{a,z}	0.93±0.04 ^{a,z}
	b*	17.13±0.22 ^{a,z}	17.09±0.19 ^{a,z}	17.33±0.24 ^{a,z}	17.13±0.19 ^{a,z}	17.21±0.21 ^{a,z}
C	L*	76.01±0.56 ^{a,z}	76.81±0.54 ^{a,z}	76.16±0.42 ^{a,z}	76.21±0.36 ^{a,z}	76.33±0.29 ^{a,z}
	a*	0.93±0.01 ^{a,z}	0.91±0.06 ^{a,z}	0.94±0.02 ^{a,z}	0.90±0.01 ^{a,z}	0.92±0.03 ^{a,z}
	b*	16.36±0.12 ^{a,z}	16.22±0.21 ^{a,z}	16.43±0.23 ^{a,z}	16.21±0.19 ^{a,z}	16.23±0.21 ^{a,z}

Texture analysis results for chicken balls are shown as in Table 2. Results regarding hardness, cohesiveness, springiness and chewiness value during storage time were determined to be similar for all groups, therefore, the mean values have been given.

Table 2: Textural properties of experimental chicken balls.

Group	Hardness (kg)	Cohesiveness(mm/mm)	Springiness (mm/mm)	Chewiness (kg/mm)
	M ± SD			
A	3.73±0.22	0.67±0.01	12.56±0.42	31.33±1.56
B	3.96±0.19	0.65±0.04	12.93±0.46	35.21±1.41
C	3.52±0.28	0.69±0.02	12.87±0.52	32.56±1.82

Discussion

The changes in total viable count (TVC) of the chicken meat balls are given Fig. 1a. The initial value of TVC (day 0) for fresh chicken meat was ca. 4.3 log cfu/g, indicative of good quality chicken meat (19). TVC reached a value of 7 log cfu/g, considered as the upper microbiological limit for good quality fresh poultry meat, as defined by the ICMSF (2), on day 6 for the control samples. Samples treated with thyme oil in groups never reached the limit of 7 log cfu/g during the 12 day storage period. The group B samples were determined to be 4.83 log cfu/g 0 day. This value decreased about 2 log cfu/g on day 3 of storage and reached 5.21 log cfu/g at the end of storage. There was no significant change to the C groups sample during storage. Thyme oil in the additive ball was more effective than in the surface ball on reducing TVC. Thyme oil resulted in a shelf-life extension of six days as compared to the control samples. In related studies, Giatrakou (7) mentioned a shelf-life extension of 2 days after the application of thyme oil 0.2% v/w on chicken, while Ergezer (20) reported that TVC in chicken balls was reduced with whey powder stored at -18 °C for three months.

Listeria spp. and *Salmonella* spp were found in one of the samples. They are also in agreement with those reported by Yavaş et al. (21), who reported no determination in ground chicken balls after 21 days of storage under modified atmosphere pressure (MAP).

Pseudomonads (Fig. 1b) are Gram-negative bacteria, comprising the main spoilage microorganisms in meat (22). In other studies, the combined use of thyme oil on chicken products stored aerobically at 4 °C, resulted in about 8.0 log cfu/g of the final population of pseudomonas (6). Deans and Richie (23) showed that thyme oil was very effective against *Pseudomonas aeruginosa*, in a study where the inhibitory properties of ten plant essential oils were tested using an agar diffusion technique. This is in agreement with Elgayyar et al. (24) who reported that oregano essential oil was less effective in inhibiting P. *aeruginosa* compared to other microorganisms.

Lactic acid bacteria (LAB) (Fig. 1c) facultative anaerobic species were found to be members of the microbial flora of the chicken product. Of all the antimicrobial treatments in this study, thyme oil on surface of ball treatment proved to be the most effective in inhibiting the growth of LAB, almost stable during the storage period. For Georgantelis et al. (25), the combined use of rosemary essential oil and chitosan on fresh pork sausages stored aerobically at 4 °C, resulted in a 2.0 log cfu/g reduction of the final population of LAB. In another study, LAB count reduced by 0.2% in the chicken balls with thyme oil during storage (6).

With respect to *Enterobacteriaceae* (Fig. 1d), considered as a hygiene indicator (26), the initial counts of 2.33 log cfu/g were indicative of good quality chicken meat. *Enterobacteriaceae* grew under essential oil applications at a slower rate than others. This is in agreement with the results of Choularaia et al. (27), who reported that oregano oil had a strong effect in the reduction of *Enterobacteriaceae* counts. On day 9 of storage, the use of thyme oil had practically no effect on *Enterobacteriaceae* counts ($p > 0.05$). On the same day, the *Enterobacteriaceae* counts were determined by 7.56 log cfu/g, 2.13 log cfu/g and 2.59 log cfu/g in groups A, B and C, respectively. Giatrokau et al. (7) reported that *Enterobacteriaceae* of chicken products were inhibited in the presence of thyme oil packaging.

As a result, the initial population of yeasts and moulds were very low (2.78 log cfu/g) and no population was determined on day 3 and 6 of storage for B and C group samples. The balls with added thyme oil were a lot more effective in reducing yeast populations in comparison to surface added balls ($P<0.05$). Yeast and mould were not determined in B and C group samples on day 9 of storage, whereas 5.3 log cfu/g was determined for the A group samples on the same day. Conner and Beuchat (28) reported a strong inhibitory action of oregano and thyme oils on the growth of yeast. The above results are in agreement with those of Giatrakou et al. (7), who reported that thyme oil 0.2% significantly lower yeasts-moulds counts than the control samples during the storage.

The microbiological analysis was performed on B and C groups for day 12, while group A was done until day 9.

Kayışoğlu et al. (29) reported a pH value of 6.1 for raw chicken doner kebab, this situation is similar to our finding. The addition of thyme oil resulted in a slight increase in pH values. The group A samples increased pH value during storage ($P<0.05$). Georgantelis (25) reported higher pH values for Greek-style sausages, containing chitosan and rosemary extract.

Choluaria et al. (27) who reported TBA values of 0.1–0.9 mg MDA / kg meat for chicken product after 25 days of storage. Thyme oil significantly affects the degree of lipid oxidation under present experimental conditions, given the antioxidant properties of this essential oil. Kim et al.(5) reported TBA values of 0.13–0.68 mg MDA/ kg meat for turkey and pork after seven days of storage, and these figures are similar to our findings. The thyme oil application of samples showed slightly higher values of TBA. Botsoglou et al. (4), who reported a threefold reduction in the degree of lipid oxidation (0.6-0.2) in turkey meat treated 200 mg/kg oregano oil, this is in contrast our results.

According to Choluaria et al.(27) the addition of thyme oil to chicken products results in a more acceptable odour and flavour as compared to the untreated samples.

Colour parameters (L*, a*, and b*) and TPA values in samples containing the thyme oil were not affected during storage. Changes in colour parameters and TPA values were statistically insignificant (P>0.05) in all samples.

As a result, it was determined in this study that thymol prolongs the shelf life of chicken meat balls. The effect of essential oil is already known. However, thyme oil added to chicken balls is as an effective method as applying it to the surface. Moreover, the taste of chicken balls with added thyme oil on the surface was probably better. This method may be preferred because it is safe, practical and a positive sensory feature. This method may be used to protect other foods as well.

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VPLIV TIMIJANOVEGA OLJA NA ROK UPORABNOSTI PIŠČANČJIH KROGLIC V OBDOBJU SKLADIŠČENJA

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Povzetek: Ugotavljali smo učinke timijanovega eteričnega olja na rok uporabnosti piščančjih kroglic v 12-dnevnem obdobju skladiščenja. Uporabili smo naslednje vzorce: A (kontrolni vzorci, netretirani), B (0.4% koncentracija eteričnega olja timijana dodana kroglici), C (0.4% koncentracija eteričnega olja timijana, nanesena na površino kroglice). Obstojnost vzorcev smo ugotavljali s pomočjo mikrobioloških in kemičnih analiz ter z zaznavo barve in teksture. V skupini B se je mikrobna populacija na koncu obdobja skladiščenja zmanjšala. Med opazovanimi mikroorganizmi so mlečnokislinske bakterije (LAB, angl. lactic acid bacteria) in enterobakterije najbolj občutljive. V skupini B je bilo ugotovljenih manj mikroorganizmov kot v kontrolni skupini (A) in skupini C, vendar je bilo ugotovljeno, da je vrednost LAB nižja v skupini vzorcev C. *Salmonella* spp. in *Staphylococcus* spp. nista bili odkriti v nobenem vzorcu. Skupno število bakterij vzorcev C je ostalo pod 1 mg MDA / kg v celotnem 12 dnevnem obdobju shranjevanja. pH vrednosti so se gibale med 6.4 (dan 0) in 5.9 (dan 12). Po izvedeni senzorični analizi je bilo ugotovljeno, da je skupina vzorcev C organoleptično sprejemljiva. Na osnovi senzorične analize (okus) je bilo ugotovljeno, da se je vzorcem skupin B in C podaljšal rok uporabnosti za 4 oz. 6 dni v primerjavi s kontrolnimi vzorci. Analiza piščančjih kroglic vseh vzorcev ni pokazala značilnih razlik ($p > 0,05$) v barvi in teksturi.

Ključne besede: timijanovo olje; piščančje kroglice; mikrobiologija; kemična in senzorična kakovost; analiza barve in teksture