



Effect of Rosemary Essential Oil Coated Vacuum Packaging on the Quality of Chicken Meatballs at +4°C

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<p><i>Research Article</i></p> <p>Received : 30/08/2019 Accepted : 01/11/2019</p> <p>Keywords: Chicken meatball Rosemary essential oil Quality parameters Vacuum packaging Shelf life</p>	<p>The aim of this study was to investigate preservation of the microbiological, chemical and sensory quality of chicken meatballs during storage time by using rosemary essential oil (REO) coated vacuum packaging materials at +4°C. The treatments of chicken meatballs examined in the present study were done by vacuum packaging and packaging materials were prepared by using REO coating 0.3% for group A, 0.5% group B and control group without any additive. The chicken meatballs were analyzed for microbiological (Psychrophilic total viable counts, <i>Enterobacteriaceae</i>, Lactic acid bacteria and Yeast/Mold), chemical (pH and thiobarbituric acid values) and sensory (appearance, taste and general acceptability) parameters. In the study, the microbiological quality of chicken meatballs in samples coated with vacuum packaging with REO had better shelf life compared to control group. The REO 0.3% treatment group samples resulted in a shelf life extension for 9 days compared to the control group samples with a shelf life of 5 days. Thiobarbituric acid values were found to be lower in REO coated groups compared to control. This study was shown that, vacuum packaging materials coated with REO are effective against microbial growth and lipid oxidation and improves sensory qualities of chicken meatballs.</p>

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Introduction

Chicken meat and chicken based meat products are highly consumed due to their short preparation times, low cost and higher nutritional values (Magdelaine et al., 2008). Despite high consumption of chicken based meat products (i.e. chicken meatballs); the food industry is seeking newer technologies to improve shelf life of chicken based meat products (Vasilatos and Savvaidis, 2013).

Packaging is done by incorporating active compounds to packaging materials in order to preserve food quality, to extend the shelf life of food, and to improve its safety and sensory quality properties (Vermeiren et al., 2002; Muppala et al., 2014). Antimicrobial and antioxidant packaging is applied to food to inhibit development of food borne pathogens and to reduce or eliminate spoilage (Han, 2005). Most recent practice in packaging is the combined use of packaging material and antimicrobial agents in order to control microbial development on food surfaces. In such systems, the antimicrobial materials can be prepared by incorporating the antimicrobial agent either into the packaging material or by coating the active compound on the surface of the packaging film (Suppakul et al., 2003).

Nowadays, combined use of packaging materials with natural antimicrobial agents in order to extend the shelf life of foods is suggested for increasing demands of consumers for using healthier and natural preservatives in food (Magdelaine et al., 2008). For these purposes natural essential oils from rosemary, thyme, and oregano plants have found wide use due to their antimicrobial, antifungal and antioxidant properties (Ntzimani et al., 2011; Can et al., 2014; Pavelkova et al., 2014; Liana-Ruiz-Cabello et al., 2015). Particularly rosemary (*Rosmarinus officinalis L.*) has found a frequent use in and preferred by the food industry due to its rich antioxidant contents and lower amounts of color and odor compounds (Bozin et al., 2007). Antioxidant and antimicrobial activities of rosemary essential oil (REO) are due to bioactive ingredients such as carnosol, carnosic acid, rosmanol, rosmadiol, epirosmanol, rosmadiophenol and rosmarinic acid (Riznar et al., 2006; Bozin et al., 2007).

The aim of this study was to investigate preservation of the microbiological, chemical and sensory qualities of chicken meatballs during storage time by using REO coated vacuum packaging materials at +4°C.

Materials and Methods

Preparation of Chicken Meatballs

Chicken carcasses used in the study were obtained from a local slaughterhouse and washed then drained. Skinned and deboned chicken meats were minced in 3 mm diameter meat grinder. Chicken meatballs mix was prepared by adding both 2% kitchen salt (NaCl) and 2% semolina into minced meat. Meatballs mix was portioned into 45 pieces of meatball weighing 20 g each and divided into three groups.

Preparation of Packaging Material

Preparation of packaging material was prepared by using water soluble REO (Herbalox® Seasoning). One hundred percent oil of rosemary (containing 9.2% carnosic acid) was purchased from Kalsec® Inc. (Kalamazoo, MI-USA). In order to prepare packaging material, low density polyethylene with a thickness of 12 µm, was cut into foils measuring 30 cm × 20 cm =600 cm². The plastic material was spread out and the required amount of REO solution to reach 0.3% (0.3 ml for 1 cm²) and 0.5% (0.5 ml for 1 cm²) were poured into plastic packaged for each group (Each group was contained of five chicken meatballs about 20 grams). REO solution was then well distributed over the surface with a brush.

Packaging

The chicken meatballs were packaged in the plastic material (Group A containing 0.3% REO; Group B containing 0.5% REO; Control group without REO) and then placed in vacuum packaged bags in high barrier nylon polyethylene at 99% vacuum. The chicken meatballs stored at +4°C and analyzed randomly on days 0, 3, 5, 7 and 9 of storage time in terms of microbiological, chemical and sensory parameters. Analyzes were repeated three times.

Microbiological Analysis

Ten grams of samples were aseptically weighed and homogenized in a Stomacher (Lab Blender 400, UK) for 2 min in 90 ml of sterile Maximum Recovery Diluent (MRD, Merck, 1.12535). Further decimal dilutions to 10⁻⁶ were made using same diluents. One ml from each dilution of samples was taken to inoculate on plates as twin series and at the end of incubation, plates having 30 to 300 colonies were taken into evaluation (Harrigan, 1998).

For total psychrophilic bacteria counts, Plate Count Agar (PCA, Merck, 1.05463) was used. Plates were incubated at 7±1°C for 10 days. For the *Enterobacteriaceae* counts, Violet Red Bile Glucose Agar (VRBG, Merck, 1.10275) was used. Plates were incubated 37±1°C for 24 hours. Lactic acid bacteria were determined on de Man Rogosa Sharpe Agar (MRS, Merck, 1.10660), after incubation 30±1°C for 48-72 hours. Yeast/mold counts were determined by inoculation on Potato Dextrose Agar (PDA, Merck, 1.10130) containing 10% tartaric acid and laid to incubation at 25±1°C for 3-5 days.

Chemical Analysis

The pH value was determined according to the method of AOAC (1990). The thiobarbituric acid (TBA) value is expressed according to the methods of Tarladgis et al.

(1960) and the amount of TBA was calculated as milligrams of malondialdehyde (MDA) per kg chicken meatball samples.

Sensory Evaluation

For sensory analysis, chicken meatball samples were cooked individually in a pan fried for 10 min and immediately presented to the panelists. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature, and humidity. The samples were tested by eight panelists in small aluminum trays. The quality of each sample was classified using characteristics to describe the appearance, taste and general acceptability. A hedonic scale from 1 to 5 was used to evaluate chicken meatball samples: 1 - very bad, 2 - bad, 3 - normal, 4 - good and 5 - very good (Kurtcan and Gönül, 1987).

Statistical Analysis

Analysis of the data was conducted using Statistical Analysis System (SAS) package programmed. Values between groups and within group-between days were compared. Data were subjected to variance analysis in accordance with 3 × 1 × 3 × 1 factorial design and in terms of fix effects and inter-variable interactions so that "repetition number × sampling time × test groups × number of samples examined at one instance from each test group". According to General Linear Model procedure, Fisher's smallest squares average (LSD) test was used. Standard deviation figures of all averages were calculated. P<0.05 was considered as statistically significant.

Results and Discussion

For total psychrophilic bacteria counts which are the indicator of food spoilage in cold preservation was found to be at 2.8 log₁₀ cfu/g for all groups on day 0. This value increased during storage and the highest amount of for total psychrophilic bacteria counts were found to be at 7.3 log₁₀ cfu/g for the control group at 7th day. For total psychrophilic bacteria count values for 0.3% and 0.5% REO coated groups were found to be at 5.7 and 4.8 log₁₀ cfu/g, respectively at 7th day. The chicken meatballs reached a total psychrophilic bacteria count value of 7 log₁₀ cfu/g (ICMSF, 1986) which was considered to be the upper acceptability limit for fresh meat, the control group exceeded this limit (7.3 log₁₀ cfu/g) on 7th day, whereas REO coated groups A and B didn't reach this limit for the duration of the storage at 9th day. This was possibly due to antimicrobial effects of REO. When these results were evaluated, it was determined that the control group was spoiled at 7th day of storage. When total psychrophilic bacteria counts were compared between groups, the difference between control and groups A and B in 3rd and 7th days was observed as significant (P<0.05)(Table 1).

Enterobacteriaceae count which is an indicator of hygienic quality of food products (Mexis et al., 2009) was determined to be as 1.3 log₁₀ cfu/g for all groups on day 0. *Enterobacteriaceae* can grow and produce high quantities of unpleasant odors H₂S in vacuum packaged high pH meats (Zhang et al., 2016). *Enterobacteriaceae* was found to be considerably lower in REO coated groups compared to the control group. *Enterobacteriaceae* was found to be highest at 4.2 log₁₀ cfu/g at 7th day of storage in the control

group. Meanwhile, a slow development in terms of *Enterobacteriaceae* was observed in 0.3% and 0.5% REO coated groups A and B, this value reached 3.9 and 3.1 log₁₀ cfu/g on the 9th day of storage, respectively. According to the *Enterobacteriaceae* counts, the difference between samples from control and group A and samples from group B in 5th and 7th days was observed as significant (P<0.05).

REO coated 0.5% packages (B) were found to be more effective on *Enterobacteriaceae* count. Zhang et al. (2016); found that raw chicken meats prepared by addition of 1% rosemary extract, had an *Enterobacteriaceae* count of 4.46 log₁₀ cfu/g on the 15th day of storage at +4°C. It is thought that this difference arises from the initial microbial load of chicken meat used in the study, the location from where the chicken meat was obtained along with the processing and storage methods.

The Lactic acid bacteria (LAB) counts are facultative anaerobe microorganisms that can grow in both aerobic and anaerobic conditions (Patsias et al., 2008). The LAB counts are responsible for spoilage of cold preserved and vacuum packaged chicken meats. The counts were determined to be at 1.9 log₁₀ cfu/g for all groups on day 0. The control group reached its highest value of 5.9 log₁₀ cfu/g on the 7th day, a decrease of about 3 log₁₀ cfu/g was observed in the 0.3% and 0.5% REO applied groups (P<0.05). The LAB counts obtained from REO coated groups were lower than the control group (P<0.05). Ntzimani et al. (2010); reported that LAB counts were lower in 0.2% rosemary extract coated vacuum packaged semi-cooked coated chicken meats. Zhang et al. (2016); reported that LAB counts were reached 5.47 log₁₀ cfu/g on raw chicken meats preserved with 1% rosemary extract at +4°C on the 15th day after storage. Lactic acid bacteria counts were determined to be lower in group A and B in vacuum packages were used 0.3% and 0.5% REO. This result indicating that the REO had a significant effect on the growth of LAB.

Yeast/Mold counts which are parts of natural aerobic flora and one of reasons for spoilage were initially determined to be at 2.6 log₁₀ cfu/g for all groups. Yeast/Mold counts were found to be highest at 7.0 log₁₀ cfu/g at 7th day of preservation in the control group. Yeast/Mold counts in 0.3% and 0.5% REO coated groups A and B were found to be as 3.2 and 3.1 log₁₀ cfu/g, respectively (P<0.05). According to the Yeast/Mold

counts, the difference between control and groups A and B in 5th and 7th days was observed as significant. Patsias et al. (2008) reported that the initial Yeast/Mold counts of chicken fillets stored at +4°C was 2.9-3.0 log₁₀ cfu/g and this value were increased to 6.3 log₁₀ cfu/g at 9th day storage. However it was reported that Yeast/Mold counts reach 3.0-3.6 log₁₀ cfu/g at 15th day of storage on chicken fillets when packaged in with modified atmosphere packaging. In this study, Yeast/Mold counts were found to be at 3.8-2.9 log₁₀ cfu/g at 9th day of storage at +4°C in 0.3% and 0.5% REO coated vacuum packaged chicken meatballs. Microbiological evaluation of control group at 9th day was not included as maximum tolerable levels were exceeded at 7th day. Therefore, microbiological evaluation was not conducted at this day.

The initial pH value for chicken meatballs was measured as 6.50. The pH value of the control group was found to increase from 6.50 to 6.92 at the end of the storage period. In a previous study in raw chicken meat samples in China by Zhang et al. (2016) the initial pH values were increased to chicken meat during refrigerated storage at 4 °C for 15 days and authors noted that the pH increase of the control samples may have been caused by the utilization of amino acids by bacteria, which are released during protein degradation because the stored glucose has been depleted (Zhang et al., 2016). In the current study, no significance in pH changes was observed from REO coated groups of A and B during the storage period. Statistical differences between REO coated groups A and B to control group (pH 6.92) at 7th day of storage were found to be significant (P<0.05). However, intergroup changes were insignificant (P>0.05). Coating with different REO percentages did not present any significant change in pH values of meatballs during storage period (Table 2) (P>0.05). Some researchers reported that no pH value changes were determined during storage periods of chicken meats treated with various antimicrobial agents (Naveena et al., 2013; Rocio Teruel et al., 2015). However, Zhang et al. (2016) recently showed the spice extract treatments inhibited the increase in pH to some extent during the storage period. In addition to treatment with rosemary extract had the best effect which caused the pH to reach a level of only 5.48 compared with that measured for the control (C) 6.66 sample.

Table 1 The results of microbiological analyses of chicken meatballs during storage period at + 4°C (mean±s.d)

Microorganisms (log ₁₀ cfu/g)	Group	Storage time (Day)				
		0	3	5	7	9
Total Psychrophilic Bacteria	C	2.8±0.13 ^d	4.2±0.03 ^{c,x}	5.3±0.07 ^{b,x}	7.1±0.03 ^{a,x}	-
	A	2.8±0.13 ^b	3.6±0.01 ^{b,y}	4.8±0.01 ^{ab,x}	5.7±0.01 ^{a,y}	6.9±0.01 ^a
	B	2.8±0.13 ^b	2.8±0.01 ^{b,z}	3.1±0.01 ^{b,y}	4.8±0.02 ^{a,y}	5.9±0.02 ^a
<i>Enterobacteriaceae</i>	C	1.3±0.01 ^b	2.1±0.03 ^b	3.5±0.04 ^{a,x}	4.2±0.05 ^{a,x}	-
	A	1.3±0.01 ^b	2.2±0.05 ^b	3.1±0.01 ^{a,x}	3.3±0.01 ^{a,x}	3.9±0.01 ^a
	B	1.3±0.01 ^b	1.9±0.03 ^b	2.7±0.01 ^{a,y}	2.6±0.01 ^{a,y}	3.1±0.02 ^a
Lactic Acid Bacteria	C	1.9±0.02 ^b	2.6±0.01 ^b	3.9±0.02 ^b	5.9±0.01 ^{a,x}	-
	A	1.9±0.02 ^b	2.7±0.02 ^b	3.1±0.03 ^b	2.3±0.02 ^{b,y}	4.0±0.01 ^a
	B	1.9±0.02 ^b	2.0±0.02 ^{ab}	2.6±0.01 ^a	2.9±0.03 ^{a,y}	3.3±0.01 ^a
Yeast/Mold	C	2.6±0.03 ^c	3.2±0.02 ^b	5.9±0.03 ^{b,x}	7.0±0.04 ^{a,x}	-
	A	2.6±0.03 ^a	2.8±0.02 ^a	3.7±0.02 ^{a,y}	3.2±0.03 ^{a,y}	3.8±0.01 ^{a,x}
	B	2.6±0.03 ^a	2.8±0.01 ^a	2.7±0.03 ^{a,y}	3.1±0.02 ^{a,y}	2.9±0.01 ^{a,y}

Groups: C: Control, A: REO coated 0.3% vacuum packaged, B: REO coated 0.5% vacuum packaged. a,b,c,d: Means within a row lacking a common superscript letter are different (P<0.05). x,y,z: Means within a column lacking a common superscript letter are different (P<0.05). -: Not analyzed.

Table 2 The results of chemical analyses of chicken meatballs during storage period at + 4°C (mean±s.d)

Analysis	Group	Storage time (Day)				
		0	3	5	7	9
Ph	C	6.50±0.01 ^b	6.48±0.01 ^b	6.52±0.03 ^b	6.92±0.01 ^{a,x}	-
	A	6.50±0.01	6.51±0.03	6.53±0.02	6.50±0.01 ^y	6.57±0.01
	B	6.50±0.01	6.53±0.01	6.55±0.01	6.48±0.01 ^y	6.51±0.01
TBA (mg MDA/kg)	C	0.23±0.15 ^b	0.68±0.11 ^{a,x}	0.98±0.07 ^{a,x}	1.01±0.06 ^{a,x}	-
	A	0.23±0.11	0.31±0.08 ^{xy}	0.41±0.11 ^{xy}	0.48±0.04 ^y	0.51±0.02 ^x
	B	0.23±0.14	0.25±0.06 ^y	0.28±0.07 ^y	0.32±0.01 ^y	0.30±0.01 ^y

Groups: C: Control, A: REO coated 0.3% vacuum packaged, B: REO coated 0.5% vacuum packaged. a,b: Means within a row lacking a common superscript letter are different (P<0.05). x,y: Means within a column lacking a common superscript letter are different (P<0.05). -: Not analyzed.

Table 3 The results of sensory analyses of chicken meatballs storage period at + 4°C (mean±s.d)

Analysis	Group	Storage time (Day)				
		0	3	5	7	9
Appearance	C	4.0±0.11	4.0±0.13	3.8±0.17	-	-
	A	4.0±0.11 ^a	4.0±0.11 ^a	4.0±0.13 ^a	3.8±0.01 ^b	3.4±0.26 ^b
	B	4.0±0.19	4.0±0.19	4.0±0.11	4.0±0.13	3.8±0.17
Taste	C	4.2±0.19	4.0±0.13	4.0±0.17	-	-
	A	4.2±0.19	3.8±0.51	4.0±0.42	3.6±0.33	3.4±0.12
	B	4.2±0.12	3.6±0.69	3.8±0.12	4.0±0.11	3.6±0.13
General Acceptability	C	4.0±0.21	3.8±0.14	3.8±0.13	-	-
	A	4.0±0.21	3.6±0.16	3.4±0.14	3.8±0.11	3.4±0.14
	B	4.0±0.21	3.4±0.14	3.8±0.11	3.6±0.14	3.4±0.18

Groups: C: Control, A: REO coated 0.3% vacuum packaged, B: REO coated 0.5% vacuum packaged. a,b: Means within a row lacking a common superscript letter are different (P<0.05). -: Not analyzed.

Thiobarbituric acid value is accepted as an indication of lipid oxidation which is one of reasons of food spoilage (Naveena et al., 2013). In this study, initial TBA value was found to be as 0.23 mg MDA/kg. Thiobarbituric acid values were found to be lower in REO coated groups compared to control. Statistical differences of TBA values intergroup (C, A, B) and storage period at 3, 5 and 7th days were found to be significant (P<0.05). Lipid oxidation was found to be lower in 0.5% REO coated group B during the storage period. Statistical differences were found to be significant between groups A and B with the exception of the beginning and 7th day of preservation (P<0.05). It is stated that the use of antimicrobial agents is synergistically contributing both to elimination of microorganisms and to reduction of meat oxidation in foods. Therefore, lipid oxidation can be delayed (Coma, 2012). The lipid oxidation was observed to be delayed in this study during the storage period due to the antioxidant activity of REO (Bozin et al., 2007).

Kahraman et al. (2015); reported that chicken fillets packaged under modified atmosphere and coated with rosemary extract were protected from lipid oxidation during the storage period. Thiobarbituric acid values of greater than 1 mg MDA/kg are considered not suitable for human consumption. Yu et al. (2002) reported that rosemary extracts can inhibit the lipid oxidation and color changes in cooked turkey meats during refrigerated storage. Chemical evaluation of control group at 9th day was not included as maximum tolerable levels were exceeded at 7th day. Therefore, chemical evaluation was not conducted at this day.

The control group samples were analyzed at the beginning of and at 3rd and 5th days of preservation by sensory parameters of appearance, taste and general acceptability. Samples from 0.3% and 0.5% REO coated groups A and B were analyzed by same parameters at the beginning of and on 3, 5, 7 and 9th days of storage.

Statistical analyses were conducted both on intergroup (C, A, B) and for storage period (at 0, 3, 5, 7 and 9th days) and differences were found to be as insignificant (P>0.05).

Foods can be preserved longer due to both antimicrobial and antioxidant activities of rosemary extract (Riznar et al., 2006). In this study, even though both microbiological and chemical evaluation results of 0.5% REO coated group B were found to be better than 0.3% REO coated group A, panelists accepted more group A samples (Table 3). Statistical differences between control and groups A and B for appearance, taste and general acceptability parameters were found to be as insignificant (P>0.05). However, while intergroup differences of 0.3% REO coated group A for appearance were insignificant on 0, 3 and 5th days (P>0.05) differences were significant on 7th and 9th days (P<0.05). In this study it was determined that control group samples were spoiled at 7th day of storage while samples from REO coated groups were not spoiled up to at 9th day. Since the total psychrophilic bacteria counts were exceeded the threshold value of 10⁶ cfu/g in microbial analyses after the 5th day, 7th and 9th days sensory analyses were not conducted.

Conclusion

The results of the antimicrobial and antioxidant effects of REO obtained from this study, was found to be effective on inhibiting microbial growth, reducing lipid oxidation and improving sensory quality of chicken meatballs. The REO 0.3% treatment group samples resulted in a shelf life extension of 9 days compared with the control group samples at +4°C of storage. It has been determined that 0.3% and 0.5% REO coated group samples were also effective in the reduction of lipid oxidation. According to the sensory evaluation, 0.3% REO coated group samples were more generally accepted by the panelists.

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