Effect of Gelatin Coated Rosemary Extract (*Rosmarinus Officinalis* L.) on the Quality of Refrigerated Duck Meat

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10.18805/ag.D-180

ABSTRACT

The effect of edible coating of gelatin (1, 2 and 3%) containing aqueous extract of rosemary (1% and 2%) on physiochemical (pH, total volatile nitrogen, peroxide values and water holding capacity) and microbiological (psychrotrophic) properties of duck fillet were evaluated. The pH of coated samples were significantly ($p<0.05$) lower than that of untreated sample. TVN (total volatile nitrogen) value increased with storage period in all the samples but coatings significantly decreased production of TVN compared to the control. Also the coatings decreased lipid oxidation of the meat as rosemary postponed primary oxidation of fillet, while this parameter in control group was sharply increased by time. WHC (water holding capacity) increased significantly ($p<0.05$) with storage period and level coatings. Coating had a significant reducing effect on growth of psychrotrophic bacteria during 9 days at 4°C. In conclusion, gelatin edible coating enriched with rosemary could retard chemical and microbial reactions related to spoilage of meat during refrigerated storage.

Key words: Bacteria, Chemical, Duck meat, Enrobing, Plant.

INTRODUCTION

Poultry meat is a highly perishable food commodity and can be preserved a few days in a refrigerator. So, extending its shelf life for a longer time has been of interest to researchers and producers. Shelf life of meat can be extended if microbial growth and oxidative reactions are limited. As consumer rejection of synthetic additives is becoming more common, natural preservative systems are sought. Rosemary [*Rosmarinus Officinalis* L. (*Labiatae*)] is a Mediterranean herb used for both culinary and medicinal purposes. The rosemary extract are used in various industry sectors and are commercialised, since they show a range of antibacterial, antioxidant, antifungal, anti-inflammatory properties. The antimicrobial activity of rosemary extract related with the presence of carnosic acid, carnosol and rosemarinic acid, which are phenolic diterpenes containing a phenolic group in their structure. Due to the strong antioxidant and antimicrobial activities, rosemary extracts are commonly used by the food industry to extend the shelf life of various products (Huang *et al*., 2017). Gelatin is one of the first materials applied to edible coatings (Gennadios *et al*., 1994). An edible coating has been defined as a thin, continuous layer of edible material placed on foods (Bravin *et al*., 2006).

Several studies have provided many examples of edible coatings for meat and meat products (Duan and Zhao, 2010; Khan *et al*., 2013; Sánchez-Ortega *et al*., 2014). For example, Can *et al*. (2016) investigated the effects of rosemary extract addition on sensory, chemical and microbiological properties of chicken meatball. In the study, TBA (thiobarbituric acid) value was found to be lower in samples added with rosemary extract than in other samples.

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How to cite this article: Banikarimi, K., Mirzaei, H. and Farsi, M. (2020). Effect of Gelatin Coated Rosemary Extract (*Rosmarinus Officinalis* L.) on the Quality of Refrigerated Duck Meat. Agricultural Science Digest. 40(1): 89-94.

Submitted: 24-07-2019 **Accepted:** 12-10-2019 **Published:** 06-03-2020

Due to low storage time of fresh poultry meat and the emphasis on the application of biodegradable coating rather than synthetic ones, therefore the aim of this study was to investigate preservative and antimicrobial effect of gelatin alone and in combination with aqueous extract of rosemary on duck fillets in the refrigerator condition (4°C).

MATERIALS AND METHODS

Bovine gelatin (Sigma co, Germany) solution was prepared by dissolving 1, 2 and 3 g in 100 mL of sterile distilled water, respectively. After stirring to allow complete dissolution, the solution was kept at 7°C for 15 min. Then the sample heated at 55°C for 30 min. Subsequently, the glycerol (Sigma Company, Germany) was added as a plasticizer. For active edible coating, the aqueous rosemary extract (Barij Essence Company, Iran) was mixed with gelatin solution (1 and 2%)

Table 1: Different used treatments for coating the duck meat in current study.

Treatment No	coatings	
	Gelatin	Rosemary extract
UCO	0	0
GC 1	1	0
GC2	2	0
GC 3	3	0
GRE 11	1	1
GRE 21	2	1
GRE 31	3	1
GRE 12	1	2
GRE 22	2	2
GRE 32	3	2

under magnetic stirring. Fillets from duck were randomly and equally divided into three groups: uncoated meat (UCO); meat with Gelatin coating and without rosemary (GC, 1, 2, 3 %, i.e, GC 1, GC2, GC 3); Gelatin coating (1, 2, 3 %) with 1% (GRE 11, GRE 21 and GRE 31) and Gelatin coating (1, 2, 3 %) with 2% rosemary extract (GRE 12, GRE 22 and GRE 32) (Table 1). The fillets were individually submerged in gelatin solution for 30 sec, then they were taken out and after 2 min, they were soaked in the solution for 30s again (Ojagh *et al*., 2010). The coated samples were allowed to drain for 5 minutes under a biological safety cabinet. The samples were placed in sterile bags and kept at 4°C and microbiological and physiochemical evaluation of fillets were conducted at intervals of three days (for 9 days) (Jonaidi jafari *et al*., 2017).

To determine pH value, a mixture of 10 g of meat in 100 mL of distilled water was prepared and digital pH meter was used. Nitrogen content estimate by Kjeldhal method and described as TVN mg/100 g of sample. To measuring peroxide value, twenty grams of fillet with 100 ml of chloroform/methanol solution was mixed at a portion of 2:1 and blended for 1 minute. After dewatering by potassium chloride, the aqueous phase (lower phase) was collected and used for titration by sodium thiosulfate (Jonaidi jafari *et al*., 2017). The WHC of samples were determined by homogenizing 20 g sample with 40 ml of distilled water. 20 g of the homogenate centrifuged at 4°C at 1000 g for 15 min. The WHC was determined as liquid loss and expressed as percentage of weight of liquid release. (Ismail *et al*., 2010).

Psychrotrophic counts were determined by preparing decimal dilutions in peptone water, then 0.1 ml of the appropriate dilutions were plated on plate count agar (PCA, Oxoid) and incubated aerobically at 7°C for 10 days (Ercolini *et al*., 2009). Results were expressed as log10 CFU/g.

All experiments were carried out in triplicate and analysis of variance (ANOVA) in SPSS statistics software (Version 19; SPSS Inc., Chicago, USA) were used for statistical analysis of data. To compare differences

Table 2: Change of pH of coated duck meat during storage time.

	Treatment Number								
day	UCO	GC 1	GC2	GC 3	GRE 11	GRE 21	GRE 31	GRE 12	GRE 22	GRE 32
0	6.70±0.10^{a}	6.79±0.10^{a}	6.80±0.10^{a}	6.80±0.10^{a}	6.80±0.10^{a}	6.80±0.10^{a}	6.90±0.10^{a}	6.90±0.10^{a}	6.85±0.10^{a}	6.84±0.10^{a}
3	6.20±0.10^{b}	6.40±0.10^{b}	6.40±0.10^{b}	6.50±0.10^{b}	6.40±0.10^{b}	6.20±0.10^{b}	6.40±0.10^{b}	6.30±0.10^{b}	6.28±0.10^{b}	6.20±0.10^{b}
6	5.52±0.10^{c}	5.80±0.10^{d}	6.00±0.10^{c}	6.10±0.10^{c}	6.20±0.10^{c}	5.90±0.10^{c}	6.00±0.10^{c}	5.88±0.10^{c}	5.80±0.10^{c}	5.70±0.10^{c}
9	5.80±0.10^{d}	6.00±0.10^{c}	6.10±0.10^{c}	6.20±0.10^{c}	6.20±0.10^{c}	6.00±0.10^{c}	6.00±0.10^{c}	5.80±0.10^{c}	5.70±0.10^{c}	5.50±0.10^{d}

Means in the same column indicated by different letters were significantly different (p<0.05).

Table 3: Change of TVN (mg nitrogen/100 g) of coated duck meat during storage time.

	Treatment Number								
day	UCO	GC 1	GC2	GC 3	GRE 11	GRE 21	GRE 31	GRE 12	GRE 22	GRE 32
0	16.60±0.06^{d}	16.70±0.06^{c}	16.60±0.06^{d}	16.70±0.06^{c}	16.60±0.06^{b}	16.70±0.06^{b}	16.70±0.06^{b}	16.60±0.06^{a}	16.54±0.06^{a}	16.51±0.06^{a}
3	19.12±0.58^{c}	18.60±0.58^{b}	18.60±0.58^{c}	18.70±0.58^{b}	17.30±0.58^{b}	17.20±0.58^{b}	17.70±0.58^{b}	16.80±0.58^{a}	16.75±0.58^{a}	16.70±0.58^{a}
6	23.60±0.58^{b}	20.20±0.58^{b}	20.40±0.58^{b}	20.30±0.58^{b}	17.90±0.58^{b}	17.85±0.58^{b}	17.80±0.58^{b}	17.30±0.58^{a}	17.25±0.58^{a}	17.10±0.58^{a}
9	48.70±0.58^{a}	22.30±0.58^{a}	22.50±0.58^{a}	22.60±0.58^{a}	20.10±0.58^{a}	20.10±0.58^{a}	19.80±0.58^{a}	18.20±0.58^{a}	18.07±0.58^{a}	18.00±0.58^{a}

Means in the same column indicated by different letters were significantly different (p<0.05).

between mean values, Duncan's test was used and $p<0.05$ was considered statistically significant (Raeisi *et al*., 2014).

RESULTS AND DISCUSSION

Physiochemical measurements

The results of physiochemical properties of different treatments of duck meat coated by rosemary extract and gelatin shown in Tables 2-5. The significant difference was observed in the pH of control and coated samples between storage periods. For the treated samples containing gelatin and rosemary extract, pH value was lower than that of untreated sample ($p<0.05$) during cold storage, which is attributed to the inhibitory effect of antimicrobial ingredients present in rosemary extract, which limited the growth and proliferation of meat spoilage microorganisms that utilize basic nitrogen compounds (Zhang *et al*., 2016) and also is partly attributed to the metabolite accumulation produced by lactic acid bacteria (Demeyer *et al*., 1979). The highest and lowest pH value of treated samples recorded for meats containing 3% gelatin and 1% rosemary extract, and 3% gelatin and 3% rosemary respectively that showed significantly lower pH than control ($p<0.05$) (Table 2). Many researchers demonstrated suitable effects of coatings on reducing pH value in different products (Ahmad *et al*., 2012; Jo *et al*., 2001; Lin and Chao, 2001). The pH of duck meat in this study was simillar to results reported by George *et al*. (2014) about Vigova Super M duck and Kuttanad duck (5.83-6.3), but lower than findings (6.87-7.33) reported by ismail *et al*. (2010) about 18-month-old local Java ducks.

TVN results for fillet during 9 days has been shown in Table 3. TVN value increased with storage period in all the samples. The total volatile nitrogen values of control samples were significantly higher than other samples followed by treatments without rosemary extract (treatment No, GC 1, GC2 and GC 3). During storage of meat, activity of some special proteolytic bacteria leads to degradation of non-protein nitrogen compounds and proteins (Li *et al*., 2012). Results of this study showed that coating enriched with rosemary extract had appropriate effect on decrease in production of TVN. The result of TVN in this study is in agreement with Kandeepan and Biswas (2007) who found that TVN significantly ($p<0.05$) increased with storage period in buffalo meat under chilled condition. Based on Iran Veterinary Organization instructions, the acceptable value for TVN is 28 mg/100g. In control category, TVN increased by time and its value reached 48.7 in the final day. In the gelatin and rosemary treated category, this number reached to lower than the value recommended by Iran Veterinary Organization instructions in all days which is still in acceptable. Rosemary had a significant impact on TVN in our study, which was in accordance with findings obtained by previous researchers such as Ali *et al*. (2010).

Results of peroxide value (m.eq.peroxide/1000 g oil) of coated duck meat during storage time (in refrigerator 4°C)

Table 4: Change of peroxide value (m.eq.peroxide/1000g oil) of coated duck meat during storage time.

	Treatment Number								
day	UCO	GC 1	GC2	GC 3	GRE 11	GRE 21	GRE 31	GRE 12	GRE 22	GRE 32
0	0.70 ± 0.10^{d}	0.70 ± 0.10^{d}	0.69 ± 0.10^{b}	0.69 ± 0.10^{b}	0.69 ± 0.10^{a}	0.68 ± 0.10^{a}	0.68 ± 0.10^{a}	0.66 ± 0.10^{a}	0.65 ± 0.10^{a}	0.64 ± 0.10^{a}
3	0.92 ± 0.10^{c}	0.90 ± 0.10^{c}	0.87 ± 0.10^{ab}	0.85 ± 0.10^{ab}	0.80 ± 0.10^{a}	0.69 ± 0.10^{a}	0.67 ± 0.10^{a}	0.66 ± 0.10^{a}	0.65 ± 0.10^{a}	0.64 ± 0.10^{a}
6	1.70 ± 0.10^{b}	1.10 ± 0.10^{b}	0.97 ± 0.10^{a}	0.90 ± 0.10^{a}	0.81 ± 0.10^{a}	0.70 ± 0.10^{a}	0.66 ± 0.10^{a}	0.63 ± 0.10^{a}	0.64 ± 0.10^{a}	0.61 ± 0.10^{a}
9	1.90 ± 0.10^{a}	1.30 ± 0.10^{a}	1.00 ± 0.10^{a}	0.93 ± 0.10^{a}	0.80 ± 0.10^{a}	0.70 ± 0.10^{a}	0.65 ± 0.10^{a}	0.60 ± 0.10^{a}	0.60 ± 0.10^{a}	0.58 ± 0.10^{a}

Means in the same column indicated by different letters were significantly different ($p<0.05$).

Table 5: Change of WHC of coated duck meat during storage time.

	Treatment Number									
day	UCO	GC 1	GC2	GC 3	GRE 11	GRE 21	GRE 31	GRE 12	GRE 22	GRE 32
0	62.40 ± 1.00^{a}	62.40 ± 1.00^{a}	62.40 ± 1.00^{a}	62.50 ± 1.00^{a}	62.60 ± 1.00^{a}	62.60 ± 1.00^{a}	62.70 ± 1.00^{a}	62.70 ± 1.00^{a}	62.80 ± 1.00^{a}	62.80 ± 1.00^{a}
3	62.10 ± 1.00^{a}	62.30 ± 1.00^{a}	62.40 ± 1.00^{a}	62.50 ± 1.00^{a}	62.50 ± 1.00^{a}	62.60 ± 1.00^{a}	62.70 ± 1.00^{a}	62.80 ± 1.00^{a}	63.00 ± 1.00^{a}	63.20 ± 1.00^{a}
6	61.20 ± 1.00^{ab}	62.20 ± 1.00^{a}	62.30 ± 1.00^{a}	62.40 ± 1.00^{a}	62.50 ± 1.00^{a}	62.60 ± 1.00^{a}	62.80 ± 1.00^{a}	62.90 ± 1.00^{a}	63.20 ± 1.00^{a}	63.50 ± 1.00^{a}
9	60.00 ± 1.00^{b}	62.00 ± 1.00^{a}	62.20 ± 1.00^{a}	62.30 ± 1.00^{a}	62.50 ± 1.00^{a}	62.60 ± 1.00^{a}	62.80 ± 1.00^{a}	63.00 ± 1.00^{a}	63.50 ± 1.00^{a}	63.80 ± 1.00^{a}

Means in the same column indicated by different letters were significantly different ($p<0.05$).

Table 6: Change of psychrotrophic counts (log CFU/g) of coated duck meat during storage time.

day	Treatment Number								
	UCO	GC 1	GC2	GC 3	GRE 11	GRE 21	GRE 31	GRE 12	GRE 22	GRE 32
0	4.30±0.10^{d}	4.20±0.10^{d}	4.20±0.10^{d}	4.30±0.10^{d}	4.10±0.10^{d}	4.00±0.10^{d}	4.00±0.10^{d}	3.70±0.10^{d}	3.60±0.10^{d}	3.40±0.10^{d}
3	5.10±0.10^{c}	4.70±0.10^{c}	4.70±0.10^{c}	4.60±0.10^{c}	4.50±0.10^{c}	4.30±0.10^{c}	4.30±0.10^{c}	4.00±0.10^{c}	4.10±0.10^{c}	3.70±0.10^{c}
6	6.70±0.10^{b}	5.50±0.10^{b}	5.40±0.10^{b}	5.40±0.10^{b}	5.10±0.10^{b}	4.90±0.10^{b}	4.80±0.10^{b}	4.60±0.10^{b}	4.70±0.10^{b}	4.30±0.10^{b}
9	8.20±0.10^{a}	6.60±0.10^{a}	6.50±0.10^{a}	6.40±0.10^{a}	6.10±0.10^{a}	5.70±0.10^{a}	5.70±0.10^{a}	5.70±0.10^{a}	5.50±0.10^{a}	5.20±0.10^{a}
t		02								

Means in the same column indicated by different letters were significantly different (p<0.05).

has been shown in Table 4. No remarkable variation observed in peroxide value between 2% and 3% gelatin (without rosemary, treatments No GC2 and GC 3) in all days, but in the last treatments, the difference was statistically significant up to day 6. In all days, this value in specimens containing rosemary was less than control and gelatin groups. The present study showed that rosemary postponed primary oxidation of fillet, while this parameter in control group was sharply increased by time. In this study, the lowest oxidation value (0.58) gained using the highest levels of rosemary in coating. It is reported that gelatin coating has not antimicrobial effect in meat (Gomez-estaca *et al.,* 2009) but can reduce the oxidation rate (Antinewski *et al*., 2007). Mielnik *et al*. (2003) investigate the effects of commercial rosemary antioxidants on oxidative stability of turkey meat. It is reported that low concentrations of antioxidants retarded the oxidation process during the initial 2 months while longer storage required higher level of antioxidants to prevent the development of reaction products from the oxidation process. Their results are in accordance with current study (Table 4).

Coated meat samples showed slight difference in WHC during the whole days of storage, and on 9 day of storage, control sample showed lowest WHC (60.0). While the highest WHC recorded for treatment No GRE 12 (3% gelatin and 2% rosemary extract) (63.8). There was no significant difference ($p< 0.05$) in WHC of all coated samples during the whole days of storage (Table 5). The higher values of WHC were observed in treated samples compared to control which could be attributed to the hydrophilic properties of gelatin which are water binders (Varela and Fizsman, 2011). These results were in agreement with Kandeepan and Biswas (2007) who revealed that WHC decreased with storage period in buffalo meat under chilling temperature. Weight loss were associated with the evaporation of water and volatile substances. Coating acts as sealant and prevents moisture and weight loss and improves juiciness and tenderness (Biswas and Keshri, 2003). In current study, coating could increase the WHC that this is coincidental with the lower weight loss and consequently higher profit.

Bacteria developing on meat at chilling temperatures are regarded as psychrotrophic. The evolution of psychrotrophic bacteria during the storage period shown in Table 6. Coating inhibited the growth of these bacteria observing significant ($p < 0.05$) reductions in counts of 3 log cfu/g at day 9 of analysis, compared with control samples. During the storage, significant differences were observed between days whitin every treatment (Table 6). Meat coated with higher levels of gelatin and rosemary extract were more effective in inhibited the growth of psychrotrophic bacteria.

Studies have demonstrated that the control of microorganisms is improved when a greater concentration of antimicrobial agents is used (Zinoviadou *et al*., 2009). In

this study, the initial count of psychrotrophic microorganisms in the control sample was 4.3 log CFU.g^{-1}, but increase the level of rosemary extract in duck met led to decreasing the psychrotrophic count (3.4 log CFU.g^{-1} for treatment No GRE 32) (Table 6). The coating containing 3% gelatin and 2% rosemary extract was the most effective because it showed counts lower than alls during the whole 9 days of storage period (Table 6). In a study, Gómez-Estaca *et al*. (2007) coated cold-smoked sardine with gelatin coatings incorporated with rosemary essential oil and stored for 20 days at 5°C. At day 16 of storage total viable bacteria were also reduced in about 1.5 log cfu/g, though at the end of the storage period no reductions were observed. In this study, with the use of the antimicrobial edible coatings, a bacteriostatic effect was observed against the growth of the psychrotrophic groups. Coated samples kept populations of psychrotrophic bacteria under the recommended value during storage time than uncoated samples, so an increase in the shelf-life of duck breast could be expected (Table 6).

CONCLUSION

The present study shows that gelatin and rosemary extract can improve the chemical and microbial properties of duck fillet and increase its shelf life. The shelf life of uncoated samples was 6 days (based on TVN and psychrotrophic count), while it was observed to be more than 6 days for samples treated by gelatin and rosemary extract. In addition, combination of gelatin and rosemary due to their synergetic effect can improve the chemical and microbial properties. Thus, the use of this kind of coatings would be considered as an emerging technology with the aim of extending the shelf-life of refrigerated poultry meat. In conclusion, gelatin edible coating enriched with rosemary extract has desirable potential to preserve fresh duck meat and could retard chemical reactions related to spoilage of meat during refrigerated storage.

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