

# Effect of Calcium Alginate Edible Coatings on Microbial and Chemical Properties of Lamb Meat during Refrigerated Storage

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## Article type

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## Abstract

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**Background:** Edible films and coatings have potential benefits for food packaging, particularly meat products. These coatings prevent weight loss and texture changes during shelf life by reducing moisture loss in fresh and frozen meat products. In this study, effect of calcium alginate edible coating on microbial and chemical properties of refrigerated lamb meat over a period of 5 days was investigated.

**Methods:** The control and coated samples were analyzed for microbial properties including total microbial count, psychrophilic bacteria count and chemical characteristics such as total volatile nitrogen (TVN) and moisture content. The experiments were conducted in triplicates and data were analyzed using SPSS software (version 16.0) by two-way ANOVA and general linear model with repeated-measurements.

**Results:** There was a significant ( $p < 0.05$ ) difference between the samples coated with calcium alginate and control samples in total microbial count during 5 days; but, there was no significant difference in psychrophilic bacteria count and moisture content. Besides, a significant reduction ( $p < 0.05$ ) was observed for the coated and control samples in TVN content on day 3. After 5 days of storage at  $4 \pm 1$  °C, TVN levels did not exceed the maximum standard limit (16.5 mg/100 g) in all coated samples.

**Conclusion:** The results of this study confirmed effectiveness of calcium alginate in improving the microbial and chemical properties and shelf life extension of lamb meat.

## Introduction

The aim of food packaging is to maintain quality and safety of products from the time of manufacturing to the time of consumption. Due to their features, plastic materials have been used in food industry for over 50 years. However, from environmental point of view, these materials almost cannot be recycled. Also, economic issues must be considered because of the dependence of plastic materials production on petroleum products and high price of these products and oil derivatives. Therefore, edible films and coatings based on proteins, polysaccharides, and lipids have been introduced to packaging industry (Cutter, 2006).

Through improvement in edible coatings' production technology, most researches have focused on producing composite coatings. For instance a composite coating formed by a mixture of protein and lipid has features of each one of its ingredients (Galus and Lenart, 2013; Kester and Fennema, 1986; McHugh and Krochta, 1994; Wang et al., 2010; Wu et al., 2002). Main functions of edible films and coatings include controlling water vapor and gas transmission, maintaining volatile aromatic compounds, carrying additives and reducing microbial load. Accordingly, edible films and coatings have potential benefits for food packaging, particularly fresh and frozen meat products. Weight loss and texture changes during shelf-life can

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be prevented using these coatings by reducing moisture loss in fresh and frozen meat products. Also, these coatings help to maintain nutrients by preventing exudation of meat extract from meat pieces. Besides, there is no need to use absorbent pads in packages. Furthermore, food stability is affected by transferring of some gases like oxygen and carbon dioxide through packaging. The first stage of spoilage in many food products involves oxidation of lipids, vitamins, aromatic compounds and pigments that can be prevented by edible coatings. Edible films and coatings are able to control permeability of oxygen and carbon dioxide and reduce lipid and myoglobin oxidation rates and microbial count. Volatile flavor loss and foreign odor pick up could be reduced by coating products. As a result, changes in odor and flavor of the products are inhibited (Cutter, 2006; Gontard et al., 1994; Krochta and De Mulder-Johnston, 1997). Lazarus et al. (1976) found that lamb carcass samples coated with calcium alginate had significantly lower weight loss compared to the control samples during 24 h and a significant decrease was observed in total microbial count on days 5 and 7. In a similar study, no significant inhibitory effect on coliforms was observed in the beef samples coated with calcium alginate; however, weight loss decreased significantly compared to the control samples (Williams et al., 1978). Song et al. (2011) reported that calcium alginate coating containing different kinds of antioxidants could extend shelf-life of bream (*Megalobrama amblycephala*) during 21 days of storage at refrigerated temperature ( $4\pm 1$  °C). Also, other similar studies confirmed effectiveness of calcium alginate coating in improving microbial and chemical properties and extending shelf-life of different sorts of meat (Keshri and Sanyal, 2009; Neetoo et al., 2010; Siragusa and Dickson, 1993).

The main objective of this study was to investigate the effect of calcium alginate edible coating on microbial and chemical properties of fresh lamb meat at refrigerated temperature ( $4\pm 1$  °C).

## Materials and methods

### Preparation of calcium alginate edible coatings

Solution No. 1 contained 5 g of sodium alginate powder, 45 g of maltodextrin and 210 ml of distilled water. Solution No.2 contained 2.75 g of calcium chloride, 0.9 g of carboxy methyl cellulose, and 49 ml of distilled water.

### Sample preparation

Fresh lamb meats (six one-kilogram packages, containing four 250 g lamb meat pieces in disposable polyethylene trays including an absorbent pad and covered with cellophane) were purchased from Zarrin Gousht-e Jam packaging company. All the lamb meats were stored in the refrigerator until being coated. After preparing the solutions, all

the lamb meats were taken out of the packages and 12 pieces were randomly selected and coated with the solutions by immersion method. The selected lamb meat pieces were immersed in solution No. 1 for 1 min. In order to obtain a uniform layer of coating on the muscle's surface, the pieces were re-immersed for 1 min. Then, they were kept out and allowed to drip excess solution, followed by immersion in solution No. 2 for 1 min. The coated samples were dried in laboratory conditions ( $23\pm 2$  °C and  $50\pm 5$  % relative humidity) for 15 min. Afterwards, the coated lamb meat pieces were put into 4 disposable trays in a way that each tray contained 3 pieces and were covered with cellophane as secondary packaging. The remaining uncoated lamb meat pieces were considered as control group. Packaging of the control and coated samples were the same, except that the control packages contained absorbent pads in order to make them look exactly the same as the muscle packages being sold in the market. Both coated and control samples were stored in the refrigerator ( $4\pm 1$  °C) over a period of 5 days. The samples were analyzed on days 0, 1, 3 and 5.

### Microbiological analysis

Sampling, sample preparation, preparing required dilutions, enumeration of total microorganisms and enumeration of psychrophilic bacteria were performed according to Iran's national standards (ISIRI, 1971; ISIRI, 2000; ISIRI, 2004; ISIRI, 2008a; ISIRI, 2008b).

### Chemical analysis

Total volatile nitrogen (TVN) and moisture content were determined according to AOAC (1997) and Iran's national standard (ISIRI, 2003), respectively.

### Statistical analysis

The experiments were conducted in triplicate. Data analysis was done using SPSS software (version 16.0) by two-way ANOVA analysis of variance and general linear model with repeated-measurements. State of being significant was defined  $p<0.05$  in the present study.

## Results

Means of total microbial count (log CFU/g) of the coated and control samples during 0, 1, 3 and 5 days of storage in the refrigerator are shown in Table 1. According to the results of analysis of variance, there was significant difference ( $p<0.05$ ) in total microbial count between the coated and control samples on days 1 and 5 (Table 1).

The means of psychrophilic bacteria count (log CFU/g) for coated and control samples during 0, 1, 3 and 5 days of storage in the refrigerator are presented in Table 1. There was no significant difference ( $p>0.05$ ) in psychrophilic bacteria count between the coated and control samples.

Table 1 represents TVN means of the coated and control samples after 0, 1, 3 and 5 days of storage in the refrigerator. Statistical analysis showed significant difference ( $p<0.05$ ) in TVN values between the coated and control samples on day 3 but there was no significant difference in other days of storage.

The means of moisture content for the coated and control samples after 0, 1, 3 and 5 days of storage in the refrigerator are shown in Table 1. According to the results of analysis of variance, there was no significant difference in moisture content between the coated and control samples (Table 1).

**Table 1:** Microbial and chemical properties (Mean±SD) of fresh lamb meat samples stored at refrigerated temperature according to coating status

Variable	Group	Days			
		0	1	3	5
TMC <sup>a</sup> (log CFU/g)	Coated	5.33±0.12	7.41±0.09*	8.58±0.11	9.72±0.12*
	Control	5.47±0.15	7.43±0.15*	9.78±0.11	12.12±0.08*
PBC <sup>b</sup> (log CFU/g)	Coated	5.75±0.17	8.26±0.19	10.37±0.09	11.31±0.11
	Control	6.12±0.20	8.29±0.22	9.84±0.39	11.27±0.36
TVN <sup>c</sup> (mg/100 g)	Coated	5.60±1.40	6.07±0.81	5.60±1.40*	14.93±4.04
	Control	5.57±2.00	6.77±1.07	17.73±2.14*	16.80± 6.10
Moisture (%)	Coated	75.41±0.21	77.72±3.90	76.37±1.46	75.12±0.25
	Control	75.16±0.52	79.77±7.42	75.59±0.39	75.07±0.23

a-Total microbial count

b-Psychrophilic bacterial count

c-Total volatile nitrogen

\*shows significant difference ( $p<0.05$ ) between treatments (in triplicate)

## Discussion

In the present study, total microbial count linearly increased during the storage period; but, level of total microbial count of the coated samples was lower than the control at the end of the storage. Similar studies have confirmed these results (Hamzeh and Rezaei, 2012; Keshri and Sanyal, 2009; Song et al., 2011; Williams et al., 1978). According to the standard of International Commission on Microbiological Specifications for Foods (ICMSF, 1986), the maximum limit of total microbial count in fresh meat is  $10^7$  CFU/g. In the current research, total microbial counts of the coated and uncoated samples exceeded the maximum limit ( $10^7$  CFU/g) on day 3. In a study on the effect of edible coatings containing different antioxidants on shelf life of bream over a 21 days storage at refrigerated temperature, total viable count of the control group reached 7.10 log CFU/g on day 15, which exceeded the ICMSF's maximum acceptable level of 7 log CFU/g for freshwater and marine fish (ICMSF, 1986; Song et al., 2011); while calcium alginate coated samples reached about 7 log CFU/g on day 17. Total viable count of the coated samples with calcium alginate containing vitamin C and calcium alginate containing tea polyphenols did not exceed the limit value

during the entire storage; they reached 5.54 and 5.63 log CFU/g, respectively, on day 21 (Song et al., 2011).

Based on the results, there was a significant decrease ( $p<0.05$ ) in total microbial count in the samples coated with calcium alginate compared to control samples. In another study, a significant decrease was also observed in total microbial count in buffalo meat patties coated with calcium alginate containing preservatives compared to the control samples (Keshri and Sanyal, 2009). Total aerobic microbial count in rainbow trout fillets coated with sodium alginate was significantly lower than the control samples (Hamzeh and Rezaei, 2012). Furthermore, alginate coatings (commercially known as Flavor-Tex) was reported with no significant effect on total aerobic microbial count of lamb carcasses stored at 4 °C (Lazarus et al., 1976). Similar results were obtained by the application of some coating for beef stored at 5 °C (Williams et al., 1978). In two previously mentioned studies, microbial growth rate decreased to some extents in the coated samples due to toxic effects of calcium chloride at 0.1 M to 0.4 M concentrations on bacteria. Similar to the present study, significant decrease on total microbial count was probably due to inhibitory effect of calcium ions present in the coatings. With respect to coating characteristics in order to maintain moisture and the fact that psychrophilic bacteria are much more

resistant to calcium chloride's inhibitory effects, no significant difference in psychrophilic bacteria count was observed between the coated and control samples in this study. However, psychrophilic bacteria count was lower in the coated samples than controls. In the present research, the overall psychrophilic bacteria count linearly increased during the storage period, which was confirmed by the findings of similar studies (Hamzeh and Rezaei, 2012; Keshri et al., 2009). Organic acids immobilized calcium alginate gel coating resulted in a significantly greater reduction in viable count of *Listeria monocytogenes* attached to lean beef surfaces after 6 days compared to acid treatments without alginate (Siragusa and Dickson, 1992). Calcium alginate edible film incorporated with garlic oil showed a significant inhibitory effect on growth of *Staphylococcus aureus* and *Bacillus cereus* (Pranoto et al., 2005). In another study, alginate coatings containing sodium lactate and sodium acetate remarkably postponed growth of *L. monocytogenes* in fillets and slices of smoked salmon (Neetoo et al., 2010). Moreover, a study showed that alginate coatings containing antimicrobial substances effectively enhanced quality and safety of ready-to-use poultry products at 4 °C (Juck et al., 2010).

According to the results of this study, the coated samples showed a significant decrease in TVN values compared to the control samples during 5 days of storage at 4 °C which reduced spoilage rate in the muscle samples. Acceptable limit for TVN in raw meat is 16.5 mg/100 g and TVN level higher than the limit is considered a sign of spoilage and reduction of organoleptic qualities of meat (Pearson, 1970). In the present study, TVN means of the coated samples at the end of day 5 were lower than the acceptable limit. In another study, coatings including calcium alginate, (calcium alginate containing 5% vitamin C and calcium alginate containing 3% tea polyphenols) could maintain bream (*Megalobrama amblycephala*) quality up to 17, 19 and 21 days, respectively, with storage at 4±1 °C. TVN values in the coated samples were reported lower than those of the control sample (Song et al., 2011). Also, Hamzeh and Rezaei (2012) showed that TVN values were significantly ( $p<0.05$ ) lower in the samples coated with sodium alginate (15.63 mg/100 g) compared to the control samples (34.06 mg/100 g).

By regard to the results obtained from this study, no variation had a significant effect on moisture content variable. Alginate coatings were applied to lamb carcass stored at 4 °C which significantly maintained higher levels of moisture (Lazarus et al., 1976). The calcium alginate coating reduced shrinkage in 40 evaluated beef steaks. The calcium alginate coating increased the weight of steaks about 15 g. The coated steaks had significantly lower shrinkage values after 96 h storage compared to the uncoated steaks (Williams et al., 1978). The mechanism by which the coating retarded moisture loss was explained by Morris et al.

(1973). Trapping of water in the gel, especially at low calcium chloride concentrations, enabled the gel to act as the sacrificing agent instead of the meat tissues. This effect was observed in comparing glossy gel-like appearance of the freshly coated steaks with the coated steaks after 144 h storage, which displayed a very thin and tough layer of coating, indicating coating's moisture loss prior to tissue desiccation. The uncoated steaks had significantly lower shrinkage loss compared to the coated steaks after 144 h storage at 1 °C, demonstrating that the calcium alginate gel had ceased its function as a water sacrificing agent and rapid tissue desiccation began sometime after 96 h storage (Morris et al., 1973). In the present study, the coated samples also maintained more moisture in day 3 to day 5 (72 to 96 h); but no significant difference was observed in general. In a previous study, the coated precooked pork chops with CaCl<sub>2</sub>-gelled alginate-starch coatings stored at 4 °C for 3, 6 or 9 days had no significant moisture loss (Hargens-Madsen et al., 1995). In another study, a significant decrease in shrinkage ( $p<0.05$ ) was observed in overall moisture level of buffalo meat samples coated with sodium alginate between days 0 and 7, and days 14 and 21 (Keshri and Sanyal, 2009) that is in accordance with the results of the present research.

## Conclusion

In the current research, total microbial counts were significantly lower in the coated samples in comparison to the controls ( $p<0.05$ ) on days 1 and 5. TVN levels were reduced in the coated samples compared to the control and reduction was statistically significant on day 3. Furthermore, TVN levels of the coated samples after 5 days storage at 4 °C did not exceed the maximum standard limit. Considering that TVN level is an index for evaluating meat spoilage and bacteria role in the decay of food products, effectiveness of calcium alginate coating in improving the microbial and chemical properties and shelf life extension of lamb meat was confirmed by the results of this study.

## Conflicts of interest

The authors declare that they have no conflicts of interest in this research.

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