

The Effect of Coating with Green Tea Extract and Collagen Fiber on Quality Attributes of Vacuum Packaged Sausage

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ABSTRACT: Oxidative and microbiological degradations are the main factors that determine the quality of food and the shelf life. Color and moisture stability are important quality attributes that contribute to meat shelf life, salability and consumer acceptability. There is currently considerable interest in edible films coatings and natural antioxidants because of their potential to improve the quality and the shelf life. The objective of the present study was to investigate the effect of collagen fiber –based edible coating and green tea extract on physico chemical degradation in beef sausage stored at 4°C for two weeks in anaerobic packaging. Collagen-based edible coating with green tea extract (0.5 and 1% v/v) was used to reduce the oxidative degradation of sausages during two weeks of storage at 4 °C. Collagen fiber edible coating delayed the lipid oxidation of sausages as measured by the formation of TBARS. The moisture loss of sausages was significantly reduced by 46% with 1% tea extract coated-sausages as compared to the controls. Also, collagen and green tea extract coated-sausages were effective in maintaining the initial color of the samples.

Keywords: Coating, Collagen Fiber, Green Tea, Sausage.

Introduction

Modern trends in convenience foods have resulted in the increased consumption of precooked, refrigerated, or frozen ready-to-eat (RTE) meats and meat products such as sausages and hams due to not only their nutritional values but also their taste and flavor (Shon *et al.*, 2011). Oxidative and microbiological degradation are the main factors that determine food quality and shelf life. Extended storage of meats and meat products such as precooked beef and ground beef during refrigerated storage can cause oxidative degradation (Wu & Brewer, 1994; Wu *et al.*, 2000). Lipid oxidation is a chemical spoilage and deteriorates flavor,

color, texture and nutritional value of foods, as well as the formation of organic free radicals (Asghar *et al.*, 1988). Color and moisture stability are important quality attributes that contribute to meat shelf life, salability and consumer acceptability (Mercier *et al.*, 1998; Wu *et al.*, 2000). Various synthetic antioxidants (butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA) and tertiary butyl hydroquinone) have been used to overcome these problems (Decker, 1998).

There is currently considerable interest in edible films and coatings (included casein, collagen, chitosan, gelatin, soy protein, whey protein, calcium alginate and methyl cellulose based films) because of their potential to improve the quality and shelf

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life of foods and to reduce complexity and cost of packaging systems (Mchugh *et al.*, 1994; Shon *et al.*, 2010). These edible films or coatings can also be used as carriers for antimicrobials and antioxidants. Applications of natural products with both antioxidants and antimicrobial additives in meat products may be necessary and useful to prolong the shelf life of beef because of the health concern of consumers (Mccarthy *et al.*, 2001).

Collagen-based edible coatings also have been proposed for use on meat products other than sausages. Jones and Whitmore described a method where ground collagen was mixed with an aqueous mixture of lactic acid and glyceraldehyde, heated to about 75°C, and neutralized to pH of 7 to make a coating for hamburgers capable of withstanding cooking temperatures without melting. An edible collagen film intended for use on netted roasts, boneless hams, fish fillets, roast beef, and meat pastes, was commercialized in the U.S. in the late 1980s. According to the manufacturer, Coffi can reduce cook shrink, increase product juiciness, and allow for easy removal of elastic stretch netting after heat processing (Young *et al.*, 2004). Another study reported that both refrigerated and frozen/thawed round beef steaks wrapped in Coffi collagen film prior to standard retail packaging (permeable film overwrap) or vacuum packaging exhibited significantly less fluid exudate than unwrapped controls. Moreover, based on thiobarbituric acid analysis and on instrumental (Hunter color meter) and sensory color evaluation, the collagen films had no significant effect on meat oxidation and color (Sheu & Rosenberg, 2002).

However, synthetic antioxidants are not completely accepted by consumers due to health concerns. Therefore, some natural ingredients including herbs and spices have been studied especially in Asian countries as potential antioxidants in meat and meat products (Mccarthy *et al.*, 2001).

Compounds from herbs and spices contain many phytochemicals which are potential sources of natural antioxidants including phenolic diterpenes, flavonoids, tannins and phenolic acids ((Dawidowicz *et al.*, 2006). These compounds have antioxidant, anti-inflammatory and anticancer activities. Catechins is a predominant group of polyphenols present in green tea leaves composed of four compounds epicatechin, epicatechingallate, epigallocatechin, and epigallocatechingallate (Zhong *et al.*, 2009). These tea compounds promote health by preventing lipid oxidation and providing antibacterial, anticarcinogenic and antiviral ability (Katiyar & Mukhtar, 1996). Tea catechins were reported to reduce the formation of peroxides even more effectively than α -tocopherol and BHA in porcine lard and chicken fat (Chen *et al.*, 1998) Tea polyphenols could inhibit the formation of mutagens, which was known to be associated with the breast and colon cancer, during cooking of ground beef hamburger style meat (Weisburger *et al.*, 2002). Added tea catechins at 300 ppm level significantly reduced the TBARS values of beef, duck, ostrich, pork and chicken during 10 days of refrigerated storage. At the same concentration, tea catechins provided two to four times more antioxidative ability than α -tocopherol depending on meats from different animal species (Tang *et al.*, 2001). Green tea extract decreased the formation of TBARS and the concentration of putrescine and tyramine in a dry fermented turkey sausage. Addition of green tea, however, had no significant effects on pH, color and overall sensory quality to sausages (Bozkurt, 2006). In pork sausages, green tea powder could partly substitute nitrite, and result in lower TBARS value and decrease the volatile basic nitrogen contents as compared to the samples prepared with nitrite alone (Choi *et al.*, 2003).

The objective of the present study was to investigate the effect of collagen fiber –

based edible coating and green tea extract on physico chemical degradation in beef sausage stored at 4°C for two weeks in anaerobic packaging.

Materials and Methods

- Materials

All the chemicals were of analytical grade. Solvents were purchased from Merck Chemical Company Darmstadt, Germany. Green tea leaves were collected from Gilan region, Iran in April 2015. Collagen fiber was obtained from Royal Protein Company (Girona, Spain).

- Preparation of green tea extract

The green tea leaves were extracted with water (1:4) for two hours by using water bath (80°C). After extraction, the tea extract was concentrated to 12 brix by rotary evaporator (Heidolph, Germany) at 20 mbar vacuum and 45°C.

- Manufacture of sausages

Frozen beef meat was chopped to reduce the particle size. Salt, sodium nitrite, and half of ice water were added and chopped for 2 min to extract the salt soluble proteins. Seasonings, flavouring, and the remaining ice water were then added and the meat batter was chopped until the temperature reached 6°C. Formulation and non-meat ingredients incorporated into sausages for this study are listed in Table 1.

Table 1. Formulation of sausages

Ingredients	Amounts (%)
Meat	55
Ice water	20.584
Non-meat dry ingredients	10.5
Oil	10.5
Spices	1.5
Salt	1.5
Sodium tripolyphosphate	0.40
Sodium nitrite	0.012
Ascorbic acid	0.004
Total (%)	100

The meat batter was vacuum-packaged and stuffed into poly amide casing (Tehran navid, Iran) and cooked to an internal temperature of 72°C in a steam chamber. After cooking, the sausages were chilled immediately in an ice bath and stored in a refrigerator (4°C) for two weeks.

- Preparation of collagen fiber coating solution

A 5% (w/v) aqueous solution of collagen fiber was prepared. Subsequently, the solution was heated at 90°C for 30 min in a shaking water bath and filtered through a layer of cheese cloth. The solution was cooled at room temperature (22±1°C) and then separated into three parts. Finally, green tea extract was added to the solution at the concentration of 0.5 and 1% before coating.

Whole part of sausages (2.5 cm diameter and 6cm length) for each treatment were dipped in to the coating solution for 1 min at room temperature (22±1°C) and drained for 10s. The sausages again dipped into solution for 1 min and then dried under a laminar hood with blowing clean air flow for 2 min (22±1°C). The sausages were vacuum packaged immediately using vacuum films (200×300 mm) and stored in a refrigerator (4°C) for up to two weeks for analysis. Control samples were vacuum packaged without any coating.

- Total phenols (Folin-Ciocalteu)

Briefly, 0.2 ml of the tea extract was transferred into a flask. Then, 0.5 ml of folin-ciocalteu reagent and 0.1 ml saturated sodium carbonate solution were added into the flask. The whole mixture was taken to 10 ml of distilled water. The final mixture was maintained in a dark place for 30 min before measuring the absorbance via a UV-visible spectrophotometer (Cary-100conc, Japan) at 765 nm. Total phenols were expressed as gram of gallic acid equivalent/kg of green tea extract (g GAE/kg).

- *Proximate analysis and pH values*

Moisture, fat, ash, protein, and starch were determined in triplicate orders according to AOAC (AOAC 1990). A pH meter (Hanna Microprocessor pH meter, Portugal) was used and pH values were measured.

- *Colour evaluation using ImageJ*

The surfaces of the sausages were photographed using camera (Samsung ST6500, Korea) and the images were evaluated using ImageJ software. First of all, it was necessary to eliminate the background and the colours were measured and expressed as lightness (L^*) and, redness (a^*).

- *Measurements of antioxidative activity*

In order to measure the thiobarbituric acid reactive substances (TBARS), coated and uncoated sausages (20 g) were homogenized with DDW (50 mL) using a Biomixer (Hamilton Beach, USA) for 2 min. The homogenate (2 mL) was transferred to a centrifuge tube and 2 mL of TBA reagent was added. The solution was then heated in a 100°C water bath for 15 min and cooled in an ice bath for 10 min. The resulting mixture was centrifuged at 6000 g for 5 min at 22±1°C, the supernatant was collected and the absorbance was measured at 532 nm using a UV-visible spectrophotometer (Rotof32A, Japan) (Shon *et al.*, 2010).

- *Percent moisture loss (PML)*

PML was determined according to the modified method of Shon *et al.* (2010). The moisture content (MC, wet basis) of the samples was determined using the AOAC method (AOAC 1990). Samples (about 60 g) were oven (Binder, Germany) dried at 102°C for 5 h. The samples were then cooled in a desiccator to room temperature and reweighed (Mettler AE 160, Switzerland). The moisture content of the samples was determined during storage for two weeks at

4°C. The PML was calculated as:

$$\text{PML (\%)} = \frac{[(\text{initial MC} - \text{final MC})/\text{initial MC}] \times 100}{}$$

- *Statistical analysis*

The statistical analysis was carried out by oneway ANOVA using the Minitab 16 software (Minitab Ltd, USA) and the differences at $p < 0.05$ were considered as significant.

Results and Discussion

- *Proximate analysis and Total Phenolic Compounds*

The mean values of samples of moisture, crude fat, crude protein, ash, starch content (%) and total phenolic compounds were 58.99±0.13, 20.06±0.16, 14.19±0.98, 2.4±0.14, 4.90±0.30, and 0.189±0.025 respectively.

- *pH values*

The pH values of each treatment during storage are shown in Table 2. The loss of pH was from 0.17% to 0.7% for collagen coated with 1% green tea extract and control samples, respectively. Therefore treatment with 5% collagen +1% green tea extract had an effect on the pH after two weeks of storage ($p < 0.05$), but did not affect the collagen-coated sample ($p > 0.05$). The reduction in pH was due to the formation of lactic acid by lactic acid bacteria that consumed the added carbohydrate in the formulation as an energy source (Antara *et al.*, 2004; Shon *et al.*, 2011).

- *Percent moisture loss (PML)*

The storage time had a significant effect on moisture content (MC), while the interaction of treatment and storage time was also significant; $p < 0.05$ (Table 3). The PML for both control and collagen-coated sausages increased with increased storage time. As the results have shown, PML in control was the highest (5.37%) and, in contrast, in 1% tea extract coated-sausages

was the lowest (2.9%). This might have occurred due to the good moisture-barrier properties of collagen coating. Previous reports have demonstrated that whey protein coating reduced the moisture loss in low fat sausages (Shon & Chin 2008; Shon *et al.*, 2011) and cut beef steak (Haque *et al.*, 2009). During preparation of the coating solution, the mixture is heated to 90°C for 30 min, producing disulfide bonds with greater surface hydrophobicity. It is conceivable that the collagen coating was effective as a moisture-barrier because of its hydrophobic nature (Shon & Haque, 2007).

- Color Evaluation

The L* and a* values are presented in Table 4. The results indicated that L* value,

which reflects lightness, decreased significantly for control and collagen-coated samples during storage. But this decrease in control was larger than the others; 4.82% (Table 4). In contrast, the a* value was increased and, the results indicated that the lowest increase occurred in 1% tea extract samples (Table 4). This phenomenon might occur due to lipid oxidation. In concordance with the previous research findings, lipid oxidation might initiate the oxidation of myoglobin to metmyoglobin, and change the meat color from red to an unattractive brown. The rate of meat discoloration is closely related to the rate of myoglobin oxidation induced by lipid oxidation (Shon *et al.*, 2010; Young *et al.*, 2004).

Table 2. pH values of sasauges during storage at 4°C for two weeks under vacuum packaging.

	Storage time (weeks)		
	0	1	2
Control		5.78±0.0007	5.77±0.0028
5% C	5.84±0.0014	5.84±0.0141 ^a	5.79±0.0014 ^b
5%C+0.5%E		5.81±0.0014 ^{ab}	5.79±0.0007 ^b
5%C+1%E		5.81±0.0028 ^{ab}	5.80±0.0007 ^a

Values represent means of three replications ± standard deviations.

Control; uncoated sausage, C; Collagen fiber coated sausage, E; Green tea extract, MC; moisture content (%), PML; percent moisture loss (%).

^{a-c} Means within the same column with different letters are significantly different (P < 0.05).

Table 3. Moisture contents and percent moisture loss of sausages during storage at 4°C for two weeks under vacuum packaging

		Storage time (weeks)		
		0	1	2
MC (%)	Control	59.72±0.05 ^a	58.61±0.06 ^c	56.52±0.10 ^c
	5% C	59.65±0.05 ^{ab}	59.35±0.06 ^b	57.28±0.15 ^b
	5%C+0.5%E	59.56±0.05 ^b	59.49±0.04 ^{ab}	57.74±0.04 ^a
	5%C+1%E	59.68±0.05 ^{ab}	59.54±0.10 ^a	57.95±0.13 ^a
PML (%)	Control	—	1.75±0.26 ^a	5.37±0.07 ^a
	5% C	—	0.51±0.01 ^b	3.97±0.17 ^b
	5%C+0.5%E	—	0.13±0.03 ^c	3.06±0.03 ^c
	5%C+1%E	—	0.23±0.08 ^{bc}	2.90±0.21 ^c

Values represent means of three replications ± standard deviations.

Control; uncoated sausage, C; Collagen fiber coated sausage, E; Green tea extract, MC; moisture content (%), PML; percent moisture loss (%).

^{a-c} Means within the same row with different letters are significantly different (P < 0.05).

- Antioxidative Activity

The TBARS values for control and collagen-coated sausages, were measured (Figure 1). The TBARS formation significantly increased during two weeks of storage and this formation after two weeks was quite significant. This indicated that lipid oxidation occurred in samples during refrigerated storage under anaerobic packaging, much faster after the second

week. By considering the previous reports about threshold value for the perception of rancidity by consumers, ≥ 0.5 mg/kg MDA concentration (Shon *et al.*, 2011; Shon *et al.*, 2010), it is clear that, after the second week, all the samples will be rejected by consumers. Samples with green tea extract had lower TBARS values due to the presence of phenolic compounds (Figure 1).

Table 4. Colour determination of sausages during storage at 4°C for two weeks under vacuum packaging.

		Treatments			
	Storage time (weeks)	Control	5% C	5%C+0.5%E	5%C+1%E
L*	0	73.37±0.22 ^{aa}	72.23±0.17 ^{aa}	76.43±0.11 ^{aa}	75.49±0.01 ^{ab}
	1	72.26±0.08 ^{ab}	71.32±0.08 ^{ab}	75.82±0.10 ^{aa}	71.23±0.13 ^{ac}
	2	69.83±0.10 ^{ac}	71.84±0.08 ^{ac}	76.25±0.11 ^{ab}	75.91±0.06 ^{aa}
a*	0	2.26±0.001 ^{dc}	2.46±0.001 ^{cc}	3.06±0.001 ^{bc}	3.20±0.001 ^{ac}
	1	2.60±0.001 ^{db}	2.85±0.010 ^{cb}	3.25±0.010 ^{ab}	3.20±0.001 ^{bb}
	2	3.76±0.010 ^{aa}	3.08±0.001 ^{da}	3.41±0.001 ^{ba}	3.39±0.001 ^{ca}

Values represent means of three replications ± standard deviations.

Control; uncoated sausage, C; Collagen fiber coated sausage, E; Green tea extract, MC; moisture content (%), PML; percent moisture loss (%).

^{a-d} Means within the same row with different letters are significantly different (P < 0.05).

^{a-c} Means within the same column with different letters are significantly different (P < 0.05).

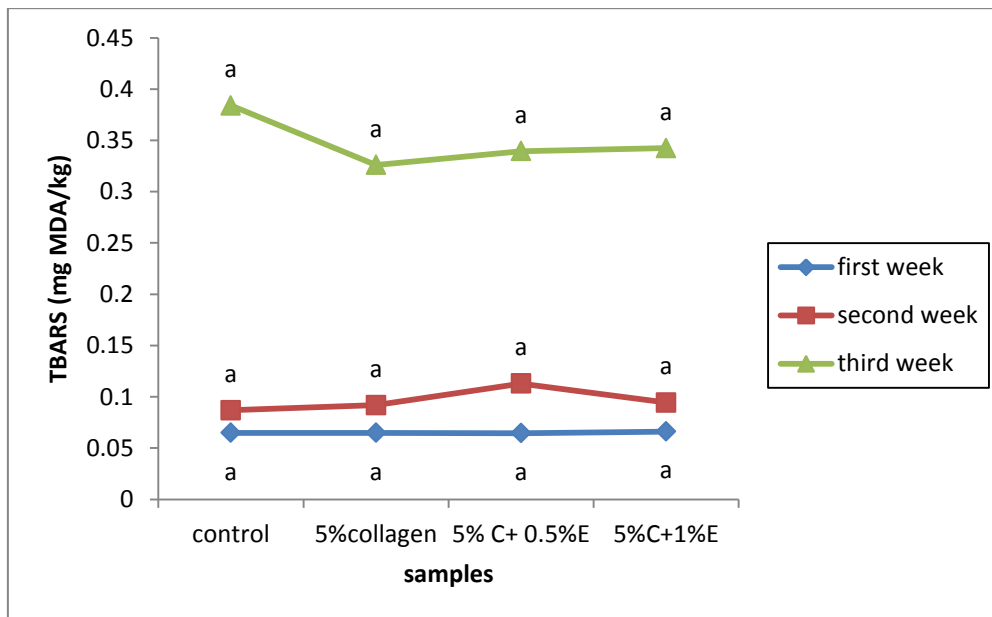


Fig. 1. Changes in TBARS (mg MDA/kg) formation in sausages with different coatings during storage at 4°C for two weeks in vacuum packaging Control; uncoated sausage, C; Collagen fiber coated sausage, E; Green tea extract

Conclusion

Collagen fiber edible coating delayed lipid oxidation of sausages as measured by the formation of TBARS. The PML of sausages was significantly reduced by 46% with 1% tea extract coating as compared to the control. The collagen and green tea extract coated-sausages were effective in maintaining the initial color of the samples. The results of this study suggest that collagen fiber and natural antioxidative coating could effectively be used for meat products to maintain the quality and the shelf life during refrigerated storage.

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