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The combined effect of pomegranate peel phenolic compounds cross-linked in the aloe vera hydrogel structure to extend the shelf-life of sheep liver

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ABSTRACT

One of the most important challenges related to fresh meat products (especially sheep liver) is the short shelf-life of the product. For this purpose, synthetic preservative compounds are used to extend their shelf life, which have many side effects on human health. In this research, the effect of using an edible coating containing phenolic compounds of pomegranate peel cross-linked in the structure of aloe vera hydrogel was studied to extend the shelf life of sheep liver. Physicochemical (pH, peroxide index, total volatile nitrogen, thiobarbituric acid index and color parameters $L^*a^*b^*$) and sensory (aroma, color, texture and overall acceptability) properties of the product in storage days of 1, 3 and 7 days and in the storage temperature of +4°C was evaluated. The results indicated that, the lowest value of monohydroperoxide formation (i.e. peroxide index) was observed in test samples coated with aleo vera gel containing phenolic compound of pomegranate peel extract that corresponding to the lowest total volatile nitrogen. Generally, in accordance to the results of sensory evaluation and quality tests, using of aleo vera gel containing bioactive compounds of pomegranate peel extract is recommended for shelf life extending of sheep liver.

1. Introduction

Meat and its products are one of the most valuable sources of protein in human nutrition. Foods, especially meat products (such as sheep liver) are exposed to physical, chemical, enzymatic and microbial spoilage during storage. On the other hand, the oxidation of fats in food occurs due to various types of reactions. As a result of the existence of free radicals and the presence of oxygen, oxidation progresses and hydroperoxides and other degradation products are produced [1]. The reaction of various types of oxygen, such as hydroxy radical (HO^{o}), superoxide anion (O_2^{o-}) and aloxy radicals (ROO^{o}), can oxidize lipids and proteins [2]. Volatile compounds resulting from breakdown, oxidation reaction and hydrolytic reaction of fats (hydroperoxides, aldehydes, ketones, fatty acids, etc.), change the smell, taste, color, texture, nutritional value and overall quality of the product and it causes the product to be undesired for consumers [3]. Among the measures methods that have been reported in postponing food spoilage, it is possible to mention the use of low temperature, keeping products in a dark environment, removing oxygen (creating a vacuum) or replacing air with a modified atmosphere in packaging, using antioxidants, using coatings and biodegradable films [4, 5].

Food coatings are often made of natural compounds of protein, polysaccharide and fat alone or in combination as a thin layer on the surface of food. The use of such coatings, in addition to their preservation properties, also minimizes the risks of using chemical additives and pollution caused by food packaging waste. Due to the desire to consume healthier food among consumers, biodegradable coatings have been receiving more and more attention in the food industry. Despite proving the advantages of such coatings, it is necessary to use complementary methods to eliminate the defects of these coatings. Due to the suitable conditions for the preparation of edible coatings, various types of antimicrobial and antioxidant materials, including bacteria, enzymes, and non-volatile (such as bioactive compounds of pomegranate peel) and volatile (edible essential oils) chemical and natural compounds can also be used to improve the effectiveness of these coatings and increase food safety during maintenance [6]. The main purpose of adding antimicrobial compounds to food coatings is to strengthen the antimicrobial property, which leads to the control of the surface growth of spoilage and disease-causing agents. Therefore, the use of such films in foods such as meat is very useful. The advantage of this method is the slow and gradual release of antimicrobial compounds into the food [7].

Aloe vera is a plant that is best known for its medical and treatment properties. The main composition of aloe vera gel contains polysaccharides and soluble sugars, along with proteins, vitamins and minerals. Aloe vera gel, which has no taste, color and smell, is used in pharmaceutical and food industries. In recent years, research on aloe vera gel as a coating for meat products has increased rapidly. Based on the research, this gel forms a protective layer against oxygen and air humidity with different mechanisms, and because of its antimicrobial compounds, it prevents the activity of microorganisms [8].

Pomegranate peel belongs to the *Punicaceae* family. Pomegranate peel is a major agricultural and industrial byproduct rich in hydrolyzable polyphenols (i.e., ellagitannins), which can be an excellent natural source of antioxidants. In fact, pomegranate peel constitutes approximately 30-40% of the total weight of the fruit and is a rich source of various bioactive compounds including flavonoids, tannins, phenolic acids and especially anthocyanins [9]. According to Xi et al. (2019), panicalagin and ellagic acid are the two main polyphenols in pomegranate peel,

and panicalagin greatly contributes to antioxidant activity [10].

Previous studies showed that there has been no research on extending the shelf life of sheep liver pieces. However, extensive research has been done on the development of shelf life of other meat products by using edible coatings, which will be mentioned below. Licciardello et al. (2018) studied the effect of edible coating (chitosan and locust bean gum) containing pomegranate peel extract on preserving the quality of fresh shrimp. The results showed that the use of pomegranate peel extract along with chitosan edible coating had a synergistic role in inhibiting microbial growth. In addition, the results showed that the amount of volatile nitrogen in shrimps coated with (chitosan+ pomegranate peel extract) after 6 days of storage was similar to the control sample (after 2 days of storage) [11]. Rahnemoon et al. (2018) studied the use of edible alginate coating enriched with pomegranate peel extract on the shelf life, physical and chemical properties, as well as microbiological contamination of chicken breast meat. The results indicated that coating with alginate and extract reduced the development of microbiological growth (Salmonella enteritidis, Escherichia coli, Staphylococcus aureus and Listeria monocytogenes) of chicken breast meat compared to other treatments. The results of the texture test showed that the samples treated with alginate coating had a more cohesive and firmer texture than the control sample, and this condition was stable during the storage period [12].

Considering that sheep liver is one of the most consumed and important internal organs of sheep, with high nutritional and economic value, improving the shelf life of this valuable product is of great importance. Therefore, in this research, in order to extend the shelf life of sheep liver, phenolic compounds extracted from pomegranate peel were cross-linked in the structure of aloe vera hydrogel. Finally, by coating the test samples, the qualitative and sensory characteristics of the product were monitored and evaluated during the storage period (7 days at +4^{o}C).

2. Material and methods

2.1. Raw material preparation

The required quantities of fresh pomegranate (*Punica granatum*) were purchased from the local market (Tehran province). Fresh pomegranate peels were separated and dried in the shade at a temperature of about 25^{o}C. According to the common method of AOAC (No. 931.04), the moisture content of dried skins was measured at 105^{o}C for 24 hours [13]. The moisture content of dried skins was reported as 4.76% (wet basis). Also, the chemical compounds used for this research were purchased from Sigma Aldrich (USA) and Merck (Germany).

2.2. Extraction of bioactive compounds of pomegranate peel

Extraction of bioactive compounds of pomegranate peel was done by solid-liquid extraction (leaching process) under atmospheric pressure conditions (1 atm) and statically. For this purpose, bioactive compounds were extracted under operating conditions (~83°C and 49 min). A ratio of 1 to 20 (dry matter to solvent) (distilled water as a solvent) was used for the extraction process [9]. The extracted extract was desolvated at a temperature of 40°C by a rotary evaporator (IK, Germany) until a constant weight was reached. Then, the concentrated extract was dried by a freeze dryer (Dena-Sanat, Tehran, Iran) at a temperature and pressure of -40^{o}C and 0.001 mbar, respectively [14].

2.3. Aloe vera hydrogel preparation and cross-linked with bioactive compounds

For this purpose, first the thick epidermis (skin) of aloe vera leaf was carefully cut with a sharp surgical blade and separated from the parenchyma (gel fillet). The parenchyma was immersed in a container containing distilled water to remove bitterness. Then, after 5 minutes, the contents were drained, and the

parenchyma was removed from the water and mixed in a home food processor (for 1 minute at 25°C) and turned into a uniform colloidal solution [8]. The treatments used to extend the shelf life of sheep liver include T(I): uncoated, T(II): coated with aloe vera hydrogel solution (pure gel) and T(III): coated with pomegranate peel bioactive compounds (2000 ppm or 2000 mg/kg) cross-linked in the structure of aloe vera hydrogel. The coated samples were placed in polypropylene packages and kept in a refrigerator at 4°C (relative humidity of 90-95%) for 7 days. The quality characteristics of the product were monitored and evaluated at intervals of 1, 3 and 7 days [12].

2.4. Sheep liver coating

For this purpose, the test samples were cut into pieces with dimensions (length, width and height of 15cm×15cm×15cm, respectively). After washing with distilled water, the samples were soaked in coating solutions (prepared in section 2-3) for 90 s. The coating procedure was repeated after 5 min and allowed to remove excess dispersion on the surface of the samples. The prepared test samples were placed at room temperature for 0.5 h to develop and stabilize the coating layer. Then the sheep livers were placed in the common storage packages of this product [12].

2.5. Product quality control chemical tests

In order to check the quality characteristics of the product during the storage period, the quality control tests of pH-value, peroxide index (PV), thiobarbituric acid index (TBA) and total volatile nitrogen (TVB-N) were used [14].

2.6. Color parameters (L^*, a^* & b^*)

The color evaluation of the test samples was measured using a Hunter lab colorimeter. Before the test, the device was calibrated with a white tile that has the standard values of L^*=67.81, a^*=19.56, and b^*=58.16, which were suggested by the manufacturer. The test sample was placed in the center of the circular glass cup provided for color analysis. The color component L^* represents the level of brightness, which ranges from 0 (black) to 100 (white). Also, the two color components a^* and b^* represent green (-120) to red (+120) and blue (-120) to yellow (+120), respectively [14, 15].

2.7. Sensory and organoleptic evaluation

Sensory evaluation of test samples (raw sample) was done by a group of semi-trained sensory evaluators, consisting of 10 people in the age range of 20 to 50 years (male and female). All evaluations were done by five-point hedonic scoring method. In this way, questionnaires were prepared and distributed among the evaluation team (panelists). In order to evaluate the sensory properties of the test samples (on the 7th day of storage at +4°C), about 100 g of the samples (uncoated and coated) were provided to the panelists in separate containers, and then the sensory properties (smell and aroma, color, context and overall acceptance) was recorded in the relevant questionnaire [15].

2.8. Statistical analysis

Statistical analysis of data was done using factorial test in the form of completely randomized design (CRD). Comparison of mean data was done using Duncan's multi-range test at 95% error probability level. SAS software version 9.1.3 (2003) of SAS Institute of America was used for statistical analysis. All qualitative tests were performed in 3 repetitions and the results were reported as (mean ± standard deviation) [14].

3. Results and discussion

3.1. pH-value & total volatile basic nitrogen (TVB-N)

Total volatile nitrogen refers to the nitrogen of protein compounds that is released as a result of the activity of proteolytic enzymes and the breakdown of the protein structure.

The formation of volatile nitrogen indicates the beginning of spoilage and its increase indicates the progress of protein spoilage in feed or food [14, 16]. The results of the mean comparison showed that the interaction effect of the edible coating type (T-I, T-II and T-III) and storage time (1, 3 and 7 days) had a significant effect ($p<0.01$) on total volatile nitrogen index and pH of sheep liver. As can be seen in Table 1, the highest total volatile nitrogen index (corresponding to the highest pH-value) is related to the treatment (T-I) and on the 7th day of storage. Observing the total volatile nitrogen and pH values of the samples after 7 days of storage showed that the use of edible coating containing the bioactive compounds of pomegranate peel cross-linked in the aloe vera hydrogel structure (T-III) was able to reduce the noted values to ~31% and ~3.5%, respectively. Licciardello et al. (2018) investigated the effect of edible coating of chitosan mixed with pomegranate peel extract on the change of volatile nitrogen of whole white shrimp during storage in the refrigerator. According to the results, it was observed that the amount of total volatile nitrogen fluctuated in the range of about 20 to 170 (mg/100 g), the lowest amount of it was related to the samples coated with chitosan cross-linked with pomegranate peel extract. Probably, this state is due to the antimicrobial property of the plant extract used on the microbial population and the bacteria growth, which confirms the results of the present study [11]. On the other hand, the results showed that the type of edible coating and storage time had a significant effect ($p<0.01$) on the pH-value of sheep liver (Table 1). According to the obtained results, it was observed that the highest pH value is related to the test samples without coating and on the 7th day of storage. El-Lahamy et al. (2018) reported that the pH value of frozen fish burger increases with storage time, which is probably due to the formation of dimethylamine from trimethylamine oxide [17].

3.2. Peroxide value (PV) & thiobarbituric acid (TBA)

The peroxide index is one of the qualitative criteria for measuring the concentration of the primary oxidation products (formation of monohydroperoxides) of fats [18]. The results of the mean comparison showed that the interaction effect of the type of edible coating and storage time on the chemical indices of peroxide and thiobarbituric acid of sheep liver is significant ($p<0.01$). As seen in Table 1, the highest amount of peroxide index (or thiobarbituric acid) is related to treatment (T-I) and on the 7th day of storage. Observing the values of peroxide and thiobarbituric acid indices of the samples after 7 days of storage showed that the use of edible coating containing the bioactive compounds of pomegranate peel cross-linked in the aloe vera hydrogel structure (T-III) could reduce the mentioned indices to ~24.62% and ~18.78%, respectively. Previous research showed that the use of bioactive compounds (phenolic compounds) in food formulations or edible coatings can have a significant effect in reducing peroxide and thiobarbituric acid indices [19].

Another indicator for measuring the progress of the oxidative reaction in protein products containing high oil is the thiobarbituric acid index. By measuring this index, the concentration of secondary metabolites of fat oxidation (such as malondialdehyde, alcohol, stane, acids, etc.) that results from the decomposition of monohydroperoxides can be measured, and this reaction causes the rancidity of fats and has a direct effect on the taste of food product [18]. According to the results presented in Table 1, it was observed that the effect of the type of edible coating and storage time on the thiobarbituric acid index was significant ($p<0.01$). Jooyandeh and Yademellat (2017) investigated the antioxidant and antimicrobial effect of

pomegranate peel extract on beef hamburger. Their results showed that the amount of thiobarbituric acid index in the samples containing pomegranate peel extract was lower than the control sample, which is consistent with the results of the present study. They reported that this condition is due to the phenolic compounds present in the pomegranate peel extract, which prevent the creation of free radicals and the propagation of free radical reactions, and it does this by trapping metal ions (such as iron). Phenolic compounds having a hydroxyl group attached to the aromatic ring are capable of donating hydrogen atoms and neutralizing free radicals. This process prevents further degradation of the oxidation products into more active oxidizing forms such as malondialdehyde [20].

Table 1 The mean values (average of 3-replication) of quality control parameters of sheep liver under the effects of different coating type and storage time at +4°C.

Type of coating(*)	Storage time (day)	Product quality control parameters(**)			
		pH	TVB-N	PV	TBA
		(-)	(mg N2/[100 g])	(meq O_2/[kg oil])	(mg MDA/kg)
T(I)					
	1	4.67^{d}	21.28^{f}	0.290^{f}	1.154^{g}
	3	4.76^{b}	29.31^{c}	0.497^{c}	1.417^{c}
	7	4.90^{a}	38.17^{a}	0.650^{a}	1.661^{a}
T(II)					
	1	4.58^{g}	20.63^{f}	0.283^{f}	1.152^{g}
	3	4.65^{e}	26.04^{d}	0.433^{d}	1.294^{e}
	7	4.76^{b}	31.55^{b}	0.570^{b}	1.506^{b}
T(III)					
	1	4.62^{f}	20.44^{f}	0.280^{f}	1.505^{g}
	3	4.66^{de}	22.40^{e}	0.397^{e}	1.506^{f}
	7	4.73^{c}	26.51^{d}	0.490^{c}	1.349^{d}

(*)The symbols of T(I), T(II) & T(III) represent control, aloe vera hydrogel alone & cross-linked phenolic compounds of pomegranate peel in aloe vera hydrogel structure, respectively.
(**)In each column, the mean values (Ave.±SD) with similar superscript letters had no significant difference (p<0.05).

3.3. Color parameters (L*, a* & b*)

The color of the produced product is one of the most obvious quality indicators for the marketability of the product [14]. The mean comparison results of the interaction effect of edible coating type and storage time on the color parameters ($L^*a^*b^*$) of sheep liver are presented in Table 2. As can be seen, the type of edible coating and storage time had a significant effect ($p<0.01$) on the color components of the sheep liver. According to the results, the lowest brightness intensity index (corresponding to the highest redness index) is related to treatment (T-I) and on the 7th day of storage. Georgantelis et al. (2007) studied the effect of rosemary extract on the color stability of beef hamburger. Their findings indicated that the color loss in the samples containing rosemary extract was less compared to the control sample and this condition was maintained during the storage period. This condition is probably due to the protective role of the phenolic compounds of the plant extract in preventing the oxidation of myoglobin pigment and its transformation into metmyoglobin [21]. The findings of Jooyandeh and Yademellat (2017) showed that meat discoloration and fat oxidation reaction are two related and synergistic phenomena. Considering that during the storage period, primary (monohydroperoxides) and secondary (unsaturated aldehydes) oxidation products are formed, which react with the iron ion present in the oxymyoglobin pigment (Fe^{+2}

ion) and with its regeneration, cause the oxidation of the above pigment and the formation of pigment metmyoglobin (the ion of the center of the porphyrin ring is converted to Fe^{+3}), which is associated with the darkening of the color of the product. They also reported that the use of pomegranate peel extract reduces the intensity of this reaction due to the delay in the oxidation reaction [20].

Table 2 The mean values (average of 3-replication) of color parameters of sheep liver under the effects of different coating type and storage time at +4°C.

Type of coating(*)	Storage time (day)	Color parameters(**)		
		L*	a*	b*
		(-)	(-)	(-)
T(I)				
	1	51.72^{d}	16.34^{e}	14.75^{f}
	3	49.64^{f}	19.05^{c}	17.02^{e}
	7	48.32^{g}	22.19^{a}	18.86^{cd}
T(II)				
	1	52.96^{bc}	17.76^{d}	18.71^{d}
	3	51.34^{de}	18.84^{c}	19.17^{cd}
	7	50.73^{e}	20.61^{b}	20.41^{b}
T(III)				
	1	54.06^{a}	15.84^{e}	19.55^{c}
	3	53.63^{ab}	19.37^{c}	20.57^{b}
	7	52.61^{c}	20.31^{b}	21.61^{a}

(*)The symbols of T(I), T(II) & T(III) represent control, aloe vera hydrogel alone & cross-linked phenolic compounds of pomegranate peel in aloe vera hydrogel structure, respectively.

(**)In each column, the mean values (Ave.±SD) with similar superscript letters had no significant difference (p<0.05).

3.4. Sensory attributes

The results of sensory evaluation of fresh raw sheep liver (coated and uncoated), after 7 days of storage at +4°C, are shown in Figure 1. As can be seen, the sheep liver coated with treatment (T-III) (edible coating containing bioactive compounds of pomegranate peel cross-linked in the structure of aloe vera hydrogel) obtained the highest score in terms of all sensory indicators (odor and aroma, color, texture and overall acceptance). Therefore, according to the results of sensory evaluation and quality control tests, the use of edible coating containing bioactive compounds of pomegranate peel cross-linked in the structure of aloe vera hydrogel is suggested for the industrial production of this product.

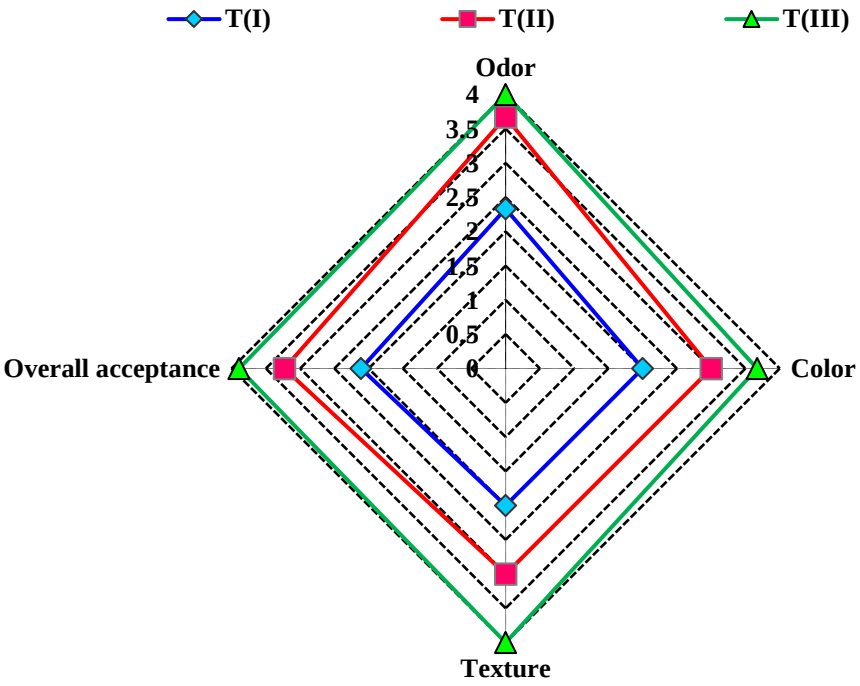

Fig 1 Scores of sensory characteristics of sheep liver coated with different edible coatings at the end of the storage period (the statistical index was the average at the statistical level of 95%).

4. Conclusion

The by-products obtained from the slaughter of livestock and poultry (especially sheep liver) are considered one of the most important sources of animal protein for human nutrition. One of the most important concerns of manufactures and sellers of these products is the short shelf life of the product during the distribution and sales chain. For this purpose, synthetic preservative compounds are used to extend their shelf life, which have many side effects on human health. Therefore, in this study, the feasibility of using a natural preservative compound (bioactive compounds of pomegranate peel) to develop the oxidative stability of sheep liver was investigated. After preparing and formulations of different edible coating (control, aloe vera hydrogel, and phenolic compounds cross-linked in the structure of aloe vera hydrogel), sheep liver pieces were coated by immersion method. The prepared samples were packed in polythene bags and kept at a temperature of 1±4^{o}C. Then the quality factors of the test samples were evaluated at different time intervals (1, 3 and 7 days). The results showed that the use of an edible coating containing the bioactive compounds of pomegranate peel cross-linked in the structure of aloe vera hydrogel (T-III) could dwindle the total volatile nitrogen, pH value, peroxide value and thiobarbituric acid value to ~31%, ~3.5%, 24.62% and 18.78%, respectively. By and large, according to the findings of this research, it is recommended to use the biological waste obtained from pomegranate peel to extend the shelf life of meat products (heart, liver, giblets, meat and fish fillets, chicken drumsticks and chicken fillets).

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چکیده

یکی از مهمترین چالش‌های مرتبط با فرآورده‌های گوشتی تازه (به‌ویژه جگر سیاه گوسفندی)، عمر نگهداری کوتاه محصول است. بدین منظور از ترکیبات نگهدارندهٔ سنتزی برای گسترش ماندگاری آن‌ها استفاده می‌شود که اثرات جانبی متعددی بر سلامت انسان دارند. در این پژوهش تأثیر بکارگیری پوشش خوراکی حاوی ترکیبات فنولیک پوست انار درهم‌تنیده شده در ساختار هیدروژل آلوئه‌ورا جهت گسترش ماندگاری جگر سیاه گوسفندی مورد مطالعه قرار گرفت. خواص فیزیکوشیمیایی (pH، شاخص پراکسید، نیتروژن فرار کل، شاخص اسید تیوباربیتوریک و پارامترهای رنگی $L^*a^*b^*$) و حسی (رایحه، رنگ، بافت و پذیرش کلی) فرآورده در روزهای نگهداری ۱، ۳ و ۷ روز و در درجه حرارت نگهداری ۴°C+ ارزیابی گردید. نتایج نشان داد که کمترین میزان تشکیل مونوهیدروپراکسیدها در نمونه‌های آزمایشی پوشش‌دهی شده با ژل آلوئه‌ورا حاوی ترکیبات فنولیک پوست انار مشاهده شد که متناظر با کمترین میزان بازهای ازتهٔ فرار بود. به‌طورکلی، با توجه به نتایج ارزیابی حسی و آزمون‌های کیفی صورت گرفته، بکارگیری پوشش خوراکی ژل آلوئه‌ورا حاوی ترکیبات زیست‌فعال پوست انار جهت گسترش ماندگاری جگر سیاه گوسفندی پیشنهاد می‌شود.