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## Investigating the effect of biodegradable edible coating based on chitosan, plantago ovata and pistacia atlantica on some microbial, chemical and sensory properties of minced meat

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### Abstract

Red meat is a rich source of protein, energy and B vitamins, amino acids are valuable and nutritious food sources. On the other hand, meat and its products, even during storage in the refrigerator, are exposed to spoilage due to microbial growth along with enzymatic and biochemical decomposition. The use of oral coatings leads to increasing the quality of the product and increasing its shelf life. In the present study, in order to cover the minced meat, chitosan solutions and 0.5, 1 and 1.5% of plantago ovata and 5.5% of pistacia atlantica were used and peroxide index tests were used. Volatile nitrogen bases, thiobarbituric acid, microbial and sensory properties were performed on the sample at intervals of 1, 3, 6, 9 and 12 days during refrigeration at 4 ° C. Evaluation of microbial test results showed that on the first day, the total number of live mesophilic and psychotrophic bacteria in Listeria-infected samples was significantly higher than other samples ( $p \leq 0.05$ ) and in other intervals. The highest total number of live mesophilic bacteria in sample 8 (meat sample infected with Listeria bacteria) and then in sample 9 (sample covered with 5.5% coriander gum and 0.5% pomegranate extract contaminated with Listeria) (10) (a sample coated with chitosan and 1% psyllium extract contaminated with Listeria) were observed ( $p \leq 0.05$ ). On the first day, the number of lactic acid bacteria, Pseudomonas and Enterobacteriaceae in listeria infected as well as control samples was significantly higher than other samples ( $p \leq 0.05$ ).

**Keywords:** Coating, meat, coriander gum, asparagus extract.

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## **1. Introduction:**

Meat and meat products as an important part of our daily diet can be considered as an excellent source of essential nutrients. In the food guide pyramid, red meat is also classified as a protein food group with chicken, fish, and eggs<sup>1,2</sup>. Undoubtedly, meat is the main source of proteins with high biological value and an excellent source of some valuable nutrients such as minerals and vitamins. Some of these nutrients (eg, iron, vitamin B12, and folic acid) are either not available in other foods or have lower bioavailability<sup>3</sup>. However, the association between meat consumption and an increased risk of serious health disorders and diseases such as colon cancer and cardiovascular disease is worrisome. Therefore, meat products are often avoided to reduce the risk of cancer, obesity and other diseases<sup>4,5</sup>. On the other hand, a variety of meat products, including hamburgers, are still very popular among consumers, especially the younger generation. The growing interest of people in ready-to-eat, fast and easy food is due to the busy life of industrial consumers<sup>6,7,8,9</sup>.

The most important challenge of meat is its perishability, which affects its health and quality. Meat is a very suitable environment for the activity of microorganisms due to its internal factors favorable for the growth of most microorganisms, especially the types of spoilage generators, and if it is not controlled by external factors, it will spoil quickly<sup>10,11,12,13,14</sup>. In order to prevent microbial and chemical spoilage of meat, various preservation methods have been proposed. These include the use of high temperatures (cooking) and low temperatures (freezing), the addition of salts and organic acids, drying, fermentation, smoking, canning, the use of modified atmospheric packaging and, more recently, the use of plant compounds<sup>15,16,17</sup>. The use of traditional preservation methods in the meat industry has an adverse effect on the attractiveness of the product. Over the years, many synthetic additives have been

used to increase shelf life at low temperatures<sup>18,19</sup>. These additives have carcinogenic and toxic properties<sup>9</sup>. Therefore, with the increasing concern of consumers about the consumption of such additives, the demand for healthier meat and meat products and natural food additives has increased. Manufacturers, on the other hand, face significant restrictions on the use of synthetic preservatives in their products<sup>20,21</sup>. So it makes sense to look for natural alternatives to chemical additives, because nature has been a good source of these compounds for a long time. In recent years, natural compounds of plant origin have been widely used in the food industry<sup>22,23,24,25</sup>.

Raw meat spoilage has been shown to occur in the food supply chain (producers, retailers, consumers) at rates of up to 20% and even higher. The increasing accumulation of packaging materials wastes, especially synthetic polymers and their long life cycle, has encouraged the food and packaging industries to explore biodegradable packaging materials<sup>26,27</sup>. So far, significant research has been done to obtain environmentally friendly or biodegradable food packaging materials<sup>28,29,30</sup>. A wide range of natural materials, including polysaccharides, proteins, fats, or a combination thereof, can be processed and used to make biodegradable and edible film and packaging coatings. On the other hand, the damage caused by the presence of oxidation-induced free radicals plays an important role in aging, cancer, heart disease, hypertension, neurological diseases and mutations.

## **2. Materials and methods:**

### **2.1. Materials:**

In the present study, pistacia atlantica will be prepared from cities in Kurdistan province of Iran. Chitosan powder was prepared from Sigma-Aldrich USA (Oakville, ON, USA). Other specifications of materials used, list of culture

medium and microbial solutions and equipment list are presented in Tables 1 and 2, respectively.

**Table 1. List of materials required in the present study**

<b>The manufacturer</b>	<b>Material</b>	<b>Row</b>
Merck - Germany	Thiobarbituric acid	1
Merck - Germany	Deionized distilled water	2
Merck - Germany	Dibutyl hydroxy toluene	3
Merck - Germany	Butylated hydroxyanisole	4
Merck - Germany	Trichloroacetic acid	5

**Table 2. List of culture media and microbial consumption solutions required in the present study**

<b>Country</b>	<b>The manufacturer</b>	<b>Device name</b>	<b>Row</b>
Germany	Merck	Nutrient-agar	1
Germany	Merck	Peptone solution 0.1%	2
Germany	Merck	Pseudomonas agar	3
Germany	Merck	Violet Red Bile Glucose (VRBG) agar	4
Germany	Merck	de Man, Rogosa and Sharpe (MRS) agar	5
Germany	Merck	Sulphite Polymyxin Sulphadiazine Agar	6
Germany	Merck	Physiological serum	7
Germany	Merck	Immersion oil	8

## **2.2. Sample preparation:**

The minced meat was prepared on the same day from a production line and packed in polypropylene trays and then transported and stored at temperature (4 ° C). After preparing homogeneous medallions with a diameter of 50 mm and a thickness of 5 mm with stainless steel blows, the minced meat was divided into two groups: 1) group inoculated without pathogenic bacteria, 2) inoculated with *L. monocytogenes* Were. In the inoculated group, minced meat was placed in everyday bags. The minced meat was soaked in the coating solutions for 30 seconds according to the designed groups. The coated minced meat was then immersed for 2 minutes, followed by a second immersion in CaCl<sub>2</sub> (Sigma

Aldrich Chemical Company) for 30 seconds. The coated samples were then placed in sterile polyethylene bags and stored at 4 ° C until testing. Coatings of different concentrations were prepared by dissolving an appropriate amount of psyllium gum powder in distilled water. The mixtures are heated and stirred to dissolve the powder effectively. The samples were divided into seven parts. Samples were coated alone with psyllium gum. Treatment was performed based on various initial experiments. In preparing the samples, 0.5, 1 and 1.5% of asparagus extract and 5.5% of gum were used. Soy lecithin (CDH Chemicals, India) was used as an emulsifier for effective oil dissolution. All samples except control (C) were given 1% calcium chloride before injection. The samples were placed in polypropylene trays and packed with transparent polyethylene packaging. Packed trays were stored at 4 ° C for further analysis and useful life studies.

**Table 3. The treatments studied in the present study**

Specifications	Code
Control (no asparagus extract)	Code 1
Sample coated with chitosan with 0.5% asparagus extract	Code 2
Sample coated with chitosan with 1% asparagus extract	Code 3
Sample coated with chitosan with 1.5% asparagus extract	Code 4
Sample coated with 5.5% coriander gum and 0.5% pomegranate extract	Code 5
Sample coated with 5.5% coriander gum and 1% asparagus extract	Code 6
Sample coated with 5.5% coriander gum and 1.5% pomegranate extract	Code 7
Sample of meat infected with <i>Listeria</i> bacteria	Code 8
Sample coated with 5.5% coriander gum and 0.5% pomegranate extract	Code 9
Chitosan-coated sample with 1% psyllium extract infected with <i>Listeria</i>	Code 10
Chitosan-coated sample with 1.5% Asparagus extract infected with <i>Listeria</i>	Code 11
Sample coated with 5.5% coriander gum and 0.5% asparagus extract infected with <i>Listeria</i>	Code 12
Sample coated with 5.5% coriander gum and 1% pomegranate extract and infected with <i>Listeria</i>	Code 13
Sample coated with 5.5% coriander gum and 1.5% pomegranate extract and infected with <i>Listeria</i> bacteria	Code 14

### **3. Tests performed on meat samples:**

#### **3.1. Lipid Oxidation (TBARS):**

Lipid oxidation was assessed based on thiobarbituric acid test (TBARS). TBARS was measured by colorimetric method based on the method of Zhang et al. (2016). For this purpose, 20 g of meat was mixed with 50 ml of 20%

trichloroacetic acid for 2 minutes. The contents of the blender were washed and mixed with 50 ml of water and then filtered through Whatman No. 1 filter paper. Then 5 ml of the extract was mixed with 0.01 M thiobarbituric acid and stored at 100 ° C for one hour. Then the absorption of the pink dye solution was measured using a UV-visible spectrophotometer at a wavelength of 532 nm. TBARS was reported as mg malondialdehyde per kilogram of meat sample.

### **3.2. TVBN measurements:**

To measure TVBN, 10 g of the sample is mixed with 190 ml of distilled water, connected to a 500 ml hot flask, and 2 g of MgO and a drop of silicone as an anti-foam solvent are added to the mixture before steam distillation. 3% aqueous solution of boric acid, methyl red and methylene blue is used as a mixture indicator to stabilize ammonia as a distillation receptor. Titration with hydrochloric acid solution (0.1 nitrogen) and TVBN are calculated as follows:

$$\% \text{ TVBN} = (V \times C \times 10) / 10$$

Where V and C indicate the volume and concentration of hydrochloric acid.

### **3.3. Measure the amount of peroxide:**

Lipids were extracted by standard method to measure the amount of peroxide (PV) by AOAC method in 30 ml of acetic acid / chloroform mixture and 0.5 ml of saturated potassium iodide mixture. After 3 minutes, distilled water (30 ml) is added to the mixture and fixed with Na<sub>2</sub>SO<sub>4</sub> (0.1 N). 0.5 ml of starch solution (1%) is used as a color marker and the titration is continued until a light color is formed. And PV is then calculated by the following formula:

$$\text{PV (meq / kg)} = (V_1 - V_0) \times T \times 100 / M$$

Where V<sub>1</sub> and V<sub>0</sub> show the volume of sodium thiosulfate solution and control (blank), respectively. M indicates the weight of the sample and T indicates the

molarity of the sodium thiosulfate solution. The amount of PV is expressed in milliequivalents of oxygen per kilogram of sample (meq / kg).

### **3.4. Microbial tests:**

Samples for microbial analysis on days 0 (after immersion), 3, 6, 9 and 12 days refrigerated with microbial counting using total colony counting methods on plate using plate count (PCA) Agar for aerobic bacteria, mesophilic aerobic bacteria and cold-blooded bacteria, MRS agar for lactic acid bacteria (LAB), Pseudomonas agar for Pseudomonas, VRBG agar for the Enterobacteriaceae family, and DRBC agar medium will be used for enumeration. Incubation conditions for 2 days at 37 ° C for total count of bacteria, Pseudomonas, and Enterobacteriaceae, 7 ° C for 10 days for cold count, 30 ° C for 2 days for LAB and 25 ° C for 5 The day will be for mold and yeast. Microbial colonies will be counted and expressed as Log 10 CFU / g of minced meat.

### **3.5. Sensory evaluation:**

Uncooked and coated meat after cooking at 185 ° C for 60 minutes based on taste, odor, color, texture and general acceptance characteristics will be examined and the results will be expressed in 9 hedonic scales. Sensory evaluation of samples was performed after 3 days of storage.

## **4. Results and discussion:**

### **4.1. Peroxide index:**

The results of comparing the mean peroxide index of the samples at intervals of 1, 3, 6, 9 and 12 days of storage at refrigerator temperature are presented in Table 4. The results of comparing the mean of the data showed that on the first day there was no statistically significant difference in the amount of peroxide index of the samples ( $p \geq 0.05$ ) and in other time intervals the highest amount of

peroxide index in sample 8 (meat sample infected with *Listeria* bacteria). Then observed in the control sample ( $p \leq 0.05$ ). In all time periods except the first day, increasing the asparagus extract led to a significant decrease in the peroxide index of the samples ( $p \leq 0.05$ ). So that the lowest amount of peroxide index belongs to samples 6 (sample covered with 5.5% coriander gum and 1% pomegranate extract) and 7 (sample covered with 5.5% coriander gum and 1.5% Pomegranate extract) ( $p \leq 0.05$ ) followed by samples 3 (sample coated with chitosan with 1% pomegranate extract) and 4 (sample coated with chitosan with 1.5% pomegranate extract). ( $P \leq 0.05$ ) and shows that samples containing coriander gum had a lower peroxide index than samples without coriander gum ( $p \leq 0.05$ ). Also, over time, the peroxide index of all samples increased significantly ( $p \leq 0.05$ ).

**Table 4. Changes in peroxide index of minced meat samples coated with chitosan, parchment gum and coriander gum during storage**

Storage time (days)					
Day 12	Day 9	Day 6	Day 3	Day 1	Code
2/81 ± 0.10 gA	1/00 ± 0.10 eB	1/23 ± 0.10 bcC	0/86 ± 0.10 bA	0/74 ± 0.10 abA	Code 1
2/28 ± 0.10 hA	1/40 ± 0.10 fB	1/11 ± 0.10 fC	0/94 ± 0.10 deA	0/71 ± 0.10 abA	Code 2
2/20 ± 0.10 hiA	1/44 ± 0.10 fB	1/10 ± 0.10 fgC	0/91 ± 0.10 efA	0/74 ± 0.10 abA	Code 3
2/21 ± 0.10 kA	1/40 ± 0.10 gB	1/08 ± 0.10 ghC	0/90 ± 0.10 fA	0/73 ± 0.10 abA	Code 4
2/22 ± 0.02 jA	1/43 ± 0.02 fgB	1/10 ± 0.02 fgC	0/93 ± 0.02 deA	0/70 ± 0.02 aA	Code 5
2/17 ± 0.06 kA	1/39 ± 0.06 hB	1/06 ± 0.06 hiC	0/79 ± 0.06 fgA	0/70 ± 0.06 aA	Code 6
2/13 ± 0.07 lA	1/30 ± 0.07 iB	1/04 ± 0.07 iC	0/76 ± 0.07 gA	0/72 ± 0.07 abA	Code 7
2/81 ± 0.01 aA	1/70 ± 0.01 aB	1/30 ± 0.01 aC	0/91 ± 0.01 aA	0/74 ± 0.01 abA	Code 8
2/70 ± 0.00 bA	1/60 ± 0.00 bB	1/20 ± 0.00 bC	0/80 ± 0.00 bA	0/70 ± 0.00 aA	Code 9
2/70 ± 0.07 cA	1/61 ± 0.07 cB	1/23 ± 0.07 bcC	0/81 ± 0.07 cA	0/74 ± 0.07 abA	Code 10
2/74 ± 0.03 dA	1/08 ± 0.03 deB	1/19 ± 0.03 dA	0/80 ± 0.03 cA	0/70 ± 0.03 aA	Code 11
2/76 ± 0.09 cdA	1/61 ± 0.09 cB	1/20 ± 0.09 cdA	0/81 ± 0.09 cA	0/73 ± 0.09 abA	Code 12
2/70 ± 0.03 eA	1/60 ± 0.03 cdB	1/19 ± 0.03 dA	0/90 ± 0.03 dA	0/74 ± 0.03 abA	Code 13
2/03 ± 0.09 fA	1/07 ± 0.09 deB	1/10 ± 0.09 eA	0/94 ± 0.09 deA	0/70 ± 0.09 aA	Code 14

Different Latin uppercase letters have a significant difference in the row and different Latin lowercase letters have a significant difference in the column ( $P < 0.05$ )



Code (1): Control (without pomegranate extract), Code (2): Sample coated with chitosan with 0.5% pomegranate extract, Code (3): Sample coated with chitosan with 1% pomegranate extract, Code (4): Sample coated with chitosan with 1.5% asparagus extract, Code (5): Sample coated with 5.5% coriander gum and 0.5% asparagus extract, Code (6): Sample Coated with 5.5% coriander gum and 1% asparagus extract, code (7): sample coated with 5.5% coriander gum and 1.5% asparagus extract, code (8): sample of meat contaminated with bacteria *Listeria*, code (9): sample coated with 5.5% coriander gum and 0.5% asparagus extract, code (10): sample coated with chitosan with 1% asparagus extract contaminated with *Listeria* bacteria, code (11): Sample coated with chitosan with 1.5% asparagus extract infected with *Listeria* bacteria, code (12): Sample coated with 5.5% coriander gum and 0.5% asparagus extract contaminated with *Listeria* bacterium, code (13): sample coated with 5.5% coriander gum and 1% asparagus extract and contaminated with *Listeria* bacteria, code (14): sample coated with 5.5% coriander gum and 5/5 1% asparagus extract infected with *Listeria* bacteria

#### **4.2. Volatile nitrogen bases:**

The results of comparing the mean volatile nitrogen bases of the samples at intervals of 1, 3, 6, 9 and 12 days of storage at refrigerator temperature are presented in Table 5. The results of comparing the mean of the data showed that on the first day there was no statistically significant difference in the amount of volatile nitrogen bases of the samples ( $p \geq 0.05$ ) and in other time periods the highest amount of volatile nitrogen bases in sample 8 (contaminated meat sample). *Listeria* bacterium) and then in sample 9 (sample coated with 5.5% coriander gum and 0.5% psyllium extract contaminated with *Listeria* bacteria) was observed ( $p \leq 0.05$ ). In all time periods except day 1, the increase of asparagus extract led to a significant decrease in volatile nitrogen bases of the samples ( $p \leq 0.05$ ). So that the lowest amount of volatile nitrogen bases belong to samples 6 (sample covered with 5.5% coriander gum and 1% pomegranate extract) and 7 (sample covered with 5.5% coriander gum and 1.5 % Of pomegranate extract) ( $p \leq 0.05$ ) and then samples 3 (sample coated with chitosan with 1% pomegranate extract) and 4 (sample coated with chitosan with 1.5% pomegranate extract) ( $P \leq 0.05$ ) and showed that samples containing coriander gum had lower volatile nitrogen bases than samples without coriander gum

( $p \leq 0.05$ ). Also, over time, volatile nitrogen bases of all samples increased significantly ( $p \leq 0.05$ ).

**Table 5. Total volatile nitrogen changes in chitosan-coated minced meat, parchment gum and coriander gum samples during storage**

Storage time (days)					
Day 12	Day 9	Day 6	Day 3	Day 1	Code
ε 7/76 ± 0.10 bA	37/84 ± 0.10 fB	23/71 ± 0.10 eC	14/70 ± 0.10 dD	9/98 ± 0.10 abE	Code 1
ε 4/80 ± 0.10 eA	37/73 ± 0.10 gB	23/28 ± 0.10 fC	12/30 ± 0.10 fD	10/17 ± 0.10 aE	Code 2
ε 3/73 ± 0.10 gA	37/17 ± 0.10 hB	23/00 ± 0.10 gC	12/88 ± 0.10 gD	9/98 ± 0.10 abE	Code 3
ε 2/47 ± 0.10 iA	37/07 ± 0.10 hiB	22/82 ± 0.10 gC	12/74 ± 0.10 gD	9/94 ± 0.10 abE	Code 4
ε 3/17 ± 0.02 hA	37/02 ± 0.02 hiB	22/91 ± 0.02 gC	12/78 ± 0.02 gD	9/80 ± 0.02 abE	Code 5
ε 2/47 ± 0.06 iA	30/74 ± 0.06 iB	21/93 ± 0.06 hC	11/90 ± 0.06 hD	10/12 ± 0.06 aE	Code 6
ε 1/08 ± 0.07 jA	30/04 ± 0.07 jB	21/28 ± 0.07 iC	11/20 ± 0.07 iD	9/89 ± 0.07 bE	Code 7
ε 7/74 ± 0.01 aA	ε 1/72 ± 0.01 aB	27/74 ± 0.01 aC	17/02 ± 0.01 aD	9/80 ± 0.01 bE	Code 8
ε 7/72 ± 0.00 bA	ε 0/79 ± 0.00 bB	24/00 ± 0.00 bC	10/08 ± 0.00 bD	10/17 ± 0.00 aE	Code 9
ε 0/97 ± 0.07 cA	ε 0/13 ± 0.07 cB	24/37 ± 0.07 bcC	10/27 ± 0.07 cD	10/08 ± 0.07 abE	Code 10
ε 0/17 ± 0.03 dA	39/99 ± 0.03 cB	24/08 ± 0.03 dC	13/90 ± 0.03 eD	10/08 ± 0.03 aAE	Code 11
ε 0/31 ± 0.09 dA	ε 0/13 ± 0.09 cB	24/27 ± 0.09 cdC	13/90 ± 0.09 eD	10/12 ± 0.09 aE	Code 12
ε 4/33 ± 0.03 fA	39/07 ± 0.03 dB	23/70 ± 0.03 eC	13/48 ± 0.03 fD	9/94 ± 0.03 abAE	Code 13
ε 3/87 ± 0.09 gA	39/10 ± 0.09 eB	23/33 ± 0.09 fC	13/30 ± 0.09 fD	10/08 ± 0.09 aE	Code 14

Different Latin uppercase letters have a significant difference in the row and different Latin lowercase letters have a significant difference in the column ( $P < 0.05$ )

Code (1): Control (without pomegranate extract), Code (2): Sample coated with chitosan with 0.5% pomegranate extract, Code (3): Sample coated with chitosan with 1% pomegranate extract, Code (4): Sample coated with chitosan with 1.5% asparagus extract, Code (5): Sample coated with 5.5% coriander gum and 0.5% asparagus extract, Code (6): Sample Coated with 5.5% coriander gum and 1% asparagus extract, code (7): sample coated with 5.5% coriander gum and 1.5% asparagus extract, code (8): sample of meat contaminated with bacteria *Listeria*, code (9): sample coated with 5.5% coriander gum and 0.5% asparagus extract, code (10): sample coated with chitosan with 1% asparagus extract contaminated with *Listeria* bacteria, code (11): Sample coated with chitosan with 1.5% asparagus extract infected with *Listeria* bacteria, code (12): Sample coated with 5.5%

coriander gum and 0.5% asparagus extract contaminated with *Listeria* bacterium, code (13): sample coated with 5.5% coriander gum and 1% asparagus extract and contaminated with *Listeria* bacteria, code (14): sample coated with 5.5% coriander gum and 5/5 1% asparagus extract infected with *Listeria* bacteria

### 4.3. Thiobarbituric acid:

The results of comparing the mean thiobarbituric acid of the samples at intervals of 1, 3, 6, 9 and 12 days of storage at refrigerator temperature are presented in Table 6. The results of comparing the mean of the data showed that on the first day there was no statistically significant difference in the amount of thiobarbituric acid in the samples ( $p \geq 0.05$ ) and in other time intervals the highest amount of thiobarbituric acid in sample 8 (meat sample infected with *Listeria* bacteria). And then in sample 9 (sample coated with 5.5% coriander gum and 0.5% psyllium extract infected with *Listeria* bacteria) was observed ( $p \leq 0.05$ ). In all time periods except day 1, the increase of asparagus extract led to a significant decrease in thiobarbituric acid of the samples ( $p \leq 0.05$ ). So that the lowest amount of thiobarbituric acid belongs to samples 6 (sample covered with 5.5% coriander gum and 1% pomegranate extract) and 7 (sample covered with 5.5% coriander gum and 1.5% Pomegranate extract) ( $p \leq 0.05$ ) followed by samples 3 (sample coated with chitosan with 1% pomegranate extract) and 4 (sample coated with chitosan with 1.5% pomegranate extract). ( $P \leq 0.05$ ) and shows that samples containing coriander gum had lower thiobarbituric acid than samples without coriander gum ( $p \leq 0.05$ ). Also, over time, thiobarbituric acid in all samples increased significantly ( $p \leq 0.05$ ).

**Table 6. Thiobarbituric acid changes in minced meat samples coated with chitosan, parchment gum and coriander gum during storage**

Storage time (days)					
Day 12	Day 9	Day 6	Day 3	Day 1	Code
$1/2 \pm 0.10$ hA	$97/03 \pm 0.10$ eA	$79/07 \pm 0.10$ gA	$43/39 \pm 0.10$ gA	$22/34 \pm 0.10$ aA	Code 1
$1/18 \pm 0.10$ iA	$97/70 \pm 0.10$ efA	$77/42 \pm 0.10$ hA	$41/74 \pm 0.10$ hA	$22/76 \pm 0.10$ aA	Code 2
$1/17 \pm 0.10$ jA	$90/07 \pm 0.10$ fgA	$77/47 \pm 0.10$ iA	$40/79 \pm 0.10$ iA	$21/97 \pm 0.10$ bA	Code 3

1/1 ± 0.10 lA	9 ± 4.2 ± 0.10 gA	73/23 ± 0.10 kA	37/06 ± 0.10 kA	22/3 ± 0.10 aA	Code 4
1/10 ± 0.2 kA	9 ± 7.5 ± 0.2 fgA	7 ± 1.8 ± 0.2 jA	38/01 ± 0.2 jA	22/3 ± 0.2 aA	Code 5
1/12 ± 0.6 mA	89/29 ± 0.6 hA	71/71 ± 0.6 lA	36/0 ± 0.6 lA	22/3 ± 0.6 aA	Code 6
1/1 ± 0.7 nA	89/6 ± 0.7 hA	6.0 ± 0.7 mA	3 ± 3.2 ± 0.7 mA	22/10 ± 0.7 abA	Code 7
1/30 ± 0.1 aA	1/11 ± 0.1 aA	81/87 ± 0.1 aA	06/2 ± 0.1 aA	22/3 ± 0.1 aA	Code 8
1/33 ± 0.5 bA	1/1 ± 0.5 abA	81/11 ± 0.5 bA	00/ ± 3 ± 0.5 bA	22/3 ± 0.5 aA	Code 9
1/32 ± 0.7 cA	1/0.9 ± 0.7 abA	79/02 ± 0.7 cA	03/80 ± 0.7 cA	22/10 ± 0.7 aA	Code 10
1/31 ± 0.3 eA	1/0.7 ± 0.3 bA	78/89 ± 0.3 dA	03/22 ± 0.3 dA	22/03 ± 0.3 aA	Code 11
1/32 ± 0.9 dA	1/0.8 ± 0.9 bA	79/1 ± 0.9 cdA	03/ ± 5 ± 0.9 cdA	22/6 ± 0.9 aA	Code 12
1/29 ± 0.3 fA	1/0.5 ± 0.3 cA	77/2 ± 0.3 eA	01/05 ± 0.3 eA	22/3 ± 0.3 aA	Code 13
1/26 ± 0.9 gA	1/0 ± 0.9 dA	7 ± 9.6 ± 0.9 fA	± 9.2 ± 0.9 fA	22/10 ± 0.9 abA	Code 14

Different Latin uppercase letters have a significant difference in the row and different Latin lowercase letters have a significant difference in the column (P < 0.05)

Code (1): Control (without pomegranate extract), Code (2): Sample coated with chitosan with 0.5% pomegranate extract, Code (3): Sample coated with chitosan with 1% pomegranate extract, Code (4): Sample coated with chitosan with 1.5% asparagus extract, Code (5): Sample coated with 5.5% coriander gum and 0.5% asparagus extract, Code (6): Sample Coated with 5.5% coriander gum and 1% asparagus extract, code (7): sample coated with 5.5% coriander gum and 1.5% asparagus extract, code (8): sample of meat contaminated with bacteria *Listeria*, code (9): sample coated with 5.5% coriander gum and 0.5% asparagus extract, code (10): sample coated with chitosan with 1% asparagus extract contaminated with *Listeria* bacteria, code (11): Sample coated with chitosan with 1.5% asparagus extract infected with *Listeria* bacteria, code (12): Sample coated with 5.5% coriander gum and 0.5% asparagus extract contaminated with *Listeria* bacterium, code (13): sample coated with 5.5% coriander gum and 1% asparagus extract and contaminated with *Listeria* bacteria, code (14): sample coated with 5.5% coriander gum and 5/5 1% asparagus extract infected with *Listeria* bacteria

#### 4.4. Evaluation of microbial test results:

The results of comparing the mean number of lactic acid bacteria in the samples at intervals of 1, 3, 6, 9 and 12 days at refrigerator temperature are presented in Table 7. The results of comparing the means of the data showed that on the first day, the number of lactic acid bacteria in the samples infected with *Listeria* and

also the control sample was significantly higher than the other samples ( $p \leq 0.05$ ) and the statistical difference was significant. The highest number of lactic acid bacteria in control samples (without chitosan coating, asparagus extract and coriander gum) and 8 (meat sample infected with *Listeria* bacteria) was not observed between the mentioned samples ( $p \geq 0.05$ ) in other time periods. And then in sample 2 (sample coated with chitosan with 0.5% asparagus extract) and observed ( $p \leq 0.05$ ). In all time periods except day 1, increasing the asparagus extract led to a significant reduction in the number of lactic acid bacteria in the samples ( $p \leq 0.05$ ). So that the lowest number of lactic acid bacteria belong to samples 6 (sample covered with 5.5% coriander gum and 1% pomegranate extract) and 7 (sample covered with 5.5% coriander gum). And 1.5% of pomegranate extract) ( $p \leq 0.05$ ) and then samples 3 (sample coated with chitosan with 1% extract of pomegranate) and 4 (sample coated with chitosan with 1.5 % Of asparagus extract) ( $p \leq 0.05$ ) and shows that samples containing coriander gum had a lower number of lactic acid bacteria than samples without coriander gum ( $p \leq 0.05$ ). Also, over time, the number of lactic acid bacteria in all samples increased significantly ( $p \leq 0.05$ ).

**Table 7. Changes in the count (log<sub>10</sub> CFU / g) of lactic acid bacteria in minced meat samples coated with chitosan, parchment gum and coriander gum during storage**

Storage time (days)					
Day 12	Day 9	Day 6	Day 3	Day 1	Code
8/37 ± 0.10 aA	7/37 ± 0.10 aA	6/39 ± 0.10 aA	0/29 ± 0.10 aA	3/6 ± 0.10 aA	Code 1
8/2 ± 0.10 aA	7/33 ± 0.10 abA	6/28 ± 0.10 abA	0/26 ± 0.10 aA	3/62 ± 0.10 aA	Code 2
8/13 ± 0.10 abA	7/31 ± 0.10 abA	6/20 ± 0.10 abcA	0/23 ± 0.10 abA	3/66 ± 0.10 aA	Code 3
8/22 ± 0.10 aA	7/21 ± 0.10 abcA	0/86 ± 0.10 efA	0/1 ± 0.10 abcA	3/60 ± 0.10 aA	Code 4
8/11 ± 0.10 abA	7/19 ± 0.10 abcA	6/20 ± 0.10 abcA	0/2 ± 0.10 abA	3/66 ± 0.10 aA	Code 5
7/80 ± 0.10 bcA	7/1 ± 0.10 abcA	6/11 ± 0.10 bcdA	0/11 ± 0.10 abcA	3/70 ± 0.10 aA	Code 6
8/3 ± 0.10 aA	6/97 ± 0.10 cdA	0/82 ± 0.10 efA	0/0 ± 0.10 bcdA	3/60 ± 0.10 aA	Code 7
8/27 ± 0.10 aA	7/22 ± 0.10 abcA	6/30 ± 0.10 abA	0/33 ± 0.10 abA	3/69 ± 0.10 aA	Code 8

Λ/Υ 1±. / . 0 aA	Υ/1 6±. / . 0 abcA	6/Υ 3±. / . 0 abcA	0 / . 6±. / . 0 bcdA	3/6 2±. / . 0 aA	Code 9
Λ/ . 7±. / . 7 abA	Υ/ . 0±. / . 7 bcdA	6/1 6±. / . 7 abcA	ξ/9 8±. / . 7 cdA	3/6 6±. / . 7 aA	Code 10
Λ/1 7±. / . 3 abA	6/8 2±. / . 3 dA	0/6 8±. / . 3 fgA	ξ/9 6±. / . 3 cdA	3/7 2±. / . 3 aA	Code 11
Λ/ . 0±. / . 9 abA	Υ/1 1±. / . 9 abcA	6/ . . ±. / . 9 cdeA	0 / . 7±. / . 9 bcdA	3/6 7±. / . 9 aA	Code 12
Υ/0 7±. / . 3 cA	6/9 8±. / . 3 cdA	0/9 3±. / . 3 defA	ξ/9 3±. / . 3 deA	3/6 3±. / . 3 aA	Code 13
Υ/0 7±. / . 9 cA	6/0 . ±. / . 9 eA	0/ξ 7±. / . 9 gA	ξ/7 7±. / . 9 eA	3/6 7±. / . 9 aA	Code 14

Different Latin uppercase letters have a significant difference in the row and different Latin lowercase letters have a significant difference in the column (P <0.05)

Code (1): Control (without pomegranate extract), Code (2): Sample coated with chitosan with 0.5% pomegranate extract, Code (3): Sample coated with chitosan with 1% pomegranate extract, Code (4): Sample coated with chitosan with 1.5% asparagus extract, Code (5): Sample coated with 5.5% coriander gum and 0.5% asparagus extract, Code (6): Sample Coated with 5.5% coriander gum and 1% asparagus extract, code (7): sample coated with 5.5% coriander gum and 1.5% asparagus extract, code (8): sample of meat contaminated with bacteria *Listeria*, code (9): sample coated with 5.5% coriander gum and 0.5% asparagus extract, code (10): sample coated with chitosan with 1% asparagus extract contaminated with *Listeria* bacteria, code (11): Sample coated with chitosan with 1.5% asparagus extract infected with *Listeria* bacteria, code (12): Sample coated with 5.5% coriander gum and 0.5% asparagus extract contaminated with *Listeria* bacterium, code (13): sample coated with 5.5% coriander gum and 1% asparagus extract and contaminated with *Listeria* bacteria, code (14): sample coated with 5.5% coriander gum and 5/5 1% asparagus extract infected with *Listeria* bacteria

#### 4.5. Sensory tests:

The results of comparing the mean color score of the samples at intervals of 1, 3, 6, 9 and 12 days of storage at refrigerator temperature are presented in Table 8. The results of comparing the means of the data showed that in all time periods except on the twelfth day, the highest color score belonged to samples 4 (sample coated with chitosan with 1.5% of asparagus extract), 5 (sample coated). Was given with 5.5% coriander gum and 0.5% pomegranate extract) and 6 (sample covered with 5.5% coriander gum and 1% pomegranate extract) (p≤0.05). On the twelfth day, the highest color score was in samples 5 (sample covered with 5.5% coriander gum and 0.5% asparagus extract) and 6 (sample covered with

5.5% coriander gum and 1% asparagus extract). Was observed ( $p \leq 0.05$ ). On the ninth and twelfth days of storage, the lowest color score belonged to sample 4 (sample coated with chitosan with 1.5% asparagus extract) and then sample 7 (sample coated with 5.5% coriander gum and 1.5% of asparagus extract ( $p \leq 0.05$ )). Also, over time, the color score of all samples decreased significantly ( $p \leq 0.05$ ).

**Table 8. Color rating of minced meat samples coated with chitosan, asparagus gum and coriander gum during storage**

Storage time (days)					
Day 12	Day 9	Day 6	Day 3	Day 1	Code
1/0.0±0.10 cA	2/0.0±0.10 cA	3/0.0±0.10 bA	4/0.0±0.10 abA	5/0.0±0.10 bA	Code 1
2/0.0±0.10 bA	3/0.0±0.10 bA	2/0.0±0.10 cA	3/0.0±0.10 bA	3/0.0±0.10 cA	Code 2
2/0.0±0.10 bA	3/0.0±0.10 bA	2/0.0±0.10 cA	3/0.0±0.10 bA	3/0.0±0.10 cA	Code 3
3/0.0±0.10 aA	4/0.0±0.10 aA	3/0.0±0.10 bA	4/0.0±0.10 abA	5/0.0±0.10 bA	Code 4
2/0.0±0.20 bA	3/0.0±0.20 bA	3/0.0±0.20 bA	4/0.0±0.20 abA	0/0.0±0.20 aA	Code 5
3/0.0±0.60 aA	4/0.0±0.60 aA	4/0.0±0.60 aA	0/0.0±0.60 aA	0/0.0±0.60 aA	Code 6
2/0.0±0.70 bA	3/0.0±0.70 bA	3/0.0±0.70 bA	4/0.0±0.70 abA	5/0.0±0.70 bA	Code 7

Different Latin uppercase letters have a significant difference in the row and different Latin lowercase letters have a significant difference in the column ( $P < 0.05$ )

Code (1): Control (without chitosan coating, asparagus extract and coriander gum), code (2): sample coated with chitosan with 0.5% asparagus extract, code (3): sample coated with chitosan to With 1% asparagus extract, code (4): sample coated with chitosan with 1.5% asparagus extract, code (5): sample coated with 5.5% coriander gum and 0.5% asparagus extract, Code (6): Sample covered with 5.5% coriander gum and 1% pomegranate extract, Code (7): Sample covered with 5.5% coriander gum and 1.5% pomegranate extract

## 5. Discussion:

### 5.1. Peroxide index:

The results of the present study showed that the highest amount of peroxide index was observed in sample 8 (sample of meat infected with *Listeria*) and then in the control sample ( $p \leq 0.05$ ). There was a significant reduction in the peroxide index of the samples, so that the lowest amount of peroxide index belonged to the samples containing 1 and 1.5% of asparagus extract, ie samples 6 (sample

covered with 5.5% coriander gum and 1% Pineapple extract) and 7 (sample coated with 5.5% coriander gum and 1.5% pomegranate extract) and then samples 3 (sample coated with chitosan with 1% pomegranate extract) and 4 (sample Coated with chitosan with 1.5% asparagus extract ( $p \leq 0.05$ )). Antioxidants compete with unoxidized lipids by donating hydrogen. Thus, by donating a hydrogen atom or free electron, they form stable compounds, or they may exert a positive effect in preventing decay by chelating metal ions (peroxidizing agents) or quenching single oxygen or removing peroxide. Some researchers believe that the antioxidant activity of extracts is due to their reducing properties, which play an important role in the absorption and neutralization of free radicals, inactivation of single and triple oxygen and decomposition of peroxides. In the present study, the reason for the lower peroxide number of samples containing higher amounts of asparagus extract can be attributed to the presence of phenolic compounds and its antioxidant activity. With increasing the concentration of the extract due to the presence of phenolic compounds, anti-radical activity has increased. At higher concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups of aromatic rings of phenolic compounds in the reaction medium, the possibility of giving hydrogen to free radical's increases, followed by the inhibitory power of the resin and the reaction of free radical chains stops.

## **5.2. Volatile nitrogen bases:**

The results of comparing the means of the data showed that on the first day there was no statistically significant difference in the amount of total volatile nitrogen of the samples ( $p \geq 0.05$ ) and in other time intervals the highest amount of total volatile nitrogen in sample 8 (contaminated meat sample). *Listeria* bacteria) was observed ( $p \leq 0.05$ ). Increase of volatile bases (ammonia and trimethylamine) is done by internal or microbial enzymes (Manat et al., 2005). In other words, the



decomposition of nitrogen compounds during meat storage leads to an increase in volatile nitrogen compounds (Gram and Huss, 1996). Volatile nitrogen compounds include compounds such as trimethylamine, dimethylamine, ammonia and other volatile nitrogen compounds. Formaldehyde nitrogen (formalin nitrogen) is used as a common indicator of the degree of protein hydrolysis. In all time periods except day 1, the increase of asparagus extract led to a significant decrease in total volatile nitrogen of the samples ( $p \leq 0.05$ ). So that the lowest amount of volatile total nitrogen belongs to the samples containing 1 and 1.5% of asparagus extract, ie treatments 3 (sample coated with chitosan with 1% extract of asparagus), 4 (sample coated with chitosan with 1.5% of asparagus extract), 6 (sample covered with 5.5% coriander gum and 1% of coriander extract) and 7 (sample covered with 5.5% coriander gum and 1.5% of coriander extract). (05/00). Also, over time, total volatile nitrogen of all samples increased significantly ( $p \leq 0.05$ ). Total volatile nitrogen bases (TVNs) is a general term that includes trimethylamines, dimethylamines, ammonia, and other nitrogenous compounds, and increases with seafood spoilage. Volatile nitrogen bases are a qualitative indicator that indicates the degree of spoilage, decomposition and breakdown of proteins and increase due to bacterial activity and internal enzymes in the tissue. Bacterial metabolism of amino acids leads to the accumulation of ammonium, monoethylamine, diethylamine, triethylamine and other volatile bases, all of which cause bad taste.

### **5.3. Thiobarbituric acid:**

The TBA index shows the mg of malondialdehyde in 1000 g of oil and indicates the secondary stages of fat oxidation and the presence of secondary oxidation compounds in the sample. Therefore, the high index in oil indicates more oxidation of oil and therefore less stability. The products of oxidation raw materials (hydroperoxides) are unstable and prone to decomposition. By-

products of oxidation include aldehydes, ketones, alcohols, hydrocarbons, organic acids, and epoxy compounds. Malondialdehyde is a component of fatty acids with three or more double bonds formed by the decomposition of polyunsaturated fatty acids during lipid oxidation. This substance is usually used as an indicator in assessing the process of lipid oxidation changes. In general, it can be stated that the extract of Asparagus plant due to its antioxidant properties can suppress free radicals or reduce their formation rate. Therefore, by stabilizing the formed free radicals, antioxidants can stop the oxidation chain and thus reduce the final amount of TBA during refrigeration.

#### **5.4. Evaluation of microbial test results:**

The results of comparing the mean of the data showed that in all time periods, the number of Pseudomonas bacteria in the samples infected with Listeria and also the control sample was significantly higher than the other samples ( $p \leq 0.05$ ) and the statistical difference No significance was observed between the mentioned samples ( $p \geq 0.05$ ). In all time periods, increasing the asparagus extract and adding coriander gum led to a significant reduction in the number of Pseudomonas bacteria in the samples ( $p \leq 0.05$ ). On the other hand, the samples infected with Listeria had a lower number of Pseudomonas bacteria, so that the lowest number of Pseudomonas bacteria belonged to sample 14 (sample covered with 5.5% coriander gum and 1.5% extract). Pseudomonas and infected with Listeria bacteria ( $p \leq 0.05$ ) and samples containing coriander gum had a lower number of Pseudomonas bacteria than samples without coriander gum ( $p \leq 0.05$ ). Also, over time, the number of Pseudomonas bacteria in all samples increased significantly ( $p \leq 0.05$ ).

#### **5.5. Evaluation of sensory test results:**

The results of comparing the mean of the data showed that in all time periods, the highest overall acceptance score was in samples 5 (sample coated with 5.5% coriander gum and 0.5% pomegranate extract) and 6 (sample coated). Was observed with 5.5% coriander gum and 1% asparagus extract ( $p \leq 0.05$ ). On the ninth and twelfth days of storage, the lowest overall acceptance score belonged to sample 1 (without chitosan coating, asparagus extract and coriander gum) and then sample 7 (sample covered with 5.5% coriander gum and 1.5% extract). ( $P \leq 0.05$ ) Also, over time, the overall acceptance score of all samples decreased significantly ( $p \leq 0.05$ ).

## **6. Conclusion:**

The results showed that in all time periods except the first day, the highest levels of peroxide index, volatile nitrogen bases, thiobarbituric acid were observed in sample 8 (meat sample infected with *Listeria*) and then in the control sample ( $p \leq 0.05$ ). And the lowest amount of the mentioned factors belong to samples 6 (sample covered with 5.5% coriander gum and 1% asparagus extract) and 7 (sample covered with 5.5% coriander gum and 1.5% extract (3 samples (sample coated with chitosan with 1% extract of asparagus) and 4 (sample coated with chitosan with 1.5% extract of asparagus))) ( $p \leq 0.05$ ). Evaluation of microbial test results showed that on the first day, the total number of live mesophilic and psychotrophic bacteria in *Listeria*-infected samples was significantly higher than other samples ( $p \leq 0.05$ ) and in other intervals. The highest total number of live mesophilic bacteria in sample 8 (meat sample infected with *Listeria* bacteria) and then in sample 9 (sample covered with 5.5% coriander gum and 0.5% pomegranate extract contaminated with bacteria *Listeria*) and 10 (sample coated with chitosan with 1% psyllium extract infected with *Listeria* bacteria) were observed ( $p \leq 0.05$ ). On day 1, the number of lactic acid, *Pseudomonas* and *Enterobacteriaceae* bacteria in *Listeria*-infected and control samples was

significantly higher than other samples ( $p \leq 0.05$ ). In other time periods, the highest number of lactic acid bacteria in control samples (without chitosan coating, asparagus extract and coriander gum) and 8 (meat sample infected with *Listeria* bacteria) and then in sample 2 (coated sample) was observed with chitosan along with 0.5% of asparagus extract ( $p \leq 0.05$ ). Sample 5 had the highest scores of texture, odor, color and overall acceptance in all time intervals.

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