

INFLUENCE OF DIFFERENT FORMULATIONS OF ALGINATE-BASED FILMS IN THEIR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY IN MEAT SLICES

MAHMOUD AMMAR MOHAMED AMMAR AND
MOHAMED HAMDY MOHAMED

Agriculture Research Center, Animal Health research institute, Assiut Regional Certified Lab., Egypt.

Received: 8 June 2022; Accepted: 26 June 2022

ABSTRACT

In the current study, the antibacterial and antioxidant effects of three formulated alginate-based edible coats on refrigerated beef were investigated. The formulated coats included 2 organic acids (OAs) based (T1 and T2) and one nisin-OAs based (T3). Control and coated beef slices were sampled immediately (zero time) and then periodically for 15 days of storage at 1°C. The antibacterial effects of coating were assessed by aerobic plate count (APC), *Enterobacteriaceae* and coliforms count. Peroxide value (POV) and catalase (CAT) activity were applied as indexes of oxidation. Moreover, the copper sulphate test and pH were used as freshness indexes. The coats appeared efficient antibacterials. By coating, APC didn't exceed 6 log cfu/g 15 for days of storage with OAs-based coats more significant. T1 and T2 were more effective against enteric bacteria where the load of *Enterobacteriaceae* and coliforms were reduced to undetectable levels for 15 days. The coats also successfully functioned as antioxidants. POV of coated slices significantly reduced where their values were 2.27, 1.87, 2.27 and 2.20 (meq/kg) for control, T1, T2 and T3, respectively after 15 days of storage. Nisin-OAs-based coats appeared the most antioxidant defense mechanism in stored beef where CAT levels were 0.005, 0.010, 0.007 and 0.044 U/g for control, T1, T2 and T3, respectively at end of 15 days of storage. According to freshness indexes, coated slices continued fresh for at least 3 days in excess to control with nisin-OAs coating the most effective. The obtained data indicate that studied coats can be effectively applied to improve the marketability of beef and preserve its quality.

Keywords: Antimicrobial, Antioxidant, alginate, coats, beef

INTRODUCTION

Livestock products, specifically, meat occupy a special intersection in the human diet. Globally they represent over 33% of protein

consumed by humans and about 16% of total food energy (FAO, 2004). Egyptians prefer beef to other types of meat where cattle are Egypt's most important source of red meat. Total slaughter in 2020 is 1.73 million heads, up by 25,000 heads compared to 2019 (GAIN, 2020). Consumers also favor fresh over frozen beef for cultural reasons. Egypt's beef production in 2020 reached 370,000 metric tons (MT) up by about 5,000 MT above 2019 estimate (GAIN, 2020).

Corresponding author: Mahmoud Ammar Mohamed Ammar
E-mail address: mahmoud2014eg@yahoo.com
Present address: Agriculture Research Center, Animal Health research institute, Assiut Regional Certified, Lab., Egypt.

Fresh meat products are commonly displayed at refrigerated temperatures (2-5 °C). However, many undesirable changes in the products can occur during refrigeration due to microbial proliferation and lipid oxidation resulting in quality deterioration, spoilage, and economic loss (Gheisari, 2011). Therefore, meat industry asks for methods to make meat safe for consumers concerning stability, transport and storage. Emerging preservation technologies such as the edible coating can serve to preserve the quality at these points.

Edible coats are single or multiple layers of a material that is applied to the surface of a food product and consumed as a part of it (Oussalah *et al.*, 2007). In case of fresh meat, the edible coating is an advanced technological protection approach that maintains its quality and safety without the use of any synthetic chemical additives (Baldwin *et al.*, 2011)

Practically, besides their barrier properties, edible coats can be used as carriers of functional ingredients such as antimicrobials and antioxidants (Bhagath and Manjula, 2019). Carbohydrates (alginates, starch, carrageenan) based edible coatings are of high interest, (Vanzela *et al.*, 2013). They can provide an advanced food protection approach to satisfy the customer requirements (Baldwin *et al.*, 2011). Of this group, alginate is the most material of interest for edible coatings. It is a naturally occurring polysaccharide used in the bio-industry (*et al.*, 2018). Also, it is commonly available, simple to manipulate, low cost (Abdallah *et al.*, 2018) and classified and generally regarded as safe material (FDA, 2018).

The Egyptian meat markets are in need of recent methods of beef marketing alternative to the traditional methods. So this study aims to evaluate the effect of different formulations of edible coats on their antibacterial and antioxidant activity on cold-stored beef.

MATERIALS AND METHODS

Preparation of beef slices

Fresh meat was obtained from the Local Mankabad slaughterhouse, Assiut Upper Egypt. With slight modification to (Dorsa *et al.*, 1998), the *longissimus dorsi* muscle was aseptically excised and transported under cooling in an

insulated tank to the laboratory. At the laboratory, the top, bottom and sides of the muscle and adipose tissue were removed and the interior portion was used to prepare enough numbers of beef slices with a thickness of 5 mm then portioned into pieces with a dimension of 10x10 cm (each weighing 50 g)

Preparation of alginate-based dipping solutions

The preparation of Na-alginate edible films was performed with modification to that previously described by Kapetanakou *et al.* (2016) and Alexanyan *et al.* (2014). Briefly, 2.5 g of Na-alginate (AVI-Chem. Laboratories, India) was added gradually to 100 mL of pre-warmed (65 °C) distilled sterile water and constantly agitated till complete dissolution. An amount of 1 ml glycerol was used as a plasticizer in order to improve the film's flexibility. After the addition of the glycerol, the forming solution was kept at 4 °C for approximately 30 min to lower the temperature. Then sodium chloride 0.15% and citric acid 0.1 % were added and that represents treatment 1 (T1). Treatment 2 (T2) contained the same ingredients of T1 plus acetic acid 0.1%. Treatment 3 (T3) contained the same ingredients of T1 plus nisin (100µg/ml). The prepared solution was clear colourless to slight yellowish. Their pH values were 5, 3.4 and 3.4 for T1, T2 and T3, respectively.

Application of treatments

Meat pieces were divided into four groups. Group 1 was packaged in plastic bags and sampled for zero time then refrigerated (control). Other three groups were dipped separately in T1, T2 or T3 solutions. Dipped pieces were drained for 1 min in sterile Petri plates. Then dipped in calcium chloride 2% solution to fix the coat and drained for 1min. Treated pieces were packaged separately in plastic bags and sampled for zero time then refrigerated at 1°C as recommended by (ESO, 2013). Refrigerated control and treated samples were sampled after 1, 2, 3, 4, 5, 6, 7, 9, 11, 13 and 15 days. At each sampling period, samples were analyzed for their bacteriological and chemical qualities.

1. Bacteriological analysis

1. 1. Preparation of samples

Ten grams sample was transferred to a stomacher bag charged with 90 ml of buffered

peptone water (BPW) and pummeled in a stomacher for 1min to obtain a dilution of 1/10, then decimal dilutions were prepared using BPW.

1. 2. Enumeration procedures

1.2.1. Aerobic plate count (APC)

Following procedures of (FAO, 1992), 1ml from each of the previously prepared dilutions were transferred into each of the marked sterile Petri-plates and thoroughly mixed with about 15 ml of previously melted and cooled ($45 \pm 1^\circ\text{C}$) standard plate count agar. After solidification, the inoculated plates were inverted and incubated at 35°C for 48h. Plates with 25-250 colonies were selected and counted. The APC was calculated and expressed as cfu/g of the sample.

1.2.2. *Enterobacteriaceae* and coliforms count

Following Herrera (2001), plates in duplicates were inoculated with one ml from the already prepared dilutions and mixed with 10 ml of violet red bile glucose (VRBG) agar tempered at $45^\circ\text{C} \pm 1^\circ\text{C}$. After solidification, the plates were overlaid by pouring 5 ml of the same media. Inoculated plates were incubated at 37°C for 24h. All colonies characterized by red-purple color and surrounded by a zone of precipitated bile were counted and recorded as total *Enterobacteriaceae* count/g. Colonies on VRBG were after that inoculated onto tubes of brilliant green bile broth tubes with inverted Durham's tubes and incubate at 35°C for 24 h. Colonies producing gas were confirmed as coliforms organisms. The number of coliforms organisms per gram of sample was calculated.

2. Chemical analysis

2.1. Determination of pH

As recommended by Januškevičienė *et al.* (2012), 5 g of minced sample was mixed with 50 ml distilled water in a conical flask and left for 30 minutes with periodical stirring then filtered using Whatman filter paper. The pH of the filtrate was assessed using an electrode of a pH-meter standardized by pH 4 and 7 buffers.

2.2 . Copper sulphate test

As described by AOAC (2012), 20 g sample was minced and transferred to a conical flask. Then 60 ml of distilled water was added and

thoroughly mixed, sealed with a glass cap and heated in a water bath (80°C) for 10 min. The hot broth was cooled and filtered through a pad of cotton wool. A volume of 2 ml of the filtrate was poured into a test tube and 3 drops of a 5% solution of copper sulphate were added. The tube was shaken and the results of the reaction were noted. The interpretation of results was evaluated according to Januškevičienė *et al.* (2012) where the score of zero corresponds clear or slightly cloudy (fresh), a score of 3 was given if flakes formed (suspected) and a score of 4 indicates bluish or greenish flakes and sediments (spoiled).

2.3. Peroxide Value (POV)

Following the procedures of Sallam *et al.* (2004), a 3g sample was transferred to a 250 ml Erlenmeyer flask with a glass stopper. To melt fat, the flask charged with the sample was heated for 3 min at 60°C in a water bath. Then the contents of the flask were thoroughly agitated for 3 min with 30 ml acetic acid chloroform solution (3:2 v/v) to dissolve the fat. Beef particles were excluded by filtering the contents of the flask through Whatman filter paper No.1. Saturated potassium iodide solution (0.5 ml) and starch solution (0.5 ml) as indicators were added to the filtrate. The filtrate was titrated against a standard solution of sodium thiosulfate until the color disappeared. POV was calculated by the following equation and expressed as milli equivalent (meq) peroxide/kg sample.

$$\text{POV (meq / kg)} = [(S \times N) / W] \times 100$$

Where (S) is the volume of titration (ml), (N) is the normality of sodium thiosulfate solution (N=0.01) and (W) is the sample weight (g).

2.4. Catalase (CAT) activity

Catalase activity was determined by measuring the decrease in hydrogen peroxide (H_2O_2) indicated by a decrease in absorbance at 510 nm according to the colorimetric method described by Aebi (1984). Catalase assay kits (Biodiagnostic) were used following manufacture instruction directions. Catalase activity (U/g) =

$$(\text{Absorbance standard} - \text{Absorbance sample}) / \text{Absorbance standard} \times 1/ \text{g tissue assayed}$$

RESULTS

Table 1: Influence of alginate-based films on aerobic plate count (APC) during refrigerated storage (1°C).

Days	APC log cfu/g										
	Control		T1			T2			T3		
	Mean values	SD	Mean values	SD	p value	Mean values	SD	p value	Mean values	SD	p value
Zero day	4.5	0.080	< 1	0.000	0.001	< 1	0.000	0.001	< 1	0.000	0.001
1 st day	4.5	0.053	3.0	0.170	0.001	4.0	0.170	0.002	3.0	0.170	0.001
2 nd day	4.6	0.029	3.6	0.080	0.001	4.5	0.136	0.14	3.3	0.080	0.001
3 rd day	4.6	0.038	3.7	0.113	0.001	4.5	0.136	0.171	3.4	0.075	0.001
4 th day	4.7	0.038	3.8	0.100	0.001	4.6	0.170	0.299	3.6	0.091	0.001
5 th day	4.8	0.031	3.8	0.060	0.001	4.7	0.175	0.188	3.7	0.080	0.001
6 th day	5.0	0.100	4.0	0.100	0.001	5.0	0.179	0.962	4.0	0.246	0.001
7 th day	6.0	0.100	4.5	0.118	0.001	5.0	0.237	0.001	4.5	0.312	0.001
9 th day	6.9	0.130	5.5	0.111	0.001	5.3	0.175	0.001	4.5	0.336	0.001
11 th day	7.0	0.173	5.3	0.153	0.001	5.5	0.225	0.001	5.2	0.238	0.001
13 th day	7.2	0.042	5.5	0.182	0.001	5.5	0.225	0.001	5.4	0.166	0.001
15 th day	7.5	0.026	6.0	0.182	0.001	5.9	0.154	0.001	5.6	0.166	0.001

T1: Basal film plus citric acid (0.1%), T2: Basal film plus citric acid (0.1%) and acetic acid (0.1%), T3: Basal film plus citric acid (0.1%) and nisin (100µg/ml).

Table 2: Influence of alginate-based films on *Enterobacteriaceae* count during refrigerated storage (1°C).

Days	<i>Enterobacteriaceae</i> log cfu/g										
	Control		T1			T2			T3		
	Mean values	SD	Mean values	SD	p value	Mean values	SD	p value	Mean values	SD	p value
Zero day	1.0	0.032	< 1	0.000	0.001	< 1	0.000	0.001	< 1	0.000	0.001
1 st day	1.5	0.155	< 1	0.000	0.001	< 1	0.000	0.001	1.0	0.020	0.001
2 nd day	1.9	0.303	< 1	0.000	0.001	< 1	0.000	0.001	1.0	0.020	0.001
3 rd day	2.7	0.254	< 1	0.000	0.001	< 1	0.000	0.001	2.3	0.169	0.012
4 th day	2.7	0.250	< 1	0.000	0.001	< 1	0.000	0.001	2.3	0.168	0.012
5 th day	2.8	0.230	< 1	0.000	0.001	< 1	0.000	0.001	2.6	0.180	0.208
6 th day	3.3	0.153	< 1	0.000	0.001	< 1	0.000	0.001	2.7	0.180	0.001
7 th day	3.3	0.115	< 1	0.000	0.001	< 1	0.000	0.001	2.8	0.190	0.001
9 th day	3.4	0.095	< 1	0.000	0.001	< 1	0.000	0.001	3.0	0.205	0.005
11 th day	3.7	0.391	< 1	0.000	0.001	< 1	0.000	0.001	3.3	0.250	0.046
13 th day	3.7	0.366	< 1	0.000	0.001	< 1	0.000	0.001	3.45	0.274	0.186
15 th day	4.0	0.431	< 1	0.000	0.001	1.7	0.241	0.001	3.48	0.270	0.051

T1: Basal film plus citric acid (0.1%), T2: Basal film plus citric acid (0.1%) and acetic acid (0.1%), T3: Basal film plus citric acid (0.1%) and nisin (100µg/ml).

Table 3: Influence of alginate-based films on Coliforms count during refrigerated storage (1°C).

Days	Coliforms log cfu/g										
	Control		T1			T2			T3		
	Mean values	SD	Mean values	SD	p value	Mean values	SD	p value	Mean values	SD	p value
Zero day	< 1	0.000	< 1	0.000	0.001	< 1	0.000	0.001	< 1	0.000	0.001
1 st day	< 1	0.000	< 1	0.000	0.001	< 1	0.000	0.001	< 1	0.000	0.001
2 nd day	1.6	0.159	< 1	0.000	0.001	< 1	0.000	0.001	1.0	0.031	0.001
3 rd day	2.3	0.100	< 1	0.000	0.001	< 1	0.000	0.001	2.2	0.095	0.114
4 th day	2.5	0.085	< 1	0.000	0.001	< 1	0.000	0.001	2.3	0.115	0.015
5 th day	2.5	0.085	2.0	0.068	0.001	< 1	0.000	0.001	2.4	0.110	0.129
6 th day	3.0	0.105	2.0	0.068	0.001	< 1	0.000	0.001	2.5	0.123	0.001
7 th day	3.1	0.150	< 1	0.000	0.001	< 1	0.000	0.001	2.5	0.129	0.001
9 th day	3.3	0.121	< 1	0.000	0.001	< 1	0.000	0.001	2.7	0.086	0.001
11 th day	3.6	0.278	< 1	0.000	0.001	< 1	0.000	0.001	3.0	0.136	0.002
13 th day	3.6	0.306	< 1	0.000	0.001	< 1	0.000	0.001	3.1	0.150	0.006
15 th day	3.8	0.285	< 1	0.000	0.001	1.7	0.247	0.001	3.2	0.150	0.005

T1: Basal film plus citric acid (0.1%), T2: Basal film plus citric acid (0.1%) and acetic acid (0.1%), T3: Basal film plus citric acid (0.1%) and nisin (100µg/ml).

Table 4: Influence of alginate-based films on pH during refrigerated storage (1°C).

Days	pH										
	Control		T1			T2			T3		
	Mean values	SD	Mean values	SD	p value	Mean values	SD	p value	Mean values	SD	p value
Zero day	5.5	0.085	5.3	0.015	0.003	5.2	0.015	0.001	5.2	0.076	0.001
1 st day	5.5	0.081	5.3	0.015	0.002	5.2	0.025	0.001	5.3	0.046	0.001
2 nd day	5.7	0.150	5.5	0.046	0.043	5.4	0.057	0.007	5.5	0.111	0.046
3 rd day	5.7	0.155	5.6	0.096	0.329	5.5	0.115	0.098	5	0.095	0.001
4 th day	5.6	0.191	5.6	0.105	0.872	5.5	0.125	0.631	5.5	0.153	0.460
5 th day	5.7	0.204	5.6	0.115	0.639	5.6	0.155	0.463	5.5	0.149	0.211
6 th day	5.8	0.150	5.8	0.150	1.000	5.7	0.157	0.528	5.8	0.136	0.979
7 th day	5.8	0.145	5.6	0.161	0.257	5.6	0.176	0.257	5.5	0.197	0.092
9 th day	6	0.100	5.4	0.265	0.005	5.6	0.171	0.041	5.5	0.191	0.017
11 th day	6.1	0.050	5.5	0.180	0.001	5.5	0.121	0.001	5.5	0.185	0.001
13 th day	6.3	0.180	5.5	0.179	0.001	5.5	0.111	0.001	5.6	0.212	0.001
15 th day	6.3	0.180	6.1	0.161	0.166	6	0.181	0.066	5.8	0.151	0.007

T1: Basal film plus citric acid (0.1%), T2: Basal film plus citric acid (0.1%) and acetic acid (0.1%), T3: Basal film plus citric acid (0.1%) and nisin (100µg/ml).

Table 5: Influence of alginate-based films on Peroxide value during refrigerated storage (1°C).

Days	Peroxide value (meq/kg)										
	Control		T1			T2			T3		
	Mean values	SD	Mean values	SD	p value	Mean values	SD	p value	Mean values	SD	p value
Zero day	0.5	0.029	0.20	0.013	0.001	0.23	0.017	0.001	0.33	0.017	0.001
1 st day	0.83	0.030	0.33	0.027	0.001	0.40	0.006	0.001	0.33	0.016	0.001
2 nd day	0.97	0.029	0.73	0.054	0.001	0.87	0.044	0.025	0.40	0.037	0.001
3 rd day	1.0	0.085	0.80	0.095	0.006	1.03	0.108	0.639	0.80	0.037	0.005
4 th day	1.17	0.151	0.80	0.095	0.012	1.00	0.064	0.160	1.07	0.202	0.419
5 th day	1.27	0.150	0.83	0.077	0.005	1.50	0.166	0.070	1.00	0.148	0.049
6 th day	1.2	0.111	1.50	0.165	0.066	1.67	0.206	0.010	1.50	0.175	0.064
7 th day	2.0	0.171	1.60	0.156	0.016	1.87	0.151	0.334	1.60	0.165	0.015
9 th day	2.03	0.050	1.77	0.156	0.031	2.00	0.083	0.798	1.80	0.165	0.048
11 th day	2.14	0.134	1.80	0.108	0.005	2.03	0.068	0.274	1.89	0.105	0.021
13 th day	2.23	0.107	1.84	0.093	0.001	2.20	0.106	0.763	2.00	0.015	0.016
15 th day	2.57	0.173	1.87	0.078	0.001	2.27	0.117	0.037	2.20	0.185	0.014

T1: Basal film plus citric acid (0.1%), T2: Basal film plus citric acid (0.1%) and acetic acid (0.1%), T3: Basal film plus citric acid (0.1%) and nisin (100µg/ml).

Table 6: Influence of alginate-based films on catalase during refrigerated storage (1°C).

Days	Catalase U / g										
	Control		T1			T2			T3		
	Mean values	SD	Mean values	SD	p value	Mean values	SD	p value	Mean values	SD	p value
Zero day	0.072	0.008	0.164	0.003	0.001	0.172	0.001	0.001	0.259	0.010	0.001
1 st day	0.171	0.010	0.160	0.004	0.149	0.141	0.002	0.002	0.180	0.013	0.231
2 nd day	0.130	0.007	0.159	0.001	0.001	0.136	0.004	0.655	0.167	0.010	0.001
3 rd day	0.108	0.002	0.139	0.001	0.001	0.123	0.003	0.001	0.153	0.005	0.001
4 th day	0.106	0.001	0.116	0.003	0.003	0.119	0.005	0.001	0.142	0.002	0.001
5 th day	0.084	0.006	0.086	0.002	0.558	0.100	0.002	0.001	0.139	0.001	0.001
6 th day	0.070	0.005	0.076	0.002	0.187	0.081	0.001	0.005	0.134	0.002	0.001
7 th day	0.040	0.002	0.040	0.002	0.500	0.056	0.003	0.001	0.080	0.003	0.001
9 th day	0.017	0.001	0.037	0.003	0.001	0.056	0.003	0.001	0.080	0.003	0.001
11 th day	0.017	0.002	0.021	0.002	0.026	0.02	0.002	0.104	0.070	0.002	0.001
13 th day	0.011	0.002	0.011	0.002	0.681	0.015	0.003	0.024	0.068	0.001	0.001
15 th day	0.005	0.001	0.010	0.002	0.002	0.007	0.001	0.128	0.044	0.002	0.001

Table 7: Influence of alginate-based films on meat freshness (copper sulphate) during refrigerated storage (1°C).

Days	Copper sulphate			
	Control	T1	T2	T3
Zero day	Clear (zero)	Clear (zero)	Clear (zero)	Clear (zero)
1 st day	Clear (zero)	Clear (zero)	Clear (zero)	Clear (zero)
2 nd day	Clear (zero)	Clear (zero)	Clear (zero)	Clear (zero)
3 rd day	Clear (zero)	Clear (zero)	Clear (zero)	Clear (zero)
4 th day	Clear (zero)	Clear (zero)	Clear (zero)	Clear (zero)
5 th day	Slight Cloudy (zero)	Clear (zero)	Clear (zero)	Clear (zero)
6 th day	Slight Cloudy (zero)	Clear (zero)	Slight Cloudy (zero)	Clear (zero)
7 th day	Slight Cloudy (zero)	Clear (zero)	Slight Cloudy (zero)	Slight Cloudy (zero)
9 th day	Flakes (3)	Slight Cloudy (zero)	Slight Cloudy (zero)	Slight Cloudy (zero)
11 th day	Flakes (3)	Slight Cloudy (zero)	Slight Cloudy (zero)	Slight Cloudy (zero)
13 th day	Flakes (3)	Flakes (3)	Flakes (3)	Slight Cloudy (zero)
15 th day	Flakes & Sediment (4)	Flakes (3)	Flakes (3)	Slight Cloudy (zero)

T1: Basal film plus citric acid (0.1%), T2: Basal film plus citric acid (0.1%) and acetic acid (0.1%), T3: Basal film plus citric acid (0.1%) and nisin (100µg/ml).

DISCUSSION

In meat industry, edible coating (EC) functions to prevent loss of moisture, changes in texture, flavor, and color and weight loss of products. Another advantage is the reduction of dripping which serves to enhance the presentation of the products. (Sánchez-Ortega *et al.*, 2014). In the current study, an additional function of EC was trialed. The ability of polysaccharide EC to carry antimicrobials and their effect on the microbial quality of meat slices was investigated. Table 1, reveals the changes in aerobic microflora of beef treated with 3 trials of EC. By using aerobic plate count (APC) as an index of aerobic bacterial contamination, the initial bacterial load was 4.5 logs cfu /g. That level of contamination immediately (zero time) reduced to

undetectable levels after application of EC where the reduction was significant for all applied edible coats (ECs). During refrigerated storage, the delayed anti-microbial effect of citric acid-based (T1) and nisin-based (T3) ECs continue significantly effective in reducing the bacterial load within 15 days of storage while citric and acetic acid combinations based EC (T2) showed fluctuated effect where significant reductions were observed within the 1st and then during the period from the 7th to the 15th days. The reduction of APC counts in coated beef slices can be attributed to the synergistic effect of the gas barrier of alginate which decreased the available oxygen required for aerobes, (Ou *et al.*, 2004) and the antimicrobials incorporated in the ECs.

Regarding the bacterial quality of beef, the applied ECs bearing natural antimicrobials used in the current study appeared good wrappers of meat and function as an additional hurdle against spoilage bacteria contaminating meat surfaces. The applied ECs could successfully prolong the shelf life of treated beef for at least 6 days extra to uncoated beef (control) where the value of APC is still within that (not more than 6 cfu/g) recommended by FAO(1992). The Egyptian Standard Organization (ESO, 2013) recommended a level of not more than 6 cfu/cm² APC for acceptable chilled meat. In the current study and due to the presence of coats we evaluated the APC per gram to explore both surface and internal contaminants. Comparing the current findings to (ESO, 2013) requirements for APC, the three applied coats could preserve the bacterial quality acceptable (not more than 6 log cfu/g) for 15 days.

The ability of ECs loaded with different antibacterials to fight meat contaminants has been trialed by other investigators. Abdallah *et al.* (2018) recorded that by alginate coating, APC in pastirma was significantly lowered than those of control at zero time and after chilling storage. Also, Alexanyan *et al.* (2014) observed a reduction in bacterial dissemination in treated beef liver treated with alginate coats loaded with organic acids. In respect, APC was found to be 1.5 times higher in control compared to veal samples treated with ECs based on sodium alginate after 9 days of refrigeration, (Baranenko *et al.*, 2013). Besides, APC was significantly reduced in alginate-coated buffalo meat patties, (Keshri and Sanyal, 2009).

Gram-negative bacteria were reported to dominate the spoilage bacteria of meat (Yousefi *et al.*, 2018). Of that division and from the food quality point of view, the most important Gram-negative group is *Enterobacteriaceae*. They are commonly constituting a part of microbiological

criteria and their presence is related to hygiene and safety of food (FAO, 1992).

The results in table 2 show the behavior of *Enterobacteriaceae* in coated and control meat slices. The three applied ECs appeared efficient in controlling the proliferation of enterobacteria. Organic acids-based coats appeared more powerful activity than the nisin-acids combination one. The load of *Enterobacteriaceae* was reduced to an undetectable level (< 1 log cfu/g) immediately after treatment (zero time) and during a period of 15 days of refrigerator storage by organic acid-based ECs (T1 and T2). In comparison, nisin plus organic acid EC (T3) was less powerful than organic acid-based ECs where the significance in reduction of *Enterobacteriaceae* was immediately and continue significant for only five days during refrigerator storage. A related study (Chidanadaiah *et al.*, 2009) found that the levels of *Enterobacteriaceae* in meat patties were reduced to undetectable levels by the application of alginate coating. In addition, Siragusa and Dickson (1993) reported that Ca-alginate EC loaded with combination organic acids was more significantly effective against *E.coli* than organic acid alone.

Nisin is considered one of the most commercially applicable bacteriocins. It possesses a small molecular size that allows its ease of release when loaded in EC (Joerger, 2007). Despite that important advantage, nisin was recorded to be more active against Gram-positive pathogens and spoilage bacteria (Arauz *et al.*, 2009). Furthermore, Bhagath and Manjula (2019) revealed that bacteriocins are of limited application as preservatives due to the problem of their adsorbance to muscle foods.

Coliforms are referred to as indicator microorganisms as their presence indicates the potential occurrence of pathogens in foods. It is accepted by many investigators that the higher their levels the greater the

possibility of pathogens (FAO, 1992). As members of *Enterobacteriaceae* coliforms behaved nearly in the same manner, (table 3). By application of ECs, their levels were reduced immediately to undetectable levels and continued undetectable for 15, 14 and one day during refrigeration for T1, T2 and T3, respectively.

The pH of meat is considered of major importance in relation to its quality. It is greatly affected by pre and post-slaughter processes. For fresh meat, their values range from 5.6 to 6.2. (Januškevičienė *et al.*, 2012). Deviation from the normal pH values of meat affects the oxidation rate, dripping and microbial decomposition, (Rahman *et al.*, 2015).

The changes in pH values in coated and in control meat slices are illustrated in table 4. The initial pH value of the control samples was 5.5. That value was within the range (5.5- 6.2) of fresh meat (Januškevičienė *et al.*, 2012). The corresponding initial values for coated samples were 5.3, 5.2 and 5.2 for T1, T2 and T3, respectively. These values revealed the immediately acidifying effect of the coating. These effects appeared significant at zero time and continue for a further 2 days for T1 and one day for T2 and T3. The acidifying effects of ECs appeared significant in many sampling periods and could preserve the pH value of coated samples lower than control during 15 days of refrigerator storage, (table 4). The acidifying effect of alginate coating was also recorded by (Mokhtar *et al.*, 2014) when coated meat patties.

By comparing the pH values of coated samples and control with that recommended by (ESO, 2013) for chilled meat, it was cleared that both organic acid-based (T1 and T2) and nisin-acids-based (T3) successfully prolonged the shelf life of treated beef for at least 5 days excessive to uncoated meat (control). That may be due to the restraint of bacterial proliferation and inhibition of proteases resulted from

lowering of pH as explored by (Fan *et al.*, 2009). Also, the findings of the current study shared the observation with Bhagath and Manjula (2019) that organic acids are the cornerstone in the formulation of coating films to prolong the keeping quality of fresh meats and their products.

Edible coats can also function to carry antioxidants to protect meat and their products from adverse effects of oxidation. In addition to the barrier effect of coating, a synergistic effect can be achieved between the barrier and antioxidants incorporated in the coat. (Parreidt *et al.*, 2018). The effects of formulated ECs regarding meat oxidation in the present study were assessed by measuring hydroperoxides production (peroxide value) and the antioxidant enzyme (catalase).

The results in table 5 cleared that at the initial (zero) sampling time the applied ECs significantly function as antioxidants. It was observed that the peroxide values (PV) were reduced from 0.5 (control) to 0.20, 0.23 and 0.33 for T1, T2 and T3 respectively. The initial value for untreated samples appeared higher than that recorded by (Rahman *et al.*, 2015).

Taking into consideration, oxidation is a chemical reaction (Chaijan and Panpipat., 2017) and the possibility of radicals to degrade lipids increase with presiding of time (Richards, 2006). That was true in the condition of the present study where a gradual increase in POV values was recorded for all treatments and the control by passing of the storage time. Moreover, Alam *et al.* (2017) and Gheisari (2011) recorded the same observation for refrigerated beef and chicken, respectively.

Comparing the POV in coated samples to control in (table 5), the reductions in values of coated samples were observed along the storage period of 15 days. These reductions appeared significant in some sampling times. The recorded POV were within the

recommended value (25 meq/K) for meat by Narasimhan *et al.* (1986). Hydroperoxides are the primary oxidation products (Domínguez *et al.*, 2019). Their reduction successively produced by applied ECs had desirable effects by retarding the loss of color that is the main factor governing the consumer purchasing of muscle foods. In a related study Song *et al.* (2011) noted that the EC prepared based on alginate could prevent oxygen diffusion when applied to the fish surface. The low oxygen diffusion retard lipid and myoglobin oxidation (Sánchez-Ortega *et al.*, 2014).

Enzymatic defense antioxidant processes in meat occur by the action of antioxidant enzymes mainly glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase, (Min *et al.*, 2008). Catalase and GPx play a major role in the peroxide clearance in the cytosol. Catalase acts in a safe manner to decompose H₂O₂ into water and molecular oxygen (Terevinto *et al.*, 2010).

The levels of CAT detected in coated and control meat slices are summarized in table 6. The initial recorded values of CAT were 0.072, 0.164, 0.172 and 0.259 U/g for control and organic acid-based ECs (T1 and T2) and nisin-acids-based coat (T3), respectively. These recorded values for all treatments appeared significant differences compared to the control. During refrigerated storage, the levels of CAT U/g gradually decreased by elapsing of storage time till reached 0.005, 0.010, 0.007 and 0.044 U/g for control, T1, T2 and T3, respectively after 15 days of storage. That observation was also reported by Gheisari (2011) for stored beef. In the current study, the most significant effect was recorded for nisin-acids-based EC (T3) where the effect continues significantly nearly during 14 days of storage. The immediate and delayed effects of nisin indicate that the processing steps of EC based on nisin did not affect its activity.

The remaining units of CAT recorded for coated meat slices were higher than those of control. Consequently, control (uncoated) beef slices are more oxidized than coated slices. The higher stored level of CAT could significantly function to resist oxidation during storage which was reflected in the low POV recorded for 15 days of refrigerated storage, table 5. This observation coordinates with that recorded by (Gheisari, 2011) for stored camel meat. The clearance of H₂O₂ by CAT enzyme helps in the maintenance of meat colour by inhibiting the oxidation of oxymyoglobin, (Chan *et al.*, 1997). That interferes with the production of H₂O₂-activated metmyoglobin which had the main role in the oxidation of meat, (Rhee, 2001). Also, related research works revealed that organic acids possess marked antioxidants properties alone or when loaded on ECs and films, (Bhagath and Manjula 2019)

The resulted changes in the primary and secondary products during storage influence the quality and nutrition constituents of stored meat and consequently its degree of freshness. In the current study, copper sulphate test was used as a freshness index. The results summarized in table 7 reveal the condition of control and coated meat slices. According to that index of freshness, the state of freshness continued for 11, 11 and 15 days for T1, T2 and T3, respectively while in control was only 7 days. These results nearly coordinate those of APC and pH indexes in the current study. By these indices, control slices were acceptable for 7 and 9 days respectively. Coated slices were acceptable for a period of 11 – 15 days by the same indexes. Thus the applied coats successfully prolonged the freshness condition of beef which is the main requirement of consumers and meat producers. This desirable effect may be due to the effect of the low pH value of applied treatments.

In a related study, the microflora on the surface of food was inhibited at a pH of 4 -

4.2 of the coating solution (Baranyi and Roberts 1994). These microflora together with endoproteases are the main ammonia producers, (Mokhtar *et al.*, 2012). Another explanation is that the low oxygen permeability achieved by the edible coating of meat produces partial inhibition of spoilage bacteria and proteolytic enzymes, (Mason and Sherratt, 2016). Also (Tunieva and Kotenkova 2017) recorded that the increase in antioxidant defense mechanism protects the meat from peroxidation which is cell toxic.

In conclusion, the applied edible coats in the current study either organic acid-based or nisin- acids based appeared powerful antimicrobial and antioxidant activity. They could efficiently serve to preserve the quality of coated beef and extend its shelf life. The obtained data indicate that these coats can be applied as hurdles to cold-stored meat. Further studies regarding their effect on the physical properties of meat are needed before their commercial applications.

REFERENCES

- Abdallah, M.R.; Mohamed, M.A. and Emara, M.T. (2018):* Application of alginate and gelatin-based edible coating materials as alternatives to traditional coating for improving the quality of pastirma Food Sci Biotechnol, 27(6)1589–1597. [Error! Hyperlink reference not valid.]
- Aebi, H. (1984):* Catalase in vitro. Methods Enzymol. 105, 121-126.
- Alam, J.; Murshed, H.M.; Rahman, S.M.E. and DH, O. (2017):* Effect of chitosan on quality and shelf life of beef at refrigerated storage. Bangladesh Animal Husbandry Association. 46 (4):230-238.
- Alexanyan, I.J.; Nugmanov, A.X.; Titova, L.M. and Nikulina, M.A. (2014):* Influence of an antimicrobial alginate covering on a period of storage and quality indicators of the cooled beef liver. Life Science Journal 11(11s). [http://www.lifesciencesite.com]
- AOAC' American Official Analytical Chemistry" (2012):* Official Methods of Analysis. 19th ed. Assoc. Off. Anal. Chem., Arlington, VA.
- Arauz, L.J.De.; Jozala, A.F.; Mazzola, P.G. and Penna, T.C.V. (2009):* Nisin biotechnological production and application: a review. Trends in Food Science and Technology, 20: 146–154.
- Baldwin, E.A.; Hagenmaier, R. and Bai, J. (2011):* Edible Coatings and Films to Improve Food Quality. 2nd ed. CRC Press, Boca Raton
- Baranenko, D.A.; Kolodyaznaya, V.S. and Zabelina, N.A. (2013):* Effect of composition and properties of chitosan-based edible coatings on microflora of meat and meat products. Acta Sci. Pol., Technol. Aliment. 12(2) 149-157. [www.food.actapol.net/]
- Baranyi, J. and Roberts, T.A. (1994):* A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23, 277-294.
- Bhagath, Y.B. and Manjula, K. (2019):* Influence of composite edible coating systems on preservation of fresh meat cuts and products: a brief review on their trends and applications. International Food Research Journal, 26(2) 377-392.
- Chaijan, M. and Panpipat, W. (2017):* Mechanism of oxidation in foods of animal origin. In Natural Antioxidants. Applications in Foods of Animal Origin; Banerjee, R., Verma, A.K., Siddiqui, M.W., Eds.; Apple Academic Press, Inc.: Boca Raton, FL, USA, 2017; pp. 1–38. ISBN 978-1-315-36591-6.
- Chan, W.K.M.; C Faustman, C.; Yin, M. and Decker, E.A. (1997):* Lipid oxidation by oxymyoglobin and metmyoglobin with involvement of H₂O₂ and superoxide anion. Meat Science, 4,181-190.

- Chidanandiah, R.C. (2009):* Effect of sodium alginate coating with preservatives on the quality of meat during refrigeration at $(4 \pm 1\text{C})$ storage. *Journal of Muscle Foods*. 3:275-292 [<https://doi.org/10.1111/j.1745-4573.2009.00147.x>]
- Domínguez, R.; Pateiro, M.; Gagaoua, M.; Barba, F.J.; Zhang, W. and Lorenzo, J. (2019):* A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants*, 8, 429; [doi:10.3390/antiox8100429]
- Dorsa, W.J.; Cutter, C.N. and Siragusa, G.R. (1998):* Long-term effect of alkaline, organic acid or hot water washes on the microbial profile of refrigerated beef contaminated with bacterial pathogen after washing. *Food Protect.* 6, 3: 300-306.
- ESO (2013):* Egyptian Standard Organization, Chilled meat standard, No.3602.Egyptian Standardization and Quality control Ministry of industry. Cairo Arab Republics of Egypt. [<http://www.alcpo.org.ly/wp-content/uploads/2017/06/newfoodstandards.pdf>]
- Fan, W.J.; Sun, J.X. and Chen, Y.C. (2009):* Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. *Food Chemistry*, 115, 66e70
- FAO (1992):* Manual of Food Quality Control. 4 Rev. 1. Microbiological Analysis (Andrews, W. edit.) FAO food and nutrition paper No. 14/4
- FAO (2004):* Proceedings "Protein sources for the animal feed industry" Experts Consultation and Workshop, Bangkok, 29 April – 3 May. [<ftp://ftp.fao.org/docrep/fao/007/y5019e/y5019e00.pdf>]
- FDA "Food and Drug Administration" (2018):* Code for Federal Regulations Title 21 Part 184—Direct Food Substances Affirmed as Generally Recognized as Safe.Avaliableonline: [<https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?r=184.1724>]
- GAIN "Global Agriculture Information Network" (2020):* Egypt livestock and product annual. GAIN Report Number EG-19014. [**Error! Hyperlink reference not valid.**]
- Gheisari, H.R. (2011):* Correlation between acid, TBA, peroxide and iodine values, catalase and glutathione peroxidase activities of chicken, cattle and camel meat during refrigerated storage. *Veterinary World*,4(4)153-157.
- Herrera, A.G. (2001):* Enterobacteriaceae /coliforms. Ch. 5 pp 29, In *Food Microbiology Protocols* 10th Ed. (Spencer, J. and De Spencer, R. edit.), Human Press, Inc. USA. [https://books.google.com/books/about/Food_Microbiology_Protocols.html?id=0J1pAAAAMAAJ]
- Januškevičienė, G.; Zaborskienė, G. and Kabašinskienė, A. (2012):* Evaluation of meat physical, chemical and technological quality. Kaunas edit.
- Joerger, R.D. (2007):* Antimicrobial films for food applications: A quantitative analysis of their effectiveness. *Packag. Technol. and Sci.* 20:231–273.
- Kapetanakou, A.E.; Karyotis, D. and Skandamis, P.N. (2016):* Control of *Listeria monocytogenes* by applying ethanol-based antimicrobial edible films on ham slices and microwave-reheated frankfurters. *Food Microbiol.*, 54, 80–90.
- Keshri, R. and Sanyal, M. (2009):* Effect of sodium alginate coating with preservatives on the quality of meat during refrigeration at $(4 \pm 1\text{C})$ storage. *Journal of Muscle Foods*, 20(3): 275-292.
- Mason, R.P. and Sherratt, S.C. (2016):* Omega-3 fatty acid fish oil dietary supplements contain saturated fats and oxidized lipids that may interfere with their intended biological benefits. *Biochemical and*

- Biophysical Research Communications. 482(1)42-429.
- Min, B.; Nam, K.C.; Cordray, J. and Ahn, D.U. (2008): Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. *J. Food Sci.* 73: 439-446.
- Mokhtar, S.; Mostafa, G.; Taha, R. and Eldeeb, G.S.S. (2012): Effect of different starter cultures on the biogenic amines production as a critical control point in fresh fermented sausages, *European Food Research and Technology*, 235: 527-535
- Mokhtar, S.M.; Youssef, K.M. and Morsy, N.E. (2014): *Journal of Agroalimentary Processes and Technologies 2014*, 20(3)282-292. [<http://journal-of-agroalimentary.ro>]
- Narasimhan, S.; Raghuvver, K.G.; Arumngam, C.; Bhat, K.K. and Sen, D.P. (1986): Oxidative rancidity of ground nut oil evaluation by sensory and chemical indices and their correlation. *J. Food Sci. Technol.* 23, 273-277.
- Ou, S.Y.; Kwok, K.C. and Kang, Y.J. (2004): Changes in in vitro digestibility and available lysine of soy protein isolate after formation of film. *J. Food Eng.* 643: 301–305.
- Oussalah, M.; Caillet, S.; Salmiéri, S.; Saucier, L. and Lacroix, M. (2007): Antimicrobial effects of alginate-based films containing essential oils on *Listeria monocytogenes* and *Salmonella typhimurium* present in bologna and ham. *J. Food Protect.* 70, 901–908.
- Parreidt, T.S.; Müller, K. and Schmid, M. (2018): Alginate-Based Edible Films and Coatings for Food Packaging Applications. *Foods*, 7, 170; [doi:10.3390/foods7100170]
- Rahman, M.H.; Hossain, M.M.; Rahman, S.M.E.; Amin, M.R. and Deog-Hwan, O. (2015): Physicochemical Deterioration and Lipid Oxidation of Beef Muscle. *Korean J. Food Sci. An.* 6: 772-782. [DOI <http://dx.doi.org/10.5851/kosfa.2015.35.6.772>.]
- Rhee, K.S. and Ziprin, Y.A. (2001): Prooxidant effect of NaCl in microbial growth controlled and uncontrolled beef and chicken. *Meat Sci.* 57:105-112.
- Richards, M.P. (2006): Lipid chemistry and biochemistry. In *Handbook of Food Science, Technology, and Engineering*; Hui, Y., Ed.; CRC Press; Taylor & Francis: Boca Raton, FL, USA, 2006; pp. 8(1)–8(21). ISBN 0849398487
- Sallam, K.I.; Ishioroshi, M. and Samejima, K. (2004): Antioxidants and antimicrobial effects of garlic in chicken sausage. *Lebensm.-Wiss. Technol.* 37: 849-855.
- Sánchez-Ortega, I.; García-Almendárez, B.E.; Santos-López, E. Amaro-Reyes, A.; Barboza-Corona, J.E. and Regalado, C. (2014): Antimicrobial Edible Films and Coatings for Meat and Meat Products Preservation. *Scientific World Journal*, Volume 2014, Article ID 248935, 18 pages. [<http://dx.doi.org/10.1155/2014/248935>.]
- Siragusa, G.R. and Dickson, J.S. (1993): Inhibition of *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli* O157:H7 on beef muscle tissue by lactic or acetic acid contained in calcium alginate gels. *Journal of Food Safety* 13(2): 147–158.
- Song, Y.; Liu, L.; Shen, H.; You, J. and Luo, Y. (2011): Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). *Food Control* 22, 608e615.
- Terevinto, A.; Ramos, A.; Castroman, G.; Cabrera, M.C. and Saadoun, A. (2010): Oxidative status, *in vitro* iron-induced lipid oxidation and superoxide dismutase, catalase and

- glutathione peroxidase activities in rhea meat. *Meat Sci.* 84:706-710.
- Tunieva, E.K. and Kotenkova, E.A. (2017): The study of effect of sodium chloride on the antioxidant activity of meat. *Foods and Raw Materials*, 5 (2)105–111. [DOI 10.21603/2308-4057-2017-2-105-111]
- Vanzela, E.S.L.; Nascimento; P.D.; Fontes, E.A.F.; Mauro, M.A. and Kimura, M. (2013): Edible coatings from native and modified starches retain carotenoids in pumpkin during drying. *J. Food Sci. Technol.* 50: 420–425
- West, R.; Lazarus, C.; Oblinger, J. and Palmer, A. (2018): In Alginate Coatings for Carcasses. Available, online:[http://agris.fao.org/agrissearch/search.do?recordID=US20130304766]
- Yousefi, M.; Azizi, M. and Ehsani, A. (2018): Antimicrobial coatings and films on meats: A perspective on the application of antimicrobial edible films or coatings on meats from the past to future. *Bali Medical Journal* 7(1): 87-96. [DOI:10.15562/bmj.v7i1.759]

تأثير التركيبات المختلفة للأفلام المستندة إلى الألبينات في نشاطها المضاد للبكتيريا والأكسدة في شرائح اللحوم

محمود عمار محمد عمار ، محمد حمدي محمد

E-mail: mahmoud2014eg@yahoo.com

Assiut University web-site: www.aun.edu.eg

في هذه الدراسة تم تحري التأثير المضاد للبكتيريا والمضاد للأكسدة لثلاثة تراكيب من الأغشية المستندة للألبينات والصالحة للأكل على اللحوم المحفوظة بالتبريد. تضمنت هذه التراكيب غشائين كلاهما احتوى على أحماض عضوية (T1, T2) والغشاء الآخر إحتوى على الناييسين والأحماض العضوية معاً (T3). تم أخذ عينات من المجموعة الضابطة للتجربة والمجموعة المعاملة بالأغلفة عند بداية التجربة (zero time) ثم على فترات لمدة ١٥ يوم من الحفظ عند ١ درجة مئوية. التأثيرات المضادة للبكتيريا تم دراستها عن طريق مؤشرات العدد البكتيري الكلي، عدد الانتيروباكتيري، وأعداد الكوليفورم. تم استخدام قيمة البروكسيد وإنزيم الكتاليز كمؤشرات للأكسدة. كما استخدم اختباري كبريتات النحاس والأس الهيدروجيني كدلائل للطازجية.

أظهرت الأغشية المستخدمة تضاد بكتيري قوى حيث لم يتجاوز العدد البكتيري الكلي ٦ لوغارتم وحدة مستعمرة للجرام خلال ١٥ يوم من الحفظ وكانت الأغشية المستندة للأحماض العضوية الأكثر معنوية. كذلك أظهرت مركبات (T1, T2) التأثير الأكثر فاعلية ضد الانتيروباكتيري والكوليفورم حيث إنخفضت أعدادها إلى (أقل من واحد لوغارتم وحدة مستعمرة للجرام) خلال ١٥ يوم من الحفظ.

أظهرت الأغشية المستخدمة أيضاً تأثيراً مضاداً للأكسدة قوي. ففي العينات المعاملة بإنخفاض مستوي البروكسيد (meq/kg) معنوياً حيث وصلت القيم إلى ٢,٢٧, ١,٨٧, ٢,٢٧ و ٢,٢٠ للمجموعة الضابطة للتجربة، T1, T2, T3 على التوالي بعد ١٥ يوم من الحفظ. كما أظهر تركيب الناييسين والأحماض العضوية التأثير المضاد للأكسدة الأقوى في اللحوم المعاملة حيث كانت مستويات الكتاليز (وحدة للجرام) ٠,٠٠٥, ٠,٠١٠, ٠,٠٠٧ و ٠,٠٤٤ للمجموعة الضابطة، T1, T2, T3 على التوالي.

حسب مؤشرات الطازجية فإن اللحوم المعاملة بالأغلفة إستمرت طازجيتها لمدة ثلاثة أيام إضافية على الأقل مقارنة بالمجموعة الضابطة حيث كانت الأغلفة المحتوية على الناييسين هي الأكثر تأثيراً. تخلصت الدراسة إلى أن البيانات المتحصل عليها تشير إلى فاعلية تطبيق الأغشية التي تم دراستها في تحسين الحالة التسويقية للحوم والحفاظ على جودتها.