# THE ROLE OF PACKAGING (MATERIALS &TREATMENTS) IN ADDITION TO SPICES EXTRACT ON STABILITY OF FROZEN BUFFALO MEAT PRODUCT

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#### Abstract

Proximate analysis, expressible water(EP), water holding capacity (WHC), pH value, total acidity, free fatty acids (FFA), thiobarbituric acid values (TBA), and microbiological examination were determined in order to evaluate the effect of packaging materials and treatments in addition to spice extracts on the shelf life of buffalo meat product stored for nine months under frozen condition. The samples were treated by spice extracts and packaged in two packaging materials low density polyethylene (LDPE) bags without vacuum, and laminated polyethylene/nylon bags under vacuum. The data showed that all the samples have expressed moisture loss during the 9 months storage period. The moisture loss and the (EP) were higher in the (LDPE) packaged samples, whereas, (WHC) values decreased with time during storage for all samples. The samples indicated an increase in the acidity values and a reduction in the pH values especially for the first four months of storage period. These changes were associated with an increase in the (FFA) values. The control samples showed the lowest pH value compared with the others, and this indicated the effect of natural antioxidants to retard the formation of (FFA). The (TBA) values for the control samples were higher than those packaged under vacuum or treated by spice extracts. Storage time had negative effect on the total bacterial counts and the coliform group for all samples. The rate of reduction was much higher in the vacuum packaged samples and the spice extracts treated samples as well especially those treated by black cumin extracts than the untreated or samples packaged without vacuum

Key words: vacuum packaging, spice extracts, buffalo meat, frozen storage

During production, processing, distribution, and storage, food undergoes deterioration from chemical and microbiological processes [22]. Oxidation is a major cause of that deterioration because of its negative effects on organoleptic qualities (flavor, color, etc.). Oxidation of lipids can also have a marked negative effect on nutritional value, and could be responsible for the production of toxic compounds [14], [15].

Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf life reduction. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors [7]. Meat products, due to fat content are highly susceptible to lipid oxidation. Moisture, prooxidant pigments, storage, handling and display conditions contribute to lipid oxidation of meat products [14], [13], [15].

Due to detrimental effects of lipid oxidation on color, flavor, texture, and nutritional value of foods; addition of synthetic antioxidants such as BHT and BHA has been effective because of their low cost, high stability, and effectiveness. However, the use of such compounds has been related to health risks resulting in strict regulations over their use in food products and this has stimulated research for alternative antioxidant sources [13]. With increased consumer concerns about the amount of chemicals in their foods, processors are looking for more natural ways to protect their products. In the last few years, there has been an increasing interest in the use of natural additives in preference to synthetic substances for the stabilization of fat-containing food stuff. Among the natural antioxidants, extracts of herbs have played an important role [16], [5], [6]. The use of antioxidants like vitamin C and E had a significant effect in reducing oxidation of lipids and pigments of meat during storage [18]. In view of the fact that natural spices are widely used in a variety of food products, it is important to know the effects they have on the keeping qualities of such products. A number of studies have been made on the bactericidal and bacteriostatic properties of spices to evaluate their effectiveness

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in preventing or retarding spoilage caused by microorganisms in addition to the antioxidant effect of spices on fats in certain foods [17].

To obtain the optimum shelf-life of fresh red meat and its products, it is necessary to limit microbial contamination. Microbial spoilage can be delayed by storage of meat at low temperature by effects on the growth rate of the organisms. Since frozen meat is highly susceptible to dehydration as a result of moisture losses and temperature fluctuations, the protection of frozen meat against fluctuations in temperature during storage is important from the standpoint of quality retention. An obvious approach is the use of suitable packaging materials to meet various criteria, such as protection against moisture migration and mechanical damage [12].

[19] Reported that vacuum packaging of meat may prolong the shelf life of retail cuts compared with those packaged in oxygen-permeable film. [24] Found that, when meat is vacuum-packaged and the contaminating flora is exposed to an atmosphere containing high levels of carbon dioxide and a low percentage of oxygen, the growth of aerobic microorganisms is depressed. Vacuum packaging helped in reducing TBA value of beef steaks during refrigerated storage [20], [8].

The main purpose of this research was to investigate the protective effects of three spice extracts individually or in a mixture with combination of two packaging materials with or without vacuum on the subjective quality characteristics of buffalo meat products during frozen storage.

# MATERIAL AND METHOD

Antioxidant extracts: Dried spices (cardamom, thyme, and black cumin) were obtained from a local market, then powdered using a mortar and pestle. Powdered spice every (2g) was extracted with (10 ml) ethanol solution (50%) on a lab line orbit shaker at 60Xg for 2h. The solution was centrifuged at 1800Xg followed by filtration using Whattman No 1 filter paper. The final concentration of the stock solution was 20 g /100 ml.

Preparation of buffalo meat product: The buffalo meat used in this study was obtained from the local market in El-Minia, Egypt, one hour after slaughter. The sample was trimmed, packed in low density polyethylene bags and held at 4±1 °C for 24 hours, cut into cubes and minced twice to obtain ground buffalo meat. Buffalo meat product was prepared according to the following recipe in (table 1).

All the ingredients were mixed well, and divided into five equal portions. Spice extracts

(individually or in mixture) were added to the first four portions in the ratio of (1ml/ 10g sample), whereas, the fifth portion was left without any additive as control.

Table 1 Formulation of buffalo meat product

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Ingredient	%			
Ground meat	64			
Minced fat	16			
Bread crumb	12			
Eggs	5			
Potato starch powder	2			
Salt	0. 99			
Black pepper powder	0.01			

Each portion was divided into small balls 10±2 g each, then formed in a finger-like shape 10±1 cm long, and packaged in single layer using two different packaging materials with or without vacuum. The samples were frozen at - 40°C for 6 hours, then stored frozen at - 18°C for 9 months.

Packaging materials and treatments: Two different packaging materials were used, commercial low density polyethylene (LDPE) 2 mil bags (1 mil = 0.001 inch) from Packaging Concepts and Design, a division of Bader Bag Co., Madison Heights, MN., USA. The second packaging material was 3 mil laminated polyethylene / nylon bags from Cryovac Co., USA.

Each portion of the buffalo meat product was divided into two equal parts. One part was packaged in a single layer in (LDPE) bags, then heat sealed under atmospheric pressure, while the other part was packed in laminated polyethylene/nylon bags and heat sealed under vacuum using a Deni Freshlock vacuum sealer.

Analytical methods: Moisture, crude protein, crude fat, ash, and carbohydrate contents were determined according to the methods of the [3].

Determination of expressible water (EP) and water holding capacity (WHC):

Expressible water (EP) was determined according to [1], whereas, the water holding capacity (WHC) was calculated.

pH measurement: A slurry was prepared by blending the meat product (5g/50ml distilled water). The pH of this slurry was measured by using the glass-electrode method according to the [2].

Determination of total acidity: The acidity was determined by titration according to [11].

Determination of free fatty acids (FFA): Free fatty acids (FFA), as measurement of enzymatic rancidity were assessed by the method described by [23].

Thiobarbituric acid (TBA) value: Frozen packaged samples were tested separately. TBA-reactive substances were measured using the method of [10]. Colorimetric absorbance at 530 nm was measured using a Spectronic 710 Spectrophotometer. Readings were converted to mg malonaldehyde /1000g meat product and reported as TBA values (mg TBA/1000g meat product).

Microbiological test: Total aerobic count, total anaerobic count, coilform, and psychrophilic count of buffalo meat product were made as (CFU/g) according to the methods described in the standard methods of [4], [21].

Statistical analysis: Data were analyzed by analysis of variance (ANOVA) to determine if treatments were significantly different [9].

### **RESULTS AND DISCUSSIONS**

Table 2 presents the chemical composition of the treated and the control buffalo meat product. The data showed that there were no variations between the samples except for moisture content which was higher in the treated samples than the controlled ones and this could be due to the effect of the addition of the spice extract solutions which contain about 50% water.

Packaging of food products in polymeric films is a technique designed to prevent moisture losses. Figure 1 show the relationship between the storage time and the moisture content for the untreated and the treated buffalo meat product stored frozen in two different packaging materials and treatments. It is shown from the results that all the samples have lost moisture during the storage period. The loss was higher in samples packaged in LDPE bags than the ones vacuum packaged in laminated PE/Nylon bags, and this was due to the higher rate of the water vapor permeability through LDPE compared to the laminated PE/Nylon bags.

The effect of storage time and packaging materials and treatments on the expressible water (EP) and water holding capacity (WHC) for buffalo meat product was shown in (Figs. 2&3).

The data revealed that the EP values increased along with storage period for all samples (control and treated with spice extracts), whereas, WHC values decreased with time during frozen storage.

The increment of EP values was higher in the LDPE packaged samples without vacuum than the others packaged under vacuum in laminated bags. The data show that the vacuum treatment reduced the rate of decline in WHC.

It is obvious that the increase in the EP values were likely related to the loss of WHC as a result of dehydration and denaturation of muscle proteins, or interaction of auto-oxidation breakdown products with proteins, or could be due to the cells which were punctured by ice crystals and volatile moisture loss through vapor permeability of packaging materials.

Figures (4&5) clearly illustrate the effect of packaging materials and treatments as well as spice extracts on the pH changes and percentage acidity (as lactic acid) in frozen buffalo meat product.

The data showed a slight decrease in the pH values and an increase in the acidity values for all samples along with storage time during the first six months of storage as a result of the increase of free fatty acids due to rancidity. The decrease in the pH values was lower in the untreated samples than the treated ones due to the effect of natural antioxidants which retarded the formation of free fatty acids.

It is also obvious that the values of pH for the product were higher than that of the pH values of meat and this could be due to the interaction effect of the other ingredients which were added during the processing of meat products.

Free fatty acids (FFA) and TBA values for the control and the treated samples packaged in two different packaging materials and treatments were illustrated in Figs. (6&7).

The data showed that the (FFA) values for the control sample were significantly (p<0.05) increased along with the storage period. It is also shown that the addition of spice extracts delayed the formation of free fatty acids specially with the use of thyme extract for the first six months of storage, then the samples suffered much more increase in its (FFA) values, and the values were much lower in the vacuum packaged samples which means that vacuum is considered as the second line of protection by retarding the formation of free fatty acids. TBA values increased over time for all samples. The increment was rapid for the control samples and the greatest change occurring between the sixth and the ninth month of storage. The values for the control samples increased significantly (p<0.01) during frozen storage, whereas the changes in the TBA values for the samples treated by spice extracts were not significantly different (p<0.05). Within each treatment the same trend was shown as in figure (7) where the vacuum packaged samples had the lowest TBA values compared to the other samples.

Meat and meat products stored in air is rapidly spoiled by bacteria which are responsible for discoloration and off odor, causing its rejection. Vacuum packaging exerts an important effect on the micro-organisms. Vacuum packaged meat is generally very stable in the cold with the low temperature and limited quantity of oxygen inhibiting bacterial growth.

Figure 8 illustrates the effect of storage time, and packaging materials and treatments on the total aerobic count of untreated and treated buffalo meat product. The data showed a negative relationship between the time of storage and the bacterial count for all samples.

This is particularly evident for the inhibiting effect of vacuum packaging and the spice extracts on the growth of aerobic microorganisms.

The same trend of reduction was observed for the anaerobic total count for the treated and the untreated samples during the storage period as shown in (Fig.9). The reduction was lower for the vacuum packaged samples compared with the samples packaged without vacuum.

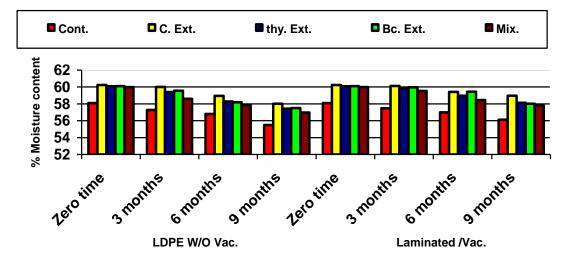
The effect of storage time in combination with packaging materials and treatments was clear on the total coliform count as shown in (Fig. 10). The data illustrated that the number of coliform decreased along with the storage period for all samples, and this effect was clear for the spice extracts treated samples and the vacuum packaged samples.

Table 2

Chemical composition of untreated and treated buffalo meat product (g/100g on dry basis).\*

Samples	Moisture content	Crude protein	Crude fat	Ash	Carbohydrate
Control	58.30±0.18	17.21±0.21	17.02±0.08	2.11±0.03	5.36±0.08
Cardamom ext.	59.84±0.10	16.74±0.08	16.41±0.12	2.03±0.06	4.98±0.11
Thyme ext.	60.11±0.18	17.11±0.15	16.71±0.21	2.07±0.01	4.00±0.08
Black cumin ext	60.10±0.17	17.45±0.21	16.28±0.18	2.01±0.10	4.16±0.12
Mixture	60.18±0.24	17.01±0.09	16.19±0.12	2.11±0.06	4.51±0.06

\*Means of three determinations ± Standard Error.



Cont. = Control. C. Ext. = Cardamom extract. Thy. Ext. = Thyme extract. Bc. Ext. = Black cumin extract.

Mix. = Mixture of extracts. LDPE W/O Vac. = Low density polyethylene without vacuum.

Laminated /Vac. = Laminated polyethylene/nylon with vacuum.

Fig. 1. Effect of storage time, Packaging materials and treatments on moisture content of untreated and treated buffalo meat product.

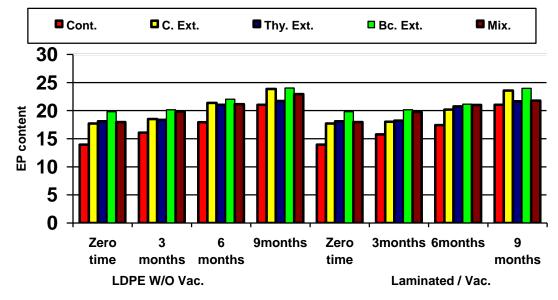


Fig. 2. Effect of storage time, packaging materials and treatments on expressible water (EP) of untreated and treated buffalo meat product.

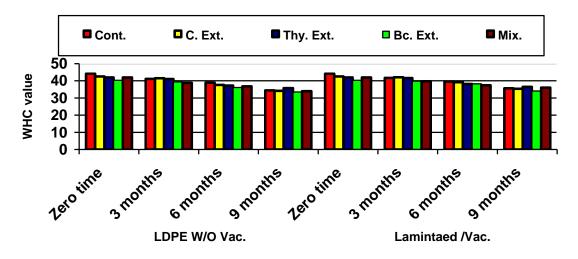


Fig. 3. Effect of storage time, packaging materials and treatments on water holding capacity (WHC) of untreated and treated buffalo meat product.

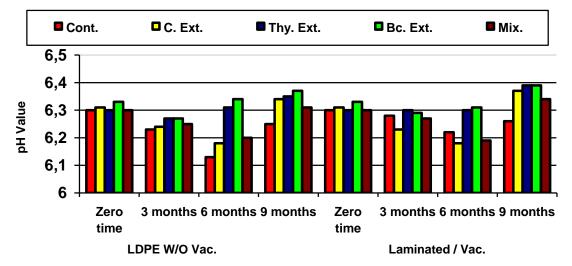


Fig. 4. Effect of storage time, packaging materials and treatments on pH value of untreated and treated buffalo meat product.

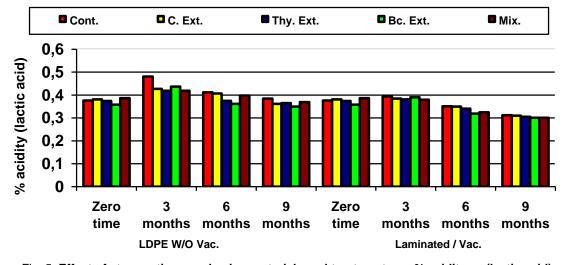


Fig. 5. Effect of storage time, packaging materials and treatments on % acidity as (lactic acid) of untreated and treated buffalo meat product.

The data also showed that some of the spice extracts treated samples have no coliform bacteria starting from the sixth month of storage which indicates the inhibiting effect of spice on the growth of coliform flora.

Total psychrophilic counts of all samples showed a reduction for the first six months of storage under frozen condition. The reduction was significant (p<0.05) for the vacuum packaged or spice extracts treated samples. After the first six months of storage, the number of the psychrophilic counts starts to increase again as shown in (Fig.11).

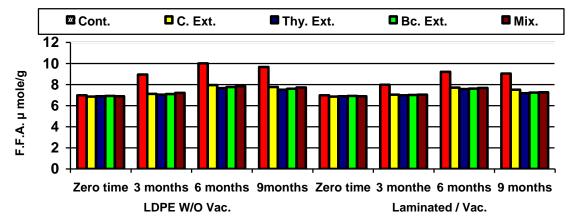


Fig. 6. Effect of storage time, packaging materials and treatments on free fatty acids content (F.F.A) as  $\mu$  mole/g of untreated and treated buffalo meat product.

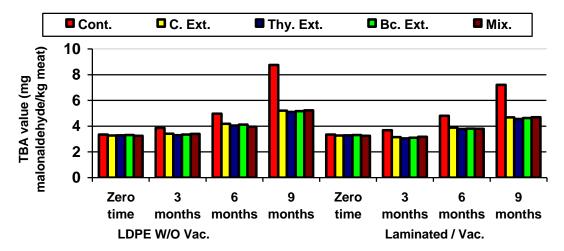


Fig. 7. Effect of storage time, packaging materials and treatments on TBA value (mg malonaldehyde/kg meat) of untreated and treated buffalo meat product.

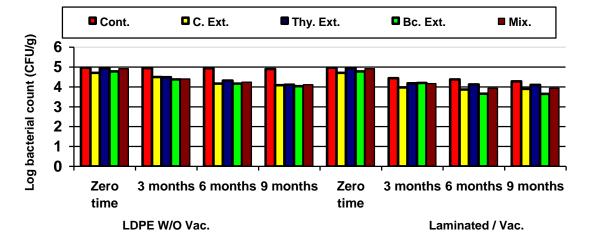


Fig. 8. Effect of storage time, packaging materials and treatments on the total aerobic plate count (CFU/g) of untreated and treated buffalo meat product.

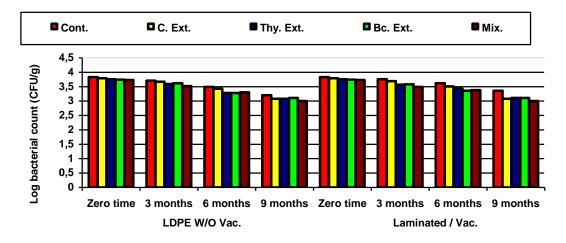


Fig. 9. Effect of storage time, packaging materials and treatments on the total anaerobic plate count (CFU/g) of untreated and treated buffalo meat product.

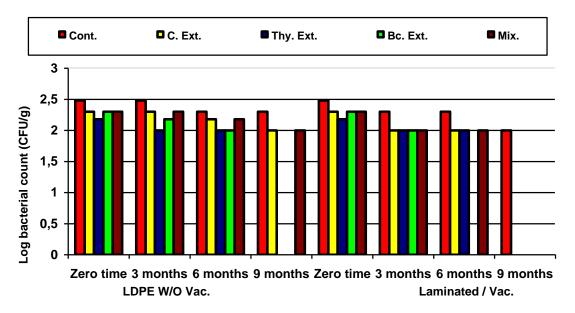


Fig. 10. Effect of storage time, packaging materials and treatments on the total coliform count (CFU/g) of untreated and treated buffalo meat product.

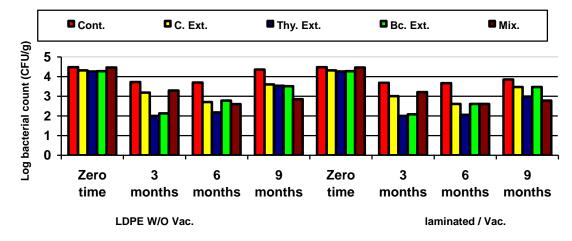


Fig. 11. Effect of storage time, packaging materials and treatments on the total psychrophilic count (CFU/g) of untreated and treated buffalo meat product.

# **CONCLUSIONS**

The application of spices extract (as natural antioxidant) and vacuum packaging technique was very effective which interacted with low storage temperature and produced impact effect resulted in inhibiting the oxidation of fat and reduction of bacterial count and coliform group for buffalo meat product.

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