

# THE COMBINED EFFECT OF EDIBLE PACKAGING AND SPICES EXTRACT ON STABILITY OF FROZEN BUFFALO MEAT PRODUCT

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## Abstract

*The chemical composition, expressible water (EW), 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 combined effect of edible packaging and two spices extract on the stability buffalo meat product stored for 10 months under frozen condition. The data showed that the treatment by spices extract as antioxidant and chitosan (as primary edible packaging) has no noticeable effect on the chemical composition of the product. The results indicated that all the samples have lost moisture during storage, and the loss was higher in the chitosan untreated samples. The data also showed that the EW values increased, whereas, WHC values decreased with time during storage for all samples. The increment of EW and the decrease of WHC were higher in the chitosan untreated samples. The data indicated a reduction in the pH values and an increase in the total acidity values especially for the first four to six months of storage, and these changes were associated with an increase in the FFA values as a result of storage. The increment in the FFA values was the highest for the control samples compared with the spices extract treated samples, and for the chitosan untreated samples compared with the treated ones. This indicated the effect of spices extract and chitosan as edible packaging to retard the formation of FFA. The data indicated that TBA values for the control samples were higher than those treated by spices extract, and for the chitosan untreated samples compared with the chitosan treated ones. The storage time had a negative effect on the total bacterial counts and the coliform group for all samples. The rate of reduction was much higher in the chitosan treated samples and the spices extract treated samples as well. The results indicated that the use of chitosan as edible film had delayed the proliferation of total plate count and coliform group.*

**Key words:** Spices extract, edible packaging, chitosan, buffalo meat product

## INTRODUCTION

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 [8]. During production, processing, distribution, and storage, food undergoes deterioration from chemical and microbiological processes. Oxidation is a major cause of that deterioration because of its negative effects

on sensorial qualities (flavour, colour, etc.). Oxidation of lipids can also have a marked negative effect on nutritional value, and could be responsible for the production of toxic compounds. Meat products, due to fat content are highly susceptible to lipid oxidation. Moisture, pro-oxidant pigments, storage, handling and display conditions contribute to lipid oxidation of meat products [14], [16]. Due to detrimental effects of lipid oxidation on colour, flavour, 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

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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 such as rosemary and sage have played an important role [17], [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 [21]. 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. Several natural antioxidants have been tested in meat systems; black pepper, propolis and extract of rosemary. Ground fresh leaves of rosemary and sage have also been reported to inhibit lipid oxidation in beef hamburgers [1], [15]. Herbs and spices have been used for their antimicrobial properties in preventing food deterioration and pathogenic diseases. A number of studies have been made on the bactericidal and bacteriostatic properties of spices to evaluate their effectiveness in preventing or retarding spoilage caused by micro-organisms in addition to the antioxidant effect of spices on fats in certain foods [19], [7]. 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 stand point 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 [11]. Edible coating of biodegradable packaging is a new technology that has been introduced in food processing in order to obtain products with longer shelf life. Several applications for meat, poultry, and sea foods have been reviewed by [13] with particular

emphasis on the reduction of lipid oxidation, weight loss, moisture loss, microbial load, and volatile flavour loss. Edible films or coatings have been investigated for their abilities to retard moisture, oxygen, aromas, and solutes transports. It is one of the most effective methods to maintain food quality [18]. Microbial growth on the surface of food is a major cause of food spoilage and food-borne illness. Therefore, the concept of using edible active coating to inhibit spoilage and pathogenic micro-organism has received considerable interest [20], [10], [24].

The objective of this investigation was to determine the potential effect of the application of two spices extract (rosemary and thyme) in combination with chitosan as primary edible packaging on stability of buffalo meat product stored for ten months under frozen condition.

## MATERIAL AND METHODS

**Spices extract:** Dried spices (rosemary and thyme) were obtained from a local market then powdered using a mortar and pestle. Powdered spice (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 Whatman No 1 filter paper. The final concentration of the stock solution was 20 g/100 ml.

**Preparation of chitosan solution:** Chitosan edible film solution was prepared by dissolving 1g of chitosan powder in 100 ml of 1% glacial acetic acid solution. The solution was then filtered through a silk screen to remove undissolved material.

**Preparation of buffalo meat product:** The buffalo meat (bottom round) used in this study was obtained from a local market in El-Minia, Egypt, one hour after slaughter. The sample was trimmed, packed in low density polyethylene bags and held at  $4\pm 1^{\circ}\text{C}$  for 24 hours, cut into cubes and minced with a meat grinder twice to obtain ground buffalo meat. Buffalo meat product was prepared according to the following recipe presented in table 1.

All the ingredients were mixed well then divided into three equal portions. Rosemary extract was added to the first portion in the

ratio of (1ml/10g sample), thyme extract was added to the second portion (same ratio), whereas, the third portion was left without any additive as control. Each portion was divided into small balls  $10 \pm 2$  g each then formed in a finger-like shape  $10 \pm 1$  cm long. The samples were frozen at  $-40^{\circ}\text{C}$  for 2 hours, and half of each treatment was immersed in chitosan solution for one minute (to form a thin film as primary package), while the other half left without primary packaging, then all samples packaged in 2 mil low density polyethylene bags (as secondary package), and stored frozen at  $-18^{\circ}\text{C}$  for 10 months as shown in figure 1.

Analytical methods: Moisture, crude protein, crude fat, ash, and carbohydrate contents were determined according to the methods of the [4].

Determination of expressible water (EP) and water holding capacity (WHC): Expressible water (EP) was determined according to [2]. Whereas, 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 [3].

Determination of total acidity: The acidity was determined by titration according to [12].

Determination of free fatty acids (FFA): Free fatty acids (FFA) as measurement of enzymatic rancidity was 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 [9]. 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 plate and coliform group of buffalo meat product were made as (CFU/g) according to the methods described in the standard methods of [5], [22].

Table 1- Share of buffalo meat product

Ingredient	Amount (%)
Ground buffalo meat	64
Minced fat	14
Bread crumb	6
Eggs	3
Modified starch	4
Crushed ice (water)	6
Onion powder	1
Salt	1
Black pepper powder	1

## RESULTS AND DISCUSSIONS

Table 2 illustrates the chemical composition of the treated and untreated buffalo meat product samples. The data showed that there were no big variations between the samples except for moisture content which was a little higher for the spices extract treated samples compared with the control one due to the percentage of water (50%) in the spices extract solutions. Table 3 illustrates the effect of storage time and chitosan film (as primary edible packaging) on the moisture content of the buffalo meat product. It is shown from the results that all the samples have lost moisture during the storage period. The loss was higher in the chitosan untreated samples compared with the treated ones. This indicated that chitosan film as primary edible package has protection effect against water vapour permeation. The effect of storage time and chitosan treatment of the expressible water (EW) and water holding capacity (WHC) for buffalo meat product was shown in tables 4 & 5. The data showed that EW values increased and WHC values decreased along with the storage time for all samples. The increment of EW and the decreasing of WHC values were much higher in the chitosan untreated samples which means that the chitosan film retarded the loss of moisture and kept the juiciness for the samples. Figures 2 & 3 clearly illustrate the effect of spices extract and chitosan edible film on the pH and total acidity values for the buffalo meat product during frozen storage.

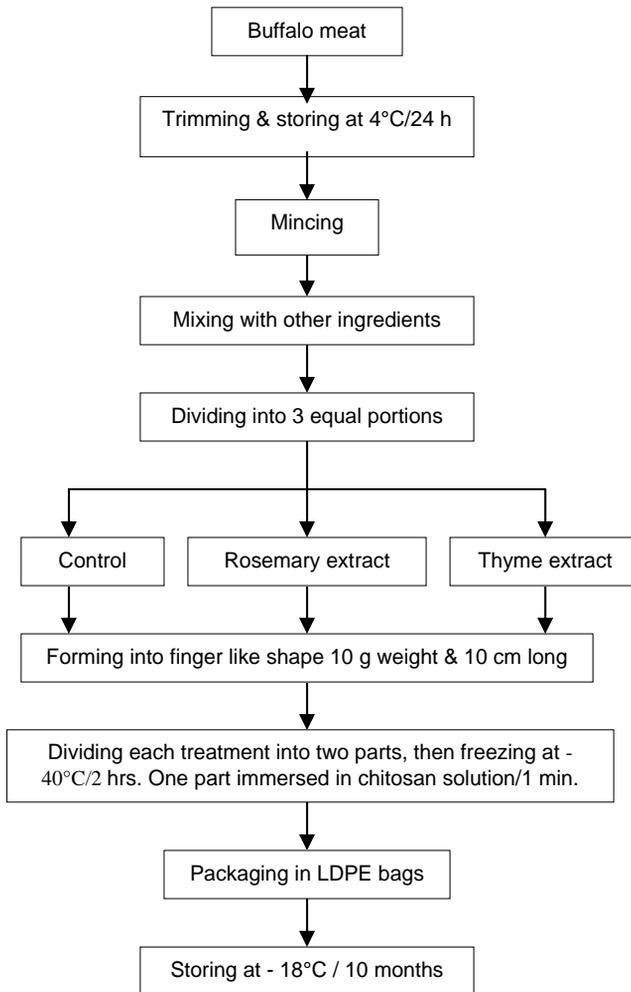


Fig. 1. Flow diagram of production, treating, and storing of buffalo meat product under frozen storing condition

Table 2 - Chemical composition of untreated and treated buffalo meat product (wet basis)\*

Compound %	Control		Rosemary extract		Thyme extract	
	W/O chitosan	With chitosan	W/O chitosan	With chitosan	W/O chitosan	With chitosan
Moisture	66.12	66.22	67.18	67.15	66.98	67.08
Protein**	16.18	16.19	16.17	16.15	16.16	16.18
Crude fat	10.13	10.17	10.09	10.11	10.08	10.05
Ash	2.31	2.32	2.48	2.47	2.49	2.51
Fibers	0.32	0.34	0.41	0.46	0.49	0.50
CHO***	4.94	4.76	3.67	3.66	3.80	3.68
Energy (Kcal)	176	175	171	171	170	170

\*Means of 3 replicates.

\*\*Total nitrogen x 6.25.

\*\*\*By difference.

Table 3 - Effect of storage time and edible packaging on the moisture content of untreated and treated buffalo meat product\*

Storage time (days)	Control		Rosemary extract		Thyme extract	
	W/O chitosan	With chitosan	W/O chitosan	With chitosan	W/O chitosan	With chitosan
Zero time	66.12	66.22	67.18	67.15	66.98	67.08
60	65.74	65.93	65.71	66.08	65.58	66.21
120	64.29	65.01	64.41	65.64	64.20	65.69
180	63.18	64.17	63.21	64.38	63.28	64.31
240	61.01	63.03	61.00	63.17	61.15	63.00
300	60.11	62.12	60.13	62.29	60.09	62.31

\*Means of 3 replicates.

Table 4 - Effect of storage time and edible packaging on the expressible water (EW) of untreated and treated buffalo meat product\*

Storage time (days)	Control		Rosemary extract		Thyme extract	
	W/O chitosan	With chitosan	W/O chitosan	With chitosan	W/O chitosan	With chitosan
Zero time	10.87	10.84	10.89	10.79	10.81	10.78
60	16.14	13.39	16.21	13.51	16.30	13.48
120	26.84	17.85	26.41	17.42	26.71	17.31
180	29.76	22.64	29.38	21.37	29.27	21.28
240	34.27	26.21	34.61	26.01	34.17	25.87
300	39.96	30.15	39.69	30.75	39.78	29.97

\*Means of 3 replicates.

Table 5 - Effect of storage time and edible packaging on the water holding capacity (WHC) of untreated and treated buffalo meat product\*

Storage time (days)	Control		Rosemary extract		Thyme extract	
	W/O chitosan	With chitosan	W/O chitosan	With chitosan	W/O chitosan	With chitosan
Zero time	66.12	66.22	67.18	67.15	66.98	67.08
60	65.74	65.93	65.71	66.08	65.58	66.21
120	64.29	65.01	64.41	65.64	64.20	65.69
180	63.18	64.17	63.21	64.38	63.28	64.31
240	61.01	63.03	61.00	63.17	61.15	63.00
300	60.11	62.12	60.13	62.29	60.09	62.31

\*Means of 3 replicates.

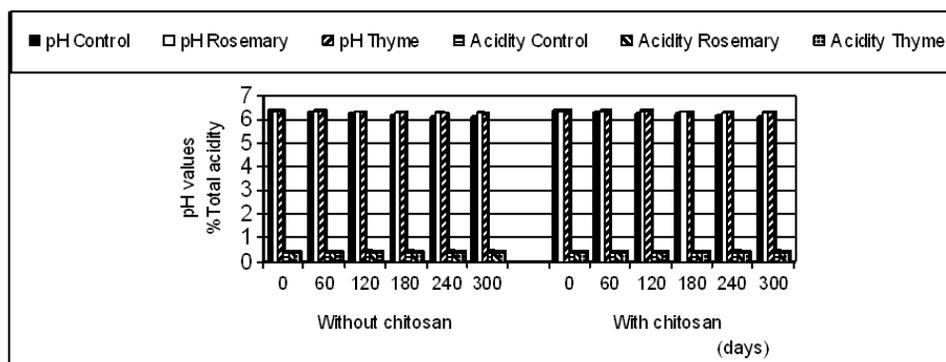


Fig. 2 Effect of storage time in combination with edible packaging (chitosan) and spices extract of the pH and % total acidity (lactic acid) values of frozen buffalo meat product

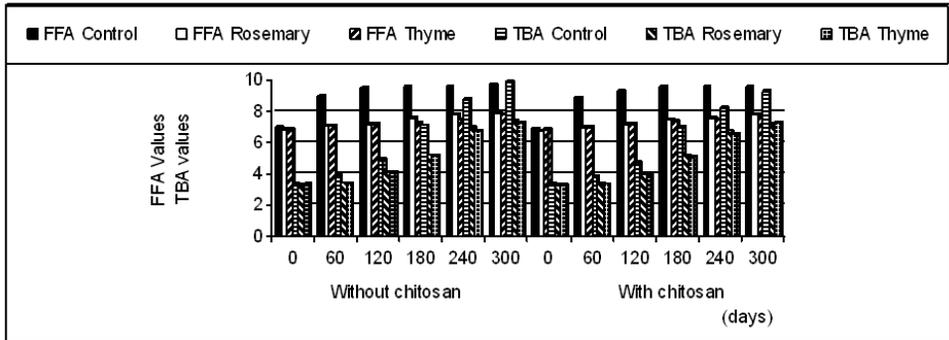


Fig. 3 Effect of storage time in combination with edible packaging (chitosan) and spices extract of the free fatty acids ( $\mu$  mole/g) and thiobarbituric acid (mg malonaldehyde/kg meat) values of frozen buffalo meat product

The data demonstrate that there is a decrease in the pH values and an increase in the acidity in the first period of storage (first four months) for all samples. The reduction of pH and increment in the total acidity values were lower in the control samples than at the spices extract treated ones and this could be due to the effect of natural antioxidants which retarded the formation of free fatty acids. It is also obvious that treatment by chitosan had negative effect on the formation of free fatty acids. Data in figures 4 show the effect of storage time in combination with spices extract and edible packaging treatment on free fatty acids and TBA values of the buffalo meat product. The data revealed that the addition of spices extract delayed the formation of FFA, and reduced the increase of TBA values along with the time of storage compared by the control ones. It is also shown that treatment by chitosan as edible film had the same trend. Same trend was shown between the two spices extract.

Figure 4 illustrates the effect of storage time and spice extract in combination with chitosan (as edible film) on the total bacterial count and coliform group of buffalo meat product. The data showed a negative relationship between the time of storage and the bacterial count for all samples. The rate of reduction was higher in the natural antioxidants treated samples and in the samples treated by chitosan as edible coating as well. This is particularly evident for the inhibiting effect of chitosan and spices extract on the growth of bacteria. The data also revealed that the number of coliform decreased along with the storage period for all samples, and this effect was clear for the spices extract treated samples and the chitosan coated samples. The data also showed that some of the spices extract treated samples coated by chitosan have no coliform bacteria starting from the fourth and fifth month of storage which indicates the inhibiting effect of chitosan and spice on the growth of coliform flora.

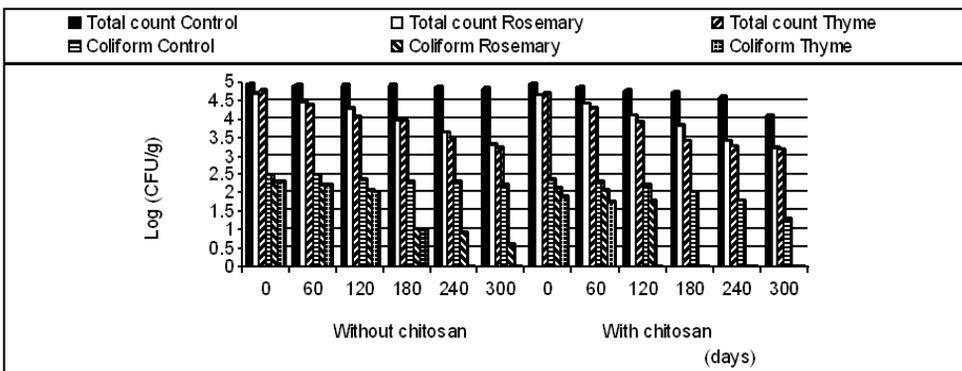


Fig. 4 Effect of storage time in combination with edible packaging (chitosan) and spices extract of the total bacterial count and coliform group count of frozen buffalo meat product

## CONCLUSIONS

The application of spices extract (as natural antioxidant) and chitosan film (as edible package and antimicrobial agent) 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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