



## Effect of Chitosan-Rosemary Incorporated Membrane on the Chemical Quality and Shelf life of Meatballs Chilled at 4°C



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### Abstract

THE study aimed to evaluate the chemical quality and shelf life of raw minced meat preserved by chitosan loaded with rosemary essential oil (EO). The control and the chitosan film incorporated with rosemary essential oil with different concentrations – preserved minced meat treatments were formed into meatballs to be stored at 4 °C for 10 days. The results showed a significant difference in the pH values of different treatments, the meat balls preserved by chitosan film incorporated with rosemary essential oil with different concentrations had the lowest value compared with control especially 0.6 % concentration, the pH recorded 6.02 at 10<sup>th</sup> day of storage in which the treatments preserved using 1 % rosemary essential oil had 6.36 pH and the control samples were spoiled. The use of rosemary essential oil and chitosan incorporated film with different concentrations of rosemary had significant reductions in the value of Thiobarbituric Acid Reactive Substances (TBARs) and peroxide value (PV) in comparison with the control treatment. The preservation of minced meat by chitosan loaded with rosemary essential oil had a highly significant impact on the total antioxidant capacity (TAC) compared to unpreserved control samples. The 0.6 % rosemary incorporated with chitosan film preserved meat balls had the highest TAC 10<sup>th</sup> day of storage (6.44). Therefore, the preservation of minced meat using chitosan loaded with rosemary essential oil improved the chemical quality and prolong the shelf life of minced meat and could be applied as a promising preservative for meat and its products.

**Keywords:** chemical quality; chitosan film; rosemary essential oil; incorporated; preservative.

### Introduction

Meat and its products are the primary source of superior proteins and fats. Therefore, meat is mainly exposed to lipid oxidation and meat product quality deterioration during processing and storage, which leads to a deterioration in the quality of meat products, with changes in the nutritional quality and organoleptic appearance, due to the formation of primary and secondary products during lipid oxidation. [1,2].

Promising approaches using the natural preservatives as organic acids (citric acid, propionic acid, sorbic acid, etc.), microbial derived

bacteriocins, plant extracts, herbs, spices and essential oils (cinnamon, basil, thyme, oregano, cloves, moringa oleifera, rosemary, etc.) as well as natural polymers (chitosan) have been used to extend the preserved meat shelf life and highly preferred in the food industry, as they increase the storage time of meat products and prevent loss of nutrients and flavor due to microbiological or chemical changes [3].

The active principles of natural herbs and plants have protective function against lipid oxidation due to having antimicrobial and antioxidant action giving it potential uses for meat preservation [4].

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Rosemary (*Rosmarinus officinalis*) is the main essential oil of great antioxidant activity containing high amount of phenolic components like carnosol, rosemary diphenol, rosmarquinone, and rosmanol which have been isolated from the oil. Rosemary is also said to have high antioxidant properties for meat [5,6].

Increased EO levels can impart off-odours to products as EOs are lipophilic in nature and difficult to disperse in water-based cleaning solutions. Therefore, alternatives have been developed, namely their integration into nanoemulsions and healthy packaging [7].

Active packaging containing rosemary may be used to maintain meat quality and safety, especially in raw beef products such as minced meat, where surface contamination is the main cause of microbial, chemical and enzymatic spoilage. However, the stability of these packaging films during processing and storage, as well as its agreement to legal standards, should be tested prior to use [8].

Selecting the proper carrier material for the essential oil delivery is crucial in active packaging because the polymer must protect the EO, this carrier should be easily released, biodegradable, biocompatible, soluble in water, and reasonably priced [9].

The most common biopolymers are starches, gums, pectin, chitosan, alginate, and cellulose [10]. Antimicrobial polysaccharide chitosan is enhanced with essential oils (EOs) to enhance its resistance against fungal infections, provided that the antimicrobial compounds are released from the polymer matrix [11].

This work aimed to improve chemical quality and shelf life of meat and overcome on using synthetic additives that cause health problems to consumers by using chitosan film loaded with rosemary oil as packaging material and compare with using rosemary EO alone.

## **Material and Methods**

### *Rosemary Essential oils*

The food grade rosemary oil used in this study was purchased from the oil pressing and extraction unit at the National Research Centre, Egypt. The oil was stored in amber-collared bottles at 4 °C until use.

### *Preparation of chitosan membrane*

Low molecular weight chitosan (Sigma-Aldrich, Saint Louis, MO) was dissolved with stirring in 1% acetic acid to yield solutions with a 2% chitosan concentration. The chitosan solution was poured into a sanitized Petri dish once it had completely dissolved. The chitosan membranes that had been cast were subjected to six cycles of freezing at -20 °C

then thawing at 20 °C, with a 0.1 °C min<sup>-1</sup> rate and a six-hour holding period [12,13].

### *Preparation of chitosan/ rosemary composite membrane*

Chitosan was dissolved under stirring in 1% acetic acid to obtain solutions of 2 wt % chitosan concentrations. After complete dissolution, different concentrations (0.2, 0.4, and 0.6%) of Rosemary oil extract were added. Then, the final solution was cast into a clean Petri dish. The casted chitosan loaded rosemary membranes were subjected to six cycles of freezing at -20 °C then thawing at 20 °C, with a 0.1 °C min<sup>-1</sup> rate and a six-hour holding period [12,13].

### *Membrane Characterization*

#### Determination of hydrolytic degradation

The chitosan and chitosan/rosemary composite dried membranes were first weighed, and then they were periodically submerged in 10 mL of 0.1 M phosphate buffered saline (PBS, pH 7.4) at 37 °C. After that, the samples were removed, and the water on the surfaces was carefully blotted away using soft filter papers. The membrane samples were then reweighed after being vacuum-dried at room temperature. Every investigation was conducted six times, and the mean and SD were determined [14].

#### Light Transmittance

An Ultra Violet-visible spectrometer (Spectrophotometer model Jasco V-630, made in Europe) was used to determine the regular light transmittance of the membranes. The wavelength range is 300 to 800 nm [15].

#### Fourier Transform Infrared Spectroscopy (FTIR)

Attenuated total reflectance was used to look into how the formulated films' functional groups were changed. Using Fourier transform infrared spectroscopy (ATR-FT-IR), the main chemical characteristics of chitosan and chitosan/rosemary composite films were determined. An FT-IR spectrophotometer (Shimadzu FTIR-8400S, Japan) was used to analyze the films using ATR-FTIR. The samples under examination were scanned between 4000 and 400 cm<sup>-1</sup>. [16].

#### Scanning electron microscopy

A scanning electron microscope (SEM; TESCAN VEGA3; operated under high vacuum conditions at an accelerating voltage of 20.0 kV using a secondary electron detector) was used to image the fracture surfaces of chitosan and chitosan/rosemary composite films.

### *Preparation of Minced beef sample*

The samples were immediately prepared and divided into three treatments of 100 g each. Then, rosemary EO was mixed with minced beef group to

achieve a final concentration of 1%. The essential oil was mixed with the minced beef samples for another 30 seconds to ensure even mixing. Each main group was subdivided into 12 equal subgroups. All the samples with oil and the control were packed in polyethylene bags, labelled, and stored at 4 °C.

Other samples of package membranes are prepared by adding a meatball (25 g) to each membrane. There are three cons. For each ounce of rosemary oil (0.2–0.4–0.6), each conc. has 3 membranes. All samples were packed in polyethylene bags, labelled, and stored at 4 °C, where they were chemically examined every 3 days. The experiment was conducted in triplicate for 10 days of storage. Then they were transported directly in insulated and iced containers to the microbiology laboratory.

#### *Chemical examination:*

##### Determination of pH

The pH value was measured by the method of [17]. Approximately 10 g of minced meat sample was mixed with 10 ml of distilled water in a blender. The homogenate was left at room temperature for 10 min under constant shaking. The pH value was measured using an electronic pH meter (Bye Model 6020, USA).

##### Determination of Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS was measured by the method of [18]. The test measures malonaldehyde (MDA), which is the byproduct of lipid peroxidation. A TBA number or value, expressed in milligrams of malonaldehyde equivalent per kilogram of sample, is typically used to indicate the degree of oxidative odor. Malanoaldehyde is released when unsaturated fatty acids in meat undergo oxidative degradation, resulting in the formation of the TBA-melanoaldehyde complex. Melanoaldehyde can be quantified as a marker of food quality and lipid oxidation once it has been produced. In actuality, 50 ml of distilled water, 10 g of the prepared meat sample, and 2.5 ml of diluted hydrochloric acid were added to a 47.5 ml distillation flask. Next, a tiny bit of an antifoaming agent was added. After 10 minutes of starting to boil, the distillation flask was heated to achieve a 50 mL distillation.

Exactly, 5 mL of a distilled solution and 5 mL of prepared thiobarbituric acid (made by dissolving 0.2883 thiobarbituric acid in 90% trichloroacetic acid to a final volume of 100 mL) were added to a tube that had a cover. After the tube was covered and placed in a water bath, it boiled for thirty-five minutes before being cooled in the water for ten minutes. Next, under wave length 538, the sample's

absorbance was determined using a spectrophotometer (UNICAM969AA Spectronic, USA).

$$\text{TBA value} = \text{absorbance of sample} \times 7.8 \\ (\text{malonaldehyde (mg) /Kg})$$

##### Determination of Peroxide value "PV":

The peroxide value (PV) was determined according to the method of [19]. Exactly 3 g of sample was weighed and placed in a glass-stoppered Erlenmeyer flask. It was then heated in a 60°C water bath for 3 min to dissolve the fat. The flask was then thoroughly shaken with 30 mL of acetic acid-chloroform solution (3:2 v/v) for 3 min to dissolve the fat. The mixture was filtered through Whatman No. 1 filter paper to remove meat particles from the filtrate. 0.5 ml of saturated potassium iodide solution and starch solution as indicator were also added to the filtrate. Titration continued with a standard solution of sodium thiosulfate. The peroxide value (PV) was determined using the following formula and is expressed as milliequivalents of peroxide per kg of sample:

$$\text{PV(meqO}_2\text{/Kg)} = \left[ \frac{S \times N}{W} \right] \times 100$$

Where, S= Volume of titration (mL), N= Normality of sodium thiosulfate solution (N=0.01), W= Weight of the sample (g).

##### *Total Antioxidant Capacity:*

Analysis of meat tissues for Total Antioxidant Capacity (T-AOC) was carried out using Ferric Reducing Antioxidant Power (FRAP) method [20]. Accurately, 0.02 ml of meat extract was placed in a test tube and the volume was adjusted to 300 µl with distilled water. Then, 1.8 mL of FRAP reagent was added and incubated at 37 °C for 10 min. The composite color was measured at 593 nm using a UV spectrophotometer (Spectronic 21D). Blank samples were prepared with 300 µL of distilled water and 1.8 mL of FRAP reagent and treated in the same way as the samples. A standard series of 1–4 µg Trolox was prepared and the volume was adjusted to 300 µl with distilled water, and all these tubes were treated in the same way as the samples.

##### *Statistical analysis*

All experiments were done in triplicate, and the results are shown as means ± SD. For the data analysis, a two-way ANOVA was employed. Using the CoStat statistical tool, Duncan's multiple range tests ( $p < 0.05$ ) were run to examine the significance degree between the treatments.

## **Results and Discussion**

### *Determination of hydrolytic degradation*

With regard to the hydrolytic degradation phenomenon, the degrading property of the membranes used in packaging applications (food preservation) is critical to the efficient execution of their biological functions. Thus, the weight loss of the chitosan and chitosan/rosemary composite membranes was estimated *in vitro* using phosphate buffer saline (PBS, pH 7.4) at 37 °C for different time points. As shown in Figure 1, the chitosan membrane doesn't have a biodegradability rate, as the weight loss was very little (it was only 7.85% after 24 hours and 8.847% after 120 hours), also the chitosan/rosemary membrane shows a slow degradation rate (it was only 7.24% after 24 hours and 8.207% after 120 hours).

### *Light Transmittance*

Packaging is one of the main required application of the material transparency. Figure 2 showed the light transmittance spectra of chitosan and chitosan/rosemary composite membranes. At the wavelength of 310 nm, the visible light transmittance of the chitosan/rosemary composite membrane exceeded that of the chitosan membrane. In addition, all membranes show a characteristic peak at the wavelength of 310 nm [21].

### *Fourier Transform Infrared Spectroscopy (FT-IR)*

The FT-IR spectra of the chitosan and chitosan/rosemary composite membranes are presented in Figure 3. The characteristic bands of chitosan were represented in the membrane spectrum; for example, a band at 3350–3540 cm<sup>-1</sup> was associated with the presence of O–H and N–H stretching groups from the intermolecular and intramolecular hydrogen bonds, while the C–H symmetric and asymmetric stretching bands appeared at 2918 and 2877 cm<sup>-1</sup> from alkyl groups, and the peaks between 1732–1657 cm<sup>-1</sup> were due to the stretching C=O [22]. The oil and chitosan bands overlapped, increasing the intensity of the amine (1540 cm<sup>-1</sup>) and carbonyl (1654 cm<sup>-1</sup>) bands. The interaction of chitosan and oil was identified as the cause of this behaviour [23-25].

### *Scanning electron microscopy*

A scanning electron microscopy analysis was performed in order to characterize the morphological changes of the chitosan and chitosan/rosemary composite membranes. Figure 5 depicts the porosity structure of the prepared membranes. It is clear that the surface of the chitosan membrane is somewhat rough, with many pores and cracks on the surface, but after adding rosemary oil, the membrane's surface becomes softer, with few or no pores; this is evident in magnifications of 500X and 1000X. The more oil there was, the softer the surface became. On

the other hand, the pores become smaller or disappear. In chitosan/rosemary membranes, chitosan and oil are connected by hydrogen bonds during the freeze-thaw process, creating a compact structure in between the pores [26,27].

### *Determination of pH*

On first day of storage (0 day), the mean pH value of control, rosemary essential oil treated meat balls and meat ball samples preserved using chitosan film incorporated with rosemary essential oil with different concentrations ranged from 5.71± 0.02 to 5.59 ± 0.09. There was significant ( $P < 0.05$ ) found in the treated meat ball from the third day of storage and afterward in comparison to control samples (Table 1). Comparing with other treatments, the pH values of the meat balls preserved using chitosan film loaded with 0.6% concentration of rosemary essential oil were better (lower). However, as the length of storage increased, the pH rose in both the treated and control groups of meat balls. The control group was spoiled from the 7 day of storage while the treated meat balls remain acceptable with the storage time increase till the 10 day of storage. The lower pH detected in meat ball samples preserved using rosemary essential oils or chitosan film loaded with rosemary oil versus the control is attributed to the antibacterial effect of rosemary [28]. As with increase the time of storage the bacterial growth was increased and the secondary metabolites resulted from protein breakdown will also increase leading to ammonia accumulation and increasing pH values [29]. Various previous studies reported that Rosemary essential oils and chitosan films had phenolic compounds with great antimicrobial activity [30,31]. Also recent study reported that chitosan film containing rosemary essential oil lowered the pH value of ground meat during cold storage increasing its shelf life [32].

### *Determination of Thiobarbituric Acid Reactive Substances*

Chemical deterioration of meat is the leading cause of off-flavor and reduced meat shelf life. Meat experts are focusing on lipid oxidation and free radicals because of their significant impact on meat quality and freshness. Recently, improvements in meat antioxidant capacity by including appropriate and safer antioxidants have received increased attention. Rosemary extract has been extensively tested and identified as a potent antioxidant that decreases TBA values in various meat products [33].

Table 2 displays that there were no significant differences ( $P > 0.05$ ) observed among all treatment samples on the zero day of storage for control, rosemary essential oil-treated meat balls, and meat ball samples preserved using chitosan film incorporated with rosemary essential oil at different concentrations. The mean ± SE TBA value of each treatment sample was 0.063±0.03, 0.057±0.03, and

0.053±0.03 mg malonaldehyde/kg, respectively. During the third day of storage, the TBA value of the control group experienced a significant ( $P < 0.05$ ) increase, reaching a mean  $\pm$  SE value of 0.52±0.04. This pattern was also observed in meat ball samples that were preserved using chitosan film loaded with rosemary essential oil at varying concentrations (0.2, 0.4 and 0.6 %); these samples displayed significantly lower mean  $\pm$  SE (0.26±0.01, 0.16  $\pm$  0.01 and 0.12  $\pm$  0.01 respectively) than control. While the sample spoiled in case of control in the storage day 7, the TBA tends to increase again to 0.60±0.06 in case of rosemary essential oil treated meat balls and 0.62±0.03, 0.47  $\pm$  0.00.03 and 0.40±0.02 in case of samples preserved using chitosan film incorporated with rosemary essential oil with different concentrations (0.2, 0.4 and 0.6 %, respectively).

By the day 10, the TBA value of the rosemary essential oil treated meat balls increased to 0.87±0.03 mg malonaldehyde/kg which is very near to the permissible limit set by the Egyptian standards (0.90 mg malonaldehyde/kg in minced meat). On the other hand, the TBA value of samples preserved using chitosan film incorporated with rosemary essential oil with different concentrations increased to 0.73±0.02 mg malonaldehyde/kg that is still below the permissible limit.

Lipid oxidation and the production of volatile chemicals in the presence of oxygen are the reasons for the increase in TBA during storage. Because of the phenomenon of electrons traveling in the benzene ring and the lack of an oxygen attack site, the compounds in the extracts function as an adequate donor of electrons and protons, and their intermediate radicals are incredibly stable. Free radicals may be neutralized by the rosemary extract's constituents, which include carnosic acid, carnosol, rosmaronin, monin rosemary, and rosemary null. Additionally, they have the ability to block metal ions like  $Fe^{2+}$ , which slows down the rate at which activated oxygen molecules are created [34].

#### *Determination of Peroxide value*

The results of PV (Table 3) showed that by increasing time, the PV increased in control, rosemary essential oil treated meat balls and meat ball samples preserved using chitosan film loaded with rosemary essential oil with different concentrations. According to the results of statistical analysis, the peroxide value was nearly the same in all samples in the day 0 of storage. In day 3 of storage, the PV value of control sample increased to 0.94±0.07, while it becomes 0.40±0.05 (increased also but still lower than the control one) in case of the rosemary essential oil treated meat balls, and 0.25±0.02 (much lower) in case of samples preserved using chitosan film incorporated with rosemary essential oil with different concentrations. By day 7 and 10 of storage, the control sample was spoiled,

and the PV values tend to increase to 1.06±0.09 and 1.60±0.07 in case of the rosemary essential oil treated meat balls, respectively. On the other hand, the PV values increased also to 0.75±0.04 and 1.2±0.04 in case of samples preserved using chitosan film incorporated with 0.6 % rosemary essential oil. Also, it is clear from the results in table 3 that the using chitosan film incorporated with rosemary essential oil in concentration of 0.6% is much better than other concentrations. The antioxidant properties of rosemary extract are responsible for the reduced PV values in treatments that contain it. The capacity of phenolic compounds like rosmarinic acid and carnosic acid and carnosol to neutralize reactive oxygen species (ROS) and chelate metal ions is linked to the antioxidant activity of rosemary extract. Numerous investigations have revealed that the quantity of antioxidant components in natural extracts influences their antioxidant activity [35,36]. The permissible PV level for consumers is 5 mEq/kg of beef [34,37].

#### *Total Antioxidant Capacity*

Natural plant and oils have stronger antioxidant activity because of their phenolic components and have a high capacity for giving hydrogen, electrons, and free electrons [38]. Rosemary one of these herbals that has the antioxidant activity. Table 4 shows no differences in total antioxidant capacity (T-AOC) in storage day 0 for the control, rosemary essential oil treated meat balls and meat ball samples preserved using chitosan film incorporated with 0.6 % rosemary essential oil, it ranged from 4.23±0.04 to 4.35±0.03. But in storage day 3, it decreased to 3.72±0.08 in case of control sample, while it was 4.04±0.06 in case of rosemary essential oil treated meat balls and 4.21±0.02 in case of samples preserved using chitosan film loaded with rosemary essential oil with different concentrations.

In storage day 7 and 10, the control sample was spoiled, while the value of T-AOC decrease to 3.20±0.02 and 2.34±0.07 in case of rosemary essential oil treated meat balls, respectively. On the other hand, it becomes 3.22±0.05 and 2.17±0.06 in case of samples preserved using chitosan film incorporated with rosemary essential oil of concentrations 0.4 and 0.6 %, respectively. From the result also, it was clear that the using chitosan film incorporated with rosemary essential oil in concentration of 0.4% is much better than other concentrations which agreed with the results obtained by [34].

So, the use of these natural additives, herbal extracts and essential oils will be promising in improving meat quality and safety [39,40]

#### **Conclusion**

The results showed that the minced meat preserved by adding 1% rosemary EO and

membranes had lower pH values compared with control minced meat samples. And also, there was improvement in the antioxidant activity with reduction in TBA and PV value with extending the shelf life in chitosan membrane incorporated with rosemary EO especially at 0.6% concentration. So, the use of these active films incorporated with essential oils will be promising in improving meat quality and safety

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**TABLE 1. Influence of rosemary essential oil and rosemary incorporated chitosan membranes on pH values of meat balls stored at 4 °C.**

Storage time	Control	Rosemary EO 1%	Rosemary incorporated chitosan membranes		
			0.2 %	0.4 %	0.6 %
Zero time	5.71± 0.02 <sup>Aa</sup>	5.67± 0.008 <sup>Aa</sup>	5.65± 0.01 <sup>Aa</sup>	5.60± 0.009 <sup>Aa</sup>	5.59± 0.009 <sup>Aa</sup>
3 <sup>rd</sup> day	6.26± 0.04 <sup>Bb</sup>	5.81± 0.02 <sup>Aa</sup>	5.84± 0.03 <sup>Aa</sup>	5.77± 0.02 <sup>Aa</sup>	5.73± 0.02 <sup>Aa</sup>
7 <sup>th</sup> day	spoiled	6.19± 0.05 <sup>Bb</sup>	6.20± 0.05 <sup>Bb</sup>	5.99± 0.04 <sup>Ba</sup>	5.90± 0.04 <sup>Ba</sup>
10 <sup>th</sup> day	spoiled	6.36± 0.07 <sup>Cc</sup>	Spoiled	6.21± 0.04 <sup>Cb</sup>	6.02± 0.04 <sup>Ba</sup>

The data were expressed as mean ± SEM. The different capital letters show a significant difference in the same row, while the other small letter shows a considerable difference in the same column at  $p < 0.05$ .

**TABLE 2. Influence of rosemary essential oil and rosemary incorporated chitosan membranes on TBA values of meat balls stored at 4 °C.**

Storage time	Control	Rosemary EO 1%	Rosemary incorporated chitosan membranes		
			0.2 %	0.4 %	0.6 %
Zero time	0.063±0.03 <sup>Aa</sup>	0.057±0.03 <sup>Aa</sup>	0.063±0.03 <sup>Aa</sup>	0.057±0.03 <sup>Aa</sup>	0.053±0.03 <sup>Aa</sup>
3 <sup>rd</sup> day	0.52±0.04 <sup>Ba</sup>	0.22±0.02 <sup>Bb</sup>	0.26±0.01 <sup>Bb</sup>	0.16±0.01 <sup>Bc</sup>	0.12±0.01 <sup>Bc</sup>
7 <sup>th</sup> day	Spoiled	0.60±0.06 <sup>Ca</sup>	0.62±0.03 <sup>Ca</sup>	0.47±0.03 <sup>Cb</sup>	0.40±0.02 <sup>Cb</sup>
10 <sup>th</sup> day	Spoiled	0.87±0.03 <sup>Da</sup>	Spoiled	0.76±0.03 <sup>Cb</sup>	0.73±0.02 <sup>Cb</sup>

The data were expressed as mean ± SEM. The different capital letters show a significant difference in the same row, while the other small letter shows a considerable difference in the same column at  $p < 0.05$ .

**TABLE 3. Influence of rosemary essential oil and rosemary incorporated chitosan membranes on peroxide values (PV) of meat balls stored at 4 °C.**

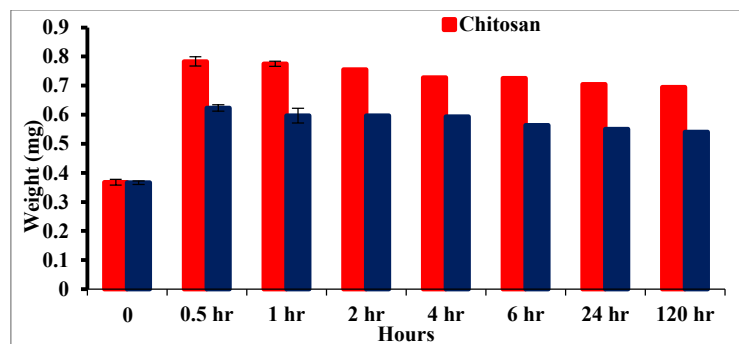
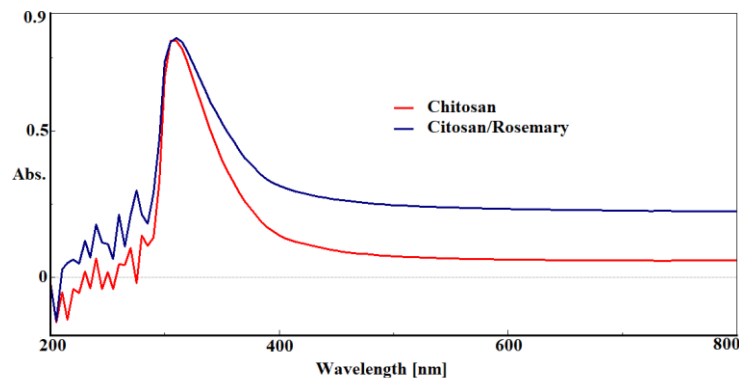
Storage time	Control	Rosemary EO 1%	Rosemary incorporated chitosan membranes		
			0.2 %	0.4 %	0.6 %
Zero time	0.12±0.01 <sup>Ab</sup>	0.11±0.01 <sup>Ad</sup>	0.12±0.01 <sup>Ac</sup>	0.11±0.01 <sup>Ad</sup>	0.10±0.01 <sup>Ad</sup>
3 <sup>rd</sup> day	0.94±0.07 <sup>Aa</sup>	0.40±0.05 <sup>Bc</sup>	0.45±0.03 <sup>Bb</sup>	0.31±0.02 <sup>Cc</sup>	0.25±0.02 <sup>Cc</sup>
7 <sup>th</sup> day	spoiled	1.06±0.09 <sup>Ab</sup>	1.10±0.04 <sup>Ba</sup>	0.85±0.05 <sup>Cb</sup>	0.75±0.04 <sup>Cb</sup>
10 <sup>th</sup> day	spoiled	1.60±0.07 <sup>Aa</sup>	spoiled	1.36±0.04 <sup>Ba</sup>	1.2±0.04 <sup>Ca</sup>

The data were expressed as mean ± SEM. The different capital letters show a significant difference in the same raw, while the other small letter shows a considerable difference in the same column at  $p < 0.05$ .

**TABLE 4. Influence of rosemary essential oil and rosemary incorporated chitosan membranes on the total antioxidant capacity (T-AOC) of meat balls stored at 4 °C.**

Storage time	Control	Rosemary EO 1%	Rosemary incorporated chitosan membranes		
			0.2 %	0.4 %	0.6 %
Zero time	4.23±0.04 <sup>A</sup>	4.35±0.03 <sup>A</sup>	4.34±0.03 <sup>A</sup>	4.24±0.03 <sup>A</sup>	4.29±0.03 <sup>A</sup>
3 <sup>rd</sup> day	3.72±0.08 <sup>B</sup>	4.04±0.06 <sup>A</sup>	3.99±0.01 <sup>A</sup>	4.15±0.03 <sup>A</sup>	4.21±0.02 <sup>A</sup>
7 <sup>th</sup> day	Spoiled	3.20±0.02 <sup>A</sup>	2.96±0.07 <sup>B</sup>	3.22±0.05 <sup>A</sup>	3.35±0.05 <sup>A</sup>
10 <sup>th</sup> day	Spoiled	2.34±0.07 <sup>A</sup>	spoiled	2.17±0.06 <sup>B</sup>	2.44±0.06 <sup>A</sup>

The data were expressed as mean ± SEM. The different capital letters show a significant difference in the same raw, while the other small letter shows a considerable difference in the same column at  $p < 0.05$ .

**Fig. 1. Hydrolytic degradation phenomena for the chitosan and chitosan/rosemary composite membranes.****Fig. 2. UV-Vis spectra of the chitosan and chitosan/rosemary composite membranes**



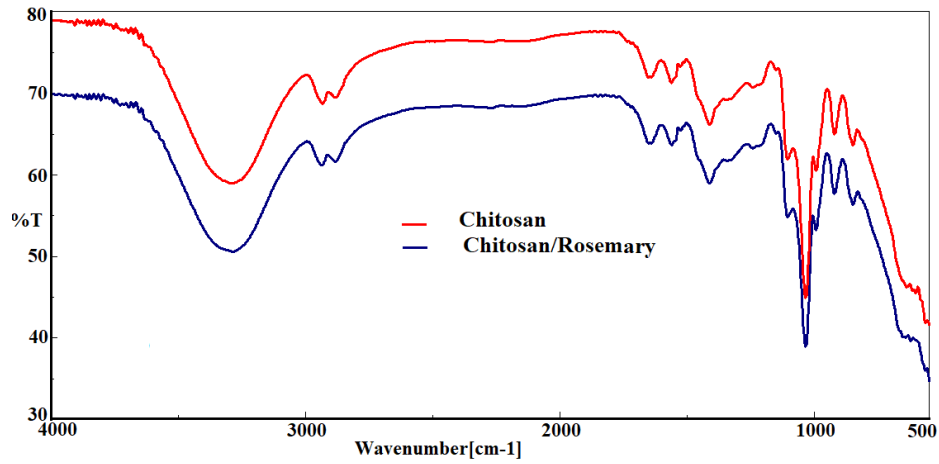


Fig. 3. FT-IR of the chitosan and chitosan/rosemary composite membranes.

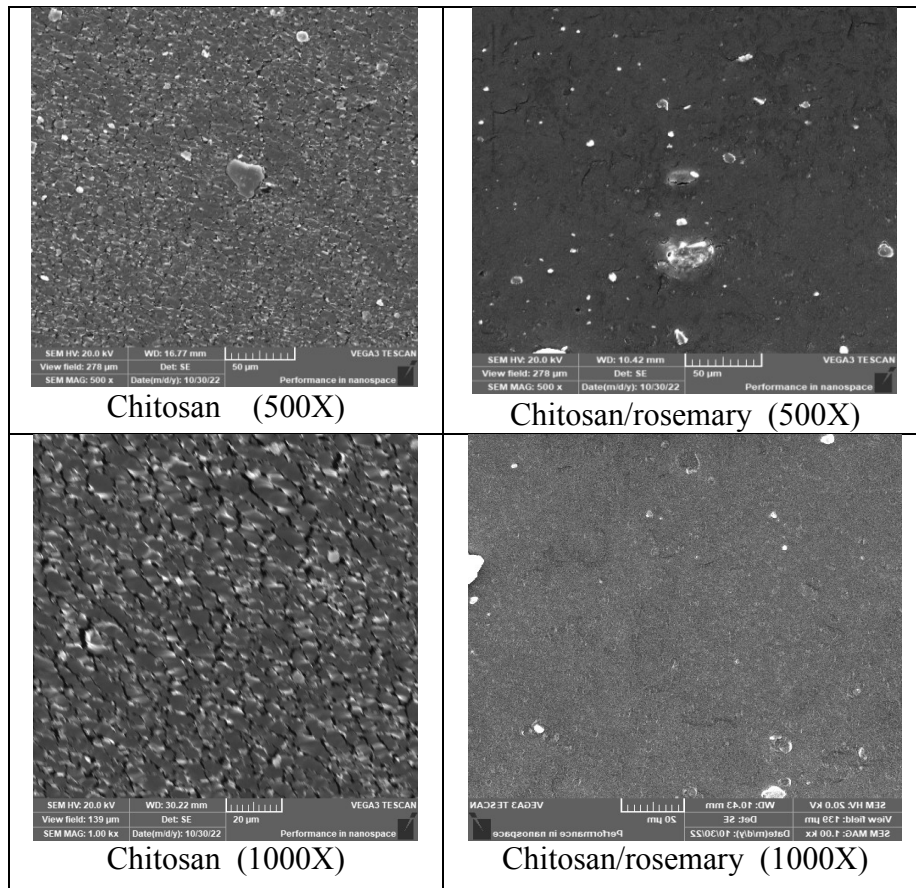


Fig. 4. SEM images of the chitosan and chitosan/rosemary composite membranes.



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## تأثير غشاء الشيتوزان المدمج بإكليل الجبل على الجودة الكيميائية وفترة الصلاحية لكرات اللحم المبردة عند 4 درجة مئوية

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### الخلاصة

هدفت الدراسة إلى تقييم الجودة الكيميائية ومدة الصلاحية للحوم المفرومة النيئة المحفوظة بمادة الشيتوزان المحملة بزيت إكليل الجبل العطري. تم دمج طبقة التحكم والشيتوزان مع زيت إكليل الجبل العطري بتركيزات مختلفة - تم تشكيل معالجات اللحم المفروم المحفوظ في كرات اللحم ليتم تخزينها في درجة حرارة 4 درجة مئوية لمدة 10 أيام. أظهرت النتائج وجود فرق معنوي في قيم الأس الهيدروجيني للمعاملات المختلفة، وكانت كرات اللحم المحفوظة بغشاء الشيتوزان الممزوجة بزيت إكليل الجبل العطري بتركيز مختلف أقل قيمة مقارنة مع المعاملة الضابطة وخاصة التركيز 0.6%، وسجل الأس الهيدروجيني 6.02 عند اليوم العاشر بينما كانت المعالجات المحفوظة باستخدام زيت إكليل الجبل العطري بنسبة 1% تحتوي على 6.36 درجة حموضة وفسدت عينات التحكم. أدى استخدام زيت إكليل الجبل العطري والطبقة المدمجة من الشيتوزان مع تركيزات مختلفة من إكليل الجبل إلى انخفاض كبير في قيمة المواد التفاعلية لحمض الثيوباربيتوريك (TBARS) وقيمة البيروكسيد (PV) مقارنة بمعاملة التحكم. كان لحفظ اللحم المفروم بواسطة الشيتوزان المحمل بزيت إكليل الجبل الأساسي تأثير كبير للغاية على إجمالي قدرة مضادات الأكسدة (TAC) مقارنة بعينات المعاملة الضابطة غير المحفوظة. يحتوي 0.6% من إكليل الجبل المدمج مع كرات اللحم المحفوظة بغشاء الشيتوزان على أعلى TAC عند اليوم العاشر (6.44). لذلك، فإن حفظ اللحم المفروم باستخدام الشيتوزان المحمل بزيت إكليل الجبل العطري أدى إلى تحسين الجودة الكيميائية وإطالة العمر الافتراضي للحوم المفرومة ويمكن استخدامه. يستخدم كمادة حافظة واعدة للحوم ومنتجاتها.

**الكلمات المفتاحية:** الجودة الكيميائية، فيلم الشيتوزان، زيت إكليل الجبل العطري، إدماج، مادة حافظة.