



Effects of edible chitosan coating containing *Salvia rosmarinus* essential oil on quality characteristics and shelf life extension of rabbit meat during chilled storage

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Abstract

Rabbit meat is one of the most consumed meats throughout the world and is extremely susceptible to spoilage due to its high protein and moisture content. Natural preservatives with antioxidant and antibacterial properties are needed to maintain meat quality and increase its shelf life. This study examined the effect of 1% chitosan (CH), 0.2% rosemary (*Salvia rosmarinus*) essential oil (REO), and their combination on pH, total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) levels, and the microbial profile of rabbit meat stored over 12 days at 4 °C. During this time, changes in appearance, odor, and texture were also noted. The shelf lives of samples treated with only 1% CH coating, or in combination with 0.2% REO, were longer than those of untreated samples. These results showed that samples treated with both CH and REO were still acceptable until the 12th day and resulted in significantly lower meat demerit scores in these treated groups compared with untreated meat. In all groups, the pH, TVB-N, and TBA values increased over time, but these values were significantly higher ($p < 0.05$) in untreated samples. Microbial analysis results showed that chilled rabbit meat samples treated with combined 1% CH and 0.2% REO reduced Enterobacteriaceae, *Pseudomonas*, and Psychrotrophic counts. The experimental results demonstrated that using CH coating in combination with REO improved the quality of rabbit meat and could be an effective approach to reduce rabbit meat deterioration during chilled storage.

Keywords Chilling · Chitosan coating · Quality characteristics · Rabbit meat · Rosemary

Introduction

Rabbit meat is a common food product around the world. Recently, its consumption has increased in numerous European and Middle Eastern countries, especially in Egypt [1–3]. It is a healthy food for human consumption since its meat has low levels of cholesterol, fat, and sodium; and it is rich in polyunsaturated fatty acids and protein. Moreover, it has essential micro and macronutrients, such as potassium, phosphorus, and selenium, in addition to vitamin B12 [4, 5].

However, the most significant challenge of meat is its quick decomposition, which compromises its quality and safety. Through numerous stages of slaughter and transportation, the meat of healthy rabbits may become contaminated with various bacteria, resulting in spoilage and food poisoning [6]. Numerous pathogens, including *Staphylococcus aureus* (*S. aureus*), *Salmonella* sp, and *Escherichia coli* (*E. coli*) showed high multi-antibiotics resistance indices (MAR) [7, 8]. Contamination occurs from the point of slaughter until the time of meat consumption. Additionally, various contamination sources throughout the slaughter chain should be considered. These include carcass contamination via animal skin, equipment, unhygienic measurements, and lack of personal hygiene [9, 10]. Growth of spoilage microorganisms in meat can accelerate the degradation of protein and increase fat rancidity, thus resulting in the loss of nutritional and negative impacts on organoleptic properties such as meat discoloration, development of off-flavor texture changes, off-odor, and reduced shelf life [11, 12].

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Consumers today are becoming more interested in a healthy lifestyle. Thus, there is a trend toward using natural antioxidant and antimicrobial agents as a substitute for chemical food additives as they are perceived to improve meat quality with little to no side effects [13–15]. In this regard, innovative preservation technologies and refrigeration methods, such as the use of high hydrostatic pressures [16, 17], irradiation [18, 19], and active packaging techniques [20] have been established to suppress microbial growth and preserve the ultimate quality of meat and meat products, as meat quality and safety are major concerns for both consumers and meat producers. Additionally, plant extracts and essential oils (EOs) demonstrated significant promise in a variety of food-related applications due to their antibacterial and antioxidant properties [21–24]. Rosemary essential oil (REO), in particular, has high antioxidant activity and is widely used in the food industry [25]. REO has also shown a greater capacity as an antimicrobial agent against foodborne pathogenic and spoilage bacteria in meat and meat products owing to the presence of component phenolic compounds [26, 27]. However, phenolic compounds are chemically reactive substances and extremely sensitive to environmental conditions such as temperature, pH, oxygen and light. Furthermore, there are certain drawbacks with directly incorporating EOs containing such phenolic compounds into complicated food matrix, including detrimental impacts on the physicochemical stability of food and the loss of biological action of components in EOs [28]. Therefore, a potential method is required to overcome these challenges, such as edible films and coating techniques that shield bioactive chemicals from undesirable reactions and restrict their probable reactivity with nutrients, resulting in improved food stability during storage and processing [29]. In this sense, the application of natural antimicrobial and/or antioxidant edible coatings has become one of the most promising agents for the efficient preservation of food [30].

Chitosan (CH) coatings have drawn much attention and have been evaluated for food industry applications since they are effective in releasing lipophilic bioactive compounds and have a synergistic effect with EOs because of their inherent antimicrobial properties [31]. Chitosan coating is commonly used as an antimicrobial agent either alone or in combination with other natural components due to its antimicrobial properties, high biodegradability, and nontoxicity [32–34]. Furthermore, active chitosan coating seems to be an efficient strategy to maintain meat quality and increase its shelf life by preventing bacterial growth on the meat surface. Although chitosan has been recently reviewed, in particular for its antimicrobial and antioxidant properties in meat systems, either alone or in combination with different essential oils, there are limited reports on the application of active food coating in certain types of meat and meat

products [35, 36]. In addition, there are no definitive data on the application of edible coatings using chitosan containing REO in rabbit meat. Thus, the objective of this study was to assess the antioxidant and antimicrobial activities of CH coating and rosemary (*Salvia rosmarinus*) essential oil (REO) on the quality and shelf life of rabbit meat during storage at 4 °C for 12 days.

Materials and methods

Sample preparation

Pure rosemary essential oil was obtained by expression at the National Research Center, Dokki, Giza, Egypt. Essential oil was dissolved in sterile distilled water with the required concentration of Tween 80 (emulsifying agent) to obtain a concentration of 0.2%. Low molecular weight chitosan from powdered crab shells was used. One gram of chitosan was dissolved in 100 ml of 1% (w/v) glacial acetic acid in order to prepare a concentration of 1% and was then stirred with a magnetic stirrer (WiseStir® MSH-20 A, DAIHAN Scientific Ltd., Korea) for 3 h at 55 °C [37].

A total of 6 kg of healthy domestic rabbit meat (60 loin pieces, 100 g each) were collected directly after slaughtering. The samples were immediately transported in an ice box to the Meat Hygiene Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt. The samples were divided into four groups, including an untreated group (control) and three treated groups. The control group consisted of rabbit meat dipped in sterilized distilled water for 15 min. Among the other three groups, samples were individually dipped for 15 min in 0.2% REO (Group I), 1% CH (Group II), and 1% CH combined with 0.2% REO (Group III). The excess solution was drained off immediately after dipping, and all groups were sampled (zero time). Finally, samples were individually packed, chilled at 4 °C and were analyzed every three days in order to determine the Enterobacteriaceae count, *Pseudomonas* count, Psychrotrophic count, pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), and sensory profile.

Sensory evaluation

The sensory attributes of untreated and treated rabbit meat samples during chilled storage, including changes in color, odor, fat appearance, and flesh texture were evaluated according to the sensory quality scheme developed by Rodriguez-Calleja et al. [38] with a slight modification. From the staff members of the Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Zagazig University, Egypt, seven experienced

panelists, 25 to 50 years of age, were asked to evaluate the acceptability (total sensory evaluation score) of rabbit meat as a composite of color, odor (Fresh/boiled), contamination, wetness, fat appearance, and flesh texture. Briefly, the quality parameters, characteristics, and demerit point scales are as follows: 2 points for color (color characteristic rabbit meat=0, opaque=1, yellowish=2), 2 points for fresh or boiled odor (normal=0, neutral=1, abnormal=2), 2 points for texture (firm=0, slightly soft=1, soft=2), 3 points for wetness (normal=0, slightly dry or slightly wet=1, moderately dry or moderately wet=2, excessively dry or excessively wet=3), 2 points for Contamination (None=0, Slight=1, Moderate=2), 2 points for Fat appearance (White=0, Opaque=1, Yellowish=2), and 2 points for Rancidity (Absent=0, Slight=1, Moderate=2). The sum of points for each sample was converted to a percentage of the maximum demerit points (Maximum demerit points for fresh rabbit carcasses=17) at the end of the evaluation and reported as the demerit score.

Chemical analyses

The determination of pH was performed according to Pearson [39]. Briefly, each sample (10 g) was blended in 10 ml of neutralized distilled water, and the homogenate was left at room temperature for 10 min with continuous shaking. The pH value was determined using an electrical pH meter (Bye model 6020, USA). Total volatile basic nitrogen (TVB-N) content was carried out according to AOAC procedures [40]. The TBA content was calculated according to Nasr et al. [41].

Bacteriological analyses

The preparation of the rabbit meat samples, and serial dilutions were performed according to ISO 6887-2 [42]. Briefly, 25 g of each type of rabbit meat sample were homogenized aseptically by mixing in 225 ml of 0.1% buffered peptone water (BPW, HiMedia EDIA, M614-500G, HiMedia Laboratories, India) in a stomacher (Stomacher 400, Seward Ltd. Co., Worthing, UK) for 2.5 min at room temperature and allowed to stand for 5 min to provide a homogenate which represented a dilution of 10^{-1} . One ml of the homogenate was transferred into a sterile test tube containing 9 ml of 0.1% BPW, and then ten serial dilutions were prepared to the required dilution (10^{-8}). Enumeration of Enterobacteriaceae was done according to ISO 21528-2 [43] on violet red bile glucose agar (HiMedia Laboratories, India). Enumeration of *Pseudomonas* was performed according to ISO 13,720 [44] using *Pseudomonas* agar base (CM 559, OXOID Ltd., Basingstoke, Hampshire, UK) supplemented with cetrимide, fucidin, and cephaloridine supplements (SR

103, OXOID Ltd., Basingstoke, Hampshire, UK) and incubated for 2 days at 25 °C. Then, the greenish yellow colonies were enumerated. However, total Psychrotrophic count was carried out using standard plate count agar (CM325, OXOID Ltd., Basingstoke, Hampshire, UK) then incubated at 5–7 °C for 7–10 days [45]. The bacterial counts were expressed as the logarithm of the colony forming units per gram (log CFU/g).

Statistical analysis

Statistical analysis of the obtained data was performed using the GLM procedure of the Statistical Package for the Social Sciences (SPSS) software Version 14 for Windows (SPSS Inc., Chicago, IL, USA). The model incorporates the fixed effect of the treatments ($i=1,0.3$), and the random effect of replicate and error. One-way analysis of variance (ANOVA) with a post hoc tukey-kramer honestly test was employed to investigate the statistical differences in the sensory, chemical, and bacteriological analysis findings in the control and treated samples. Data are presented as the mean $\pm$ SE, and significance was determined at a significance level ($P < 0.05$).

Results

Sensory evaluation

The sensory attributes of chilled rabbit meat samples were improved using chitosan and rosemary compared with the control samples. Generally, samples treated with 1% CH combined with 0.2% REO (Group III) demonstrated the highest enhancement of sensory properties. As shown in Table 1, the untreated group decomposed after the 6th day of chilled storage, while the treated groups with 0.2% rosemary (Group I) and 1% chitosan (Group II) were tolerated until the 9th and 12th days of chilled storage, respectively. Furthermore, the mean demerit scores of the treated rabbit meat were significantly lower ($p < 0.05$) than those observed in the control group. On the 12th day of storage, the samples treated with CH coating supplemented with REO (Group III) had the lowest mean score (7.00 ± 0.03) when compared to the other treated groups.

Chemical analyses

The changes in pH, TVB-N, and TBA of the various treatments during chilled storage are exhibited in Table 2. At zero time, the mean values for pH, TVB-N, and TBA in samples stored at 4 °C revealed no significant differences between the treated and control groups. However, significant

Table 1 Sensory evaluation of untreated and treated rabbit meat during storage at 4 °C

Storage period	Control group (Untreated samples)		Treated groups					
			G I		G II		G III	
	Mean ± SE	Percent-age *	Mean ± SE	Percent-age *	Mean ± SE	Percent-age *	Mean ± SE	Percent-age *
Zero time	1.67 ± 0.67 ^a	9.82%	3.67 ± 0.67 ^{ab}	21.59%	3.67 ± 0.67 ^{ab}	21.59%	4.33 ± 0.33 ^b	25.47%
3rd day	1.67 ± 0.67 ^a	9.82%	4.00 ± 0.58 ^b	23.53%	4.33 ± 0.33 ^b	25.47%	4.67 ± 0.33 ^b	27.47%
6th day	7.00 ± 0.61 ^a	41.18%	5.00 ± 0.58 ^b	29.41%	5.00 ± 0.23 ^b	29.41%	5.33 ± 0.33 ^b	31.35%
9th day	D	-	8.34 ± 1.86	49.06%	7.00 ± 0.31	41.18%	6.33 ± 0.33	37.24%
12th day	D	-	11.01 ± 2.73	64.76%	7.33 ± 0.33	43.12%	7.00 ± 0.03	41.18%

G I (Group I = 0.2% REO), **G II** (Group II = 1% CH), and **G III** (Group III = 1% CH combined with 0.2% REO).

S.E.: Standard error of mean.

D: Decomposed samples (not determined).

Means within the same row carrying different superscripts are significantly different ($p < 0.05$).

Maximum demerit points for fresh rabbit carcasses are 17 (Rodriguez-Calleja et al., 2004) [20].

* The sum of demerit points obtained divided by maximum demerit points (**Sample with a total sensory evaluation score greater than 8.5 (> 50%) were deemed unacceptable for consumption**).

Table 2 Changes in physicochemical properties of the examined rabbit meat during cold storage at 4 °C

Parameters	Storage period	Control samples	Treated groups					
			G I	R%	G II	R%	G III	R%
pH	Zero time	5.75 ± 0.04 ^a	5.67 ± 0.55 ^a	16.82%	5.62 ± 0.72 ^a	25.87%	5.60 ± 0.57 ^a	29.21%
	3rd day	6.26 ± 0.57 ^a	5.89 ± 0.57 ^{ab}	57.34%	5.79 ± 0.57 ^b	66.12%	5.72 ± 0.57 ^b	71.15%
	6th day	6.81 ± 0.05 ^a	6.29 ± 0.04 ^b	69.8%	6.16 ± 0.06 ^{bc}	77.61%	6.08 ± 0.06 ^c	81.38%
	9th day	7.18 ± 0.57 ^a	6.71 ± 0.04 ^b	66.12%	6.48 ± 0.09 ^b	80.04%	6.12 ± 0.57 ^b	91.29%
	12th day	7.48 ± 0.57 ^a	6.91 ± 0.57 ^b	73.08%	6.69 ± 0.57 ^b	83.78%	6.32 ± 0.03 ^c	93.08%
Total volatile nitrogen (TVN) mg/100 g	Zero time	3.18 ± 0.21 ^a	2.89 ± 0.57 ^a	48.71%	2.73 ± 0.51 ^a	64.52%	2.57 ± 0.07 ^a	75.45%
	3rd day	11.68 ± 0.57 ^a	6.04 ± 0.47 ^{ab}	99.9%	5.49 ± 0.47 ^b	99.9%	5.28 ± 0.57 ^b	99.9%
	6th day	22.19 ± 0.63 ^a	12.31 ± 0.62 ^b	99.9%	10.34 ± 0.26 ^c	99.9%	9.28 ± 0.31 ^c	99.9%
	9th day	28.25 ± 0.57 ^a	17.75 ± 0.29 ^b	99.9%	16.00 ± 0.21 ^b	99.9%	13.39 ± 0.35 ^c	99.9%
	12th day	37.20 ± 0.51 ^a	19.11 ± 0.57 ^b	99.9%	18.00 ± 0.23 ^b	99.9%	16.06 ± 0.57 ^c	99.9%
TBA mg malonaldehyde/kg	Zero time	0.07 ± 0.02	0.05 ± 0.00 ^a	4.5%	0.04 ± 0.01 ^a	6.67%	0.04 ± 0.01 ^a	6.67%
	3rd day	0.57 ± 0.01 ^a	0.32 ± 0.01 ^{ab}	43.77%	0.25 ± 0.01 ^b	52.14%	0.16 ± 0.01 ^b	61.09%
	6th day	0.96 ± 0.02 ^a	0.60 ± 0.03 ^b	56.35%	0.52 ± 0.03 ^b	63.69%	0.35 ± 0.02 ^c	75.45%
	9th day	1.15 ± 0.01 ^a	0.79 ± 0.01 ^b	56.35%	0.76 ± 0.09 ^b	59.26%	0.74 ± 0.03 ^b	61.09%
	12th day	1.36 ± 0.01 ^a	0.87 ± 0.02 ^b	67.64%	0.84 ± 0.01 ^b	69.8%	0.78 ± 0.01 ^b	73.70%

Different superscript letters in the same rows indicate significant differences ($p < 0.05$). D: Decomposed (not determined).

$$R : \text{Reduction \%} = \frac{(\text{Mean of control} - \text{Mean of treated sample})}{\text{Mean of control}} \times 100$$

variations ($p < 0.05$) occurred between the untreated and treated groups from the 3rd day until the end of the experiment. On the 12th day of storage, the mean values of all studied chemical parameters increased in both the untreated and treated groups, but the untreated group had the highest values. Furthermore, lower values for pH, TVB-N, and TBA were observed in the samples treated with 1% CH combined with 0.2% REO (Group III) throughout the cold storage period (4 °C).

Bacteriological assessment of untreated and treated samples

The results of the antimicrobial potential of all the treatments against various spoilage bacteria are presented in Table 3. The Enterobacteriaceae count gradually increased with increased storage duration. Significant differences ($p < 0.05$) in Enterobacteriaceae count were observed between the untreated group and treated groups from day zero until the end of storage. Concerning the *Pseudomonas* count, there were no significant differences observed between the untreated and treated groups at time zero, although significant differences ($p < 0.05$) were observed from the 3rd day of chilling period. *Pseudomonas* count

Table 3 Changes in bacterial counts ($\log_{10}$ CFU/g) of the examined rabbit meat during cold storage at 4 °C

Parameters	Storage period	Control samples	Treated groups					
			G I	R%	G II	R%	G III	R%
Enterobacteriaceae counts	Zero time	4.76 ± 0.21 ^a	4.27 ± 0.22 ^{ab}	67.64%	4.25 ± 0.23 ^{ab}	69.097%	4.09 ± 0.24 ^b	78.6%
	3rd day	5.30 ± 0.02 ^a	4.28 ± 0.16 ^b	90.45%	4.19 ± 0.21 ^b	92.24%	4.03 ± 0.16 ^b	94.63%
	6th day	5.84 ± 0.43 ^a	4.92 ± 0.39 ^{ab}	87.98%	4.56 ± 0.14 ^b	94.75%	4.41 ± 0.28 ^b	96.28%
	9th day	D	5.11 ± 0.09 ^a	-	5.10 ± 0.44 ^a	-	4.84 ± 0.29 ^a	-
	12th day	D	D	-	5.40 ± 0.29 ^a	-	5.22 ± 0.18 ^a	-
Pseudomonas counts	Zero time	6.65 ± 0.13 ^a	6.51 ± 0.18 ^a	27.55%	6.48 ± 0.16 ^a	32.39%	6.45 ± 0.21 ^a	36.9%
	3rd day	7.34 ± 0.17 ^a	6.03 ± 0.29 ^b	95.1%	5.90 ± 0.07 ^b	96.37%	5.54 ± 0.27 ^b	98.41%
	6th day	7.73 ± 0.02 ^a	6.93 ± 0.17 ^b	84.15%	6.54 ± 0.10 ^c	93.54%	6.33 ± 0.14 ^c	96.02%
	9th day	D	7.00 ± 0.12 ^a	-	6.70 ± 0.06 ^{ab}	-	6.52 ± 0.12 ^b	-
	12th day	D	D	-	7.26 ± 0.24 ^a	-	6.63 ± 0.15 ^a	-
Psychotropic counts	Zero time	6.67 ± 0.84 ^a	6.62 ± 0.23 ^a	10.78%	6.57 ± 0.24 ^a	20.57%	6.49 ± 0.23 ^a	33.93%
	3rd day	7.52 ± 0.15 ^a	5.77 ± 0.45 ^b	98.22%	5.71 ± 0.44 ^b	98.45%	4.91 ± 0.45 ^b	99.75%
	6th day	7.93 ± 0.05 ^a	7.02 ± 0.15 ^b	87.69%	6.77 ± 0.03 ^{ab}	93.08%	6.30 ± 0.25 ^c	97.66%
	9th day	D	7.33 ± 0.15 ^a	-	6.96 ± 0.09 ^{ab}	-	6.78 ± 0.15 ^b	-
	12th day	D	D	-	7.53 ± 0.18 ^a	-	6.84 ± 0.14 ^b	-

Different superscript letters in the same rows indicate significant differences ($p < 0.05$). D: Decomposed (not determined).

$$R : \text{Reduction \%} = \frac{(\text{Mean of control} - \text{Mean of treated sample})}{\text{Mean of control}} \times 100$$

gradually increased in all the treatments, but the untreated group had the highest values. Moreover, Psychrotrophic counts at time zero were slightly decreased in the treated groups when compared to the untreated samples. Additionally, the counts were increased in all treated groups, but these counts remained below the untreated group counts on the 6th day. The best reduction percentages in Enterobacteriaceae count (94.63% and 96.28%), *Pseudomonas* count (98.41% and 96.02%), and Psychrotrophic count (99.75% and 97.66%) were achieved in the samples treated with 1% CH coating combined with 0.2% REO (Group III) on the 3rd and 6th days, respectively.

Discussion

Sensory evaluation

Evaluation of rabbit freshness is the primary goal of this study along with potential consumer acceptance. This is represented by many factors, such as appearance, odor, and texture. Lipid oxidation is one of the most important processes affecting foodstuff products and leads to the development of off-flavors and rancid odors, which result in food rejection by consumers [46, 47]. The sensory characteristics of the untreated samples in this study were evaluated until days 6 and 12 in the treated samples based on the perceived loss of quality based on physicochemical parameters and the microbiological outcomes.

The physical quality of rabbit meat was determined on the basis of sensory grading (lower scores represented better quality), and this grading was based on evaluation of

significant features that could result in demerit points ranging from 0 to 3 [38]. Samples with a total sensory evaluation score greater than 8.5 (> 50%) were deemed unacceptable for consumption. In the current study, the total sensory scores among all the treatments were < 17 and therefore evaluated as acceptable for consumption, with the exception of the REO treated sample on day 12, which was deemed unacceptable. The significant difference ($p < 0.05$) observed in the sensorial qualities of the untreated and treated rabbit meat samples agreed with the findings of Rodriguez-Calleja et al. [38]. This significant variation during the chilling period could be attributed to the powerful antioxidant and antibacterial properties of CH coating and REO on rabbit meat [48–50].

Chemical analyses

Variations in pH values for all sample treatments were observed during chilled storage. This variation in pH could be a factor influencing the types of microorganisms growing on rabbit meat. The untreated group had the highest pH value, which may be attributed to the increase in populations of Gram-negative bacteria, such as Enterobacteriaceae and *Pseudomonas*, which through endogenous proteolytic activity cause ammonia formation and production of volatile basic components as products of protein decomposition, and subsequently increase pH values [51]. A significant difference ($p < 0.05$) between untreated and treated groups was observed with increased storage durations. The pH increase in the untreated sample was significantly higher than in the treated samples. On the last day of the experiment, the pH value of the CH-treated samples (Group I)

did not significantly differ from the REO treated samples (Group II), but was significantly ($p < 0.05$) higher than the pH value of samples treated with CH coating combined with REO (Group III). However, the mean pH value observed on day zero ranged from 5.75 ± 0.04 in the control group to 5.60 ± 0.57 in the samples treated with 1% CH coating combined with 0.2% REO (Group III). Nearly similar pH values of 5.77 were observed by Chabela et al. [52] in central Mexico City, and 5.81 in Hungary by Dal Bosco et al. [53]. While lower pH values of 5.53 were seen in Greece by Simitzis et al. [54], and 5.49 in Spain by Alagón et al. [55]. However, a higher pH value of 5.95 was observed in Slovakia by Mertin et al. [56], and a value of 5.99 was recorded by Króliczewska et al. [57] in Poland. The potency of hydrogen analysis indicated that CH coating and REO could maintain a lower pH among the treated groups compared with the untreated group. This supported the theory of the inhibitory effects of CH coating and REO on bacterial growth, which is the main cause of pH increases. Thus, the antibacterial efficacy of CH coating and REO might be the explanation for the lower pH found in the treated samples [49, 50, 58].

The TVB-N figure is commonly used as an indicator of the rate of spoilage and the shelf life of meat. The observed results revealed that the untreated samples had significantly ($p < 0.05$) higher levels of TVB-N throughout the storage period when compared to the CH and REO treated groups. On the 12th day, the highest (37.20 ± 0.51 mg/100 g) and the lowest (16.06 ± 0.57 mg/100 g) levels of TVB-N were recorded in the control sample and the combined 1% CH and 0.2% REO treatment, respectively. The significant increase in TVB-N in the control sample during the storage period could be attributed to the rapid growth of spoilage bacteria and proteolytic enzymes, which resulted in protein degradation and the formation of free amines, trimethylamine, dimethylamine, and ammonia [59]. However, the lowest levels of TVN in samples treated with CH and REO could be attributed to the antioxidant and antimicrobial properties of REO, especially its phenolic compounds and chitosan used as coatings. Furthermore, the untreated group exceeded the permissible limit (20 mg/100 g meat) of TVN in meat and meat products set by Egyptian standards [60]. In contrast, TVB-N levels in the CH and REO treated groups were within permissible limits, suggesting that CH and REO inhibit the microbial activity in chilled rabbit meat. These findings are consistent with Baker et al. [61], who discovered that the addition of rosemary with or without sodium lactate resulted in lower values of TVB-N when compared to control samples. Likewise, Mehrabi et al. [33] observed that the change in TVB-N values in CH-coated chicken fillets during chilled storage was lower than the untreated samples. This reduction in TVB-N value in CH and REO-treated samples has been attributed to the ability

of phenolic compounds to inhibit the growth of microorganisms, thereby preventing the oxidation of lipids and degradation of proteins.

Changes in the TBA index generally showed significantly increased levels with increased time ($p < 0.05$) in all study treatments. On day zero, the TBA level (0.07 ± 0.02 mg MDA/kg) in the untreated sample reached 1.36 ± 0.005 mg MDA/kg (the highest) after 12 days of chilled storage. Previously, similar TBA results of 0.09 mg MDA/kg were reported in Italy by Dal Bosco et al. [62], 0.07 mg MDA/kg in Hungary by Mattioli et al. [63], and 0.261 to 0.278 mg MDA/kg in Egypt by Nasr et al. [41]. Jouki and Khazaei [30] reported the highest amount of TBA (1.35 mg MDA/kg) in untreated nugget samples, which is consistent with our findings. However, higher values of 2.16 mg MDA/kg were reported in Canada by Koné et al. [64]. The significant increase in TBA in the control sample during the storage period could be attributed to the highest oxidation rate of lipids and the formation of hydroperoxides and oxidized unsaturated fatty acids that decompose rapidly to form secondary oxidation products such as aldehydes and ketones [65]. The lowest changes in TBA were observed in chitosan-coated samples containing 0.2% REO. In these samples, a TBA level of 0.04 mg MDA/kg on day zero reached 0.78 ± 0.009 mg MDA/kg after 12 days of storage. The TBA values of the current study were consistent with Georgantelis et al. [66], who mentioned that the addition of a combination of chitosan and rosemary provided the greatest efficacy in retarding lipid oxidation in beef burger. Additionally, Li et al. [67] observed that the TBA value of a chitosan and rosemary treated group was significantly lower than that of a control group. According to announced Egyptian standards [60], the maximum permissible amount of TBA in meat is 0.9 mg MDA/kg. Therefore, the control sample with a TBA value of 0.96 ± 0.02 mg MDA/kg could not be consumed on the 6th day of storage, whereas all the coated treatments were within the permissible limit in terms of TBA levels until the end of the 6th day. However, on the 9th and 12th days of storage, the TBA value in the control group dramatically increased, exceeding the allowable limit, whereas the corresponding TBA values in the CH and REO treated samples remained within the recommended limits. Consistent with our findings, Fattahian et al. [36] reported a significant reduction in TBA in veal treated with CH containing *Cuminum cyminum* essential oil. Moreover, Mehrabi et al. [33] investigated the effects of CH coating (2%) combined with *Nepeta pogonosperma* extract (0.2 and 0.6%) on chicken fillet shelf life and discovered that it could significantly reduce the oxidation process of meat. Additionally, Sayadi et al. [68] found a reduction in TBA levels in turkey meat treated with CH coatings containing barberry extract and *Mentha pulegium* essential

oils. Abdollahi et al. [69] also detected that a combination of CH coating and REO effectively retarded lipid oxidation in silver carp fish (*Hypophthalmichthys molitrix*) fillets by decreasing peroxide, free fatty acid, and TBA production. Similarly, Jouki et al. [29] found that edible quince seed mucilage films containing natural preservatives were effective in retarding the production of lipid oxidation products in rainbow trout fillets during cold storage. The protective effect of the applied coatings can be attributed to either the effective oxygen barrier or the chelator ability of the chitosan [70], and/or to the REO through the ability of its phenolic compounds to donate electrons and stabilize free radicals acting as antioxidants [71, 72]. Therefore, CH coating along with REO can be considered as an excellent additive for food bio-preservatives and coatings capable of maintaining food safety and freshness [73]. Such coatings can be used to extend the shelf life of rabbit meat by retarding oxidative rancidity and microbial growth [32, 74].

Bacteriological assessment of untreated and treated samples

Changes in Enterobacteriaceae, *Pseudomonas*, and Psychrotrophic bacteria counts of untreated and treated rabbit meat are presented in Table 3. The meat presented initial Enterobacteriaceae values of 4.76, 4.27, 4.25, and 4.09 log CFU/g for the control group, Group I (0.2% REO), Group II (1% CH), and Group III (1% CH combined with 0.2% REO), respectively. Over time, Enterobacteriaceae counts increased for all treatments ($p < 0.05$), reaching a final count of 5.84 log CFU/g for untreated rabbit meat samples and a reduction varying between (87.98%) 0.92, (94.75%) 1.28, and (96.28%) 1.43 log for the samples treated with 0.2% REO, 1% CH, and their combination, respectively. Similar results were observed in fresh pork sausage samples coated with chitosan incorporated with rosemary essential oil [75]. Moreover, these results were in accordance with Eldaly et al. [32] and Petrou et al. [76], who recorded that Enterobacteriaceae counts decreased in chicken meat samples coated with chitosan. Additionally, Giatrakou et al. [77] found that the addition of chitosan to a ready to cook poultry product resulted in low Enterobacteriaceae counts. Although there were differences in nominal value between the samples treated with rosemary and those treated with chitosan, the samples treated with chitosan without rosemary showed higher values than the mixed group with the oil added. Furthermore, all treated groups were significantly lower ($p < 0.05$) than the control group due to the influence of rosemary and chitosan. The combination of rosemary and chitosan provided good activity against Enterobacteriaceae isolated from food samples. Chitosan has a stronger antibacterial activity against Gram-negative bacteria owing to

their high hydrophilicity and negative charge on the surface of their cell walls, where the antimicrobial action of chitosan can be mediated by the interactions between the positively charged chitosan and negatively charged microbial cell membranes, which induce the leakage of cellular proteins and other intracellular constituents [78]. Rosemary has antimicrobial activity against Gram-positive and Gram-negative bacteria owing to the presence of phenolic compounds, carnosol, and carnosic acid [79].

Pseudomonas counts increased with elapsed time, and higher counts belonged to the control group. There were significant variances ($p < 0.05$) in treated groups, especially in the samples treated with rosemary combined with chitosan (Group III), with reduction percentages of 98.41% and 96.02%, respectively, in the 3rd and 6th days of chilled storage. The effectiveness of chitosan against *Pseudomonas* species was described by Darmadji and Izumimoto [80] who reported significantly lower *Pseudomonas* counts in fresh minced beef containing 1% chitosan. The counts of *Pseudomonas* species nearly remained stable throughout a storage period in fresh pork sausage samples containing chitosan and rosemary [75]. Petrou et al. [76] mentioned that *Pseudomonas* species populations were significantly lower in chicken samples treated with chitosan compared to a control group. Also, Giatrakou et al. [77] showed that the addition of chitosan produced low *Pseudomonas* counts in a ready to cook poultry product. Chitosan inhibits microbial growth by chelating nutrients and essential metals, penetrating microorganism nuclei, and interfering with protein synthesis by interacting with DNA. Furthermore, chitosan coatings act as oxygen barriers and can inhibit the growth of aerobic bacteria [81].

Concerning Psychrotrophic bacteria counts, the results were in line with those reported for Enterobacteriaceae and for *Pseudomonas* (Table 3). Untreated rabbit meat presented higher counts, followed by samples treated with CH and REO. The comparatively higher Psychrotrophic values of untreated samples can result from inadequate hygienic measures adopted during slaughtering and evisceration. Samples treated with the CH coating containing REO showed lower counts. Again, it was observed that the presence of REO enhanced the antimicrobial activity of the chitosan film (with statistical significance, $p < 0.05$) with reduction percentages of 99.75% and 97.66%, respectively, during the 3rd and 6th days of storage. Nearly similar findings were obtained by Djenane et al. [25], who reported a significant reduction of Psychrotrophic bacteria, and a shelf life extension of 10 days compared to controls in beef steak treated with rosemary. While Georgantelis et al. [75] demonstrated that the lowest microbial counts were those seen in fresh traditional Greek-style pork sausage samples containing both rosemary and chitosan, indicating a synergistic effect, despite

the fact that the antibacterial effect of rosemary was inferior to that of chitosan. Additionally, Karpińska-Tymoszczyk [82] observed that the addition of ground rosemary to turkey meatballs had a beneficial effect on suppressing of the growth of Psychrotrophic bacteria. Furthermore, the significant ($p < 0.05$) reduction of Psychrotrophic bacteria in chitosan-treated samples may be attributed to the coating film of chitosan that acted as an oxygen barrier around the bacterial cell and consequently prevented bacterial growth [83]. Additionally, Barbosa et al. [84] found that rosemary exhibits antibacterial activity against Psychrotrophic bacteria in vitro. Other studies have similarly demonstrated the inhibitory impact of rosemary and chitosan [34, 67, 85–87]. Concurrently, chitosan can be beneficial in the food industry by improving food quality and extending shelf life [88, 89].

Due to the unique composition of meat, particularly rabbit meat with a higher ultimate pH, it comprises an excellent substrate for the development of spoilage bacteria [90]. Therefore, bacteriological testing as well as chemical indicators are critical for determining the quality and shelf life of rabbit meat. In the present study, rabbit meat showed a relatively high ultimate pH, with values exceeding 6.0. According to the Egyptian guidelines [60], 10^7 CFU/g is the maximum acceptable limit for raw meats, which corresponds to a value of 7 log CFU/g. Furthermore, the ICMSF (International Commission for Microbial Specifications in Food) recommends 10^6 – 10^7 CFU/g for fresh meat [91]. However, 5×10^6 (6.7 log CFU/g) was established by the European legislation as the maximum acceptable limit for raw meats [92]. By the third day of storage with objective signs of spoilage, the psychrotrophic count of untreated rabbit meat samples had reached 7.52 log CFU/g (above the acceptable limit of 7 log CFU/g). According to the results, the bacterial load in control samples exceeded the acceptable limit of meat spoilage (10^6 CFU/g) reported by Barbin et al. [93] in porcine meat during refrigerated storage. Moreover, the *Pseudomonas* counts displayed similar changes and increased significantly to 7.34 log CFU/g on the 3rd day of chilled storage. On day 4, no further analyses were performed as the counts eventually increased in the untreated samples and exceeded the permissible standard limits [60, 91, 92]. In comparison to previous reports, the findings of the current study were similar to those reported by Bobbitt [94], who evaluated the shelf life of rabbit carcasses stored at 4 °C and estimated a shelf life of only 3 days for boxed rabbit carcasses, but lower than those reported by Rodríguez-Calleja et al. [90], who reported a shelf life of 6–7 days under aerobic refrigeration. Unlike control samples, the treatments (REO, CH, and CH+REO) and storage time had significant effects on bacterial count ($P < 0.05$), whereas the psychrotrophic count of samples coated with CH+REO was less than the mentioned standard limit on

the 12th day of storage. In all treated groups, a similar antimicrobial effect was observed against the *pseudomonas*. *Pseudomonas* count was significantly lower than the control ($P < 0.05$). Additionally, the lowest Enterobacteriaceae counts belonged to the treated groups compared to the untreated ones at the end of storage time (Table 3). The long shelf life of rabbit meat samples treated with chitosan and REO can be attributed to the antimicrobial and antioxidant properties of the edible CH coatings and the content of phenolic compounds in REO. Thus, the results of the present study confirm the synergistic effect of chitosan coating with REO on inhibiting bacterial growth.

Conclusion

The shelf life of fresh rabbit meat is highly dependent on many intrinsic and extrinsic factors, such as the storage temperature, pH, microbial contamination by pathogens, lipid oxidation, and color changes. If these factors are controlled; the shelf life can be extended. Due to rapid microbial growth, the untreated samples spoiled and had a slimy appearance and off-odor after 6 days of storage. According to the findings of this study, CH coating and REO enhanced the shelf life of rabbit meat, which was supported by the results of the sensory, chemical, and bacteriological evaluations. The results showed that the CH coating, either alone or in combination with REO, had the best results in controlling microbial growth and effectively delaying the oxidative deterioration of fresh rabbit meat. The results indicate that the treated samples, particularly those treated with CH coating combined with REO (CH+REO), had an acceptable sensory score and the lowest bacterial counts throughout the storage period due to their antioxidant and antimicrobial properties that helped in preserving the quality characteristics and sensory attributes of rabbit meat stored under chilled conditions at 4 °C for 12 days. Thus, the combination of CH coating with REO might have valuable economic potential worldwide for enhancing rabbit meat preservation and shelf life without the use of harmful synthetic chemical additives, ensuring food safety while reducing harmful environmental consequences. However, more research is needed to discover all the properties of CH coating and REO employed for different types of meat.

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Declarations

Conflict of interest None of the authors have any conflicts of interest to declare.

Ethical approval Our research did not contain any animal experiments or human subjects.

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