

Research Article

The Effect of Chitosan and Rosemary Essential Oil on the Quality Characteristics of Chicken Burgers during Storage

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In this study, the effects of chitosan film (1 and 2%) containing rosemary essential oil (1%) in free and nanoliposome forms were investigated on the physicochemical, microbial, organoleptic, and oxidation characteristics of chicken burgers during storage (day 1, day 10, and day 20) at 4°C. The results showed that chitosan and rosemary essential oil decreased pH, total volatile basic nitrogen compounds (TVB-N), and oxidation parameters (peroxide value and thiobarbituric acid) in the chicken burgers ($p < 0.05$). Compared to the control group (C), the growth of mesophilic and psychrophilic bacteria, Enterobacteriaceae, Pseudomonas, *Staphylococcus aureus*, and mold and yeast in all treated groups (E1, E2, CH1, CH2, CHE1, CHE2, CHE3, and CHE4) was delayed during storage. The color and hardness of the chicken burger were affected significantly ($p < 0.05$). Regarding sensory characteristics, the control group obtained the highest score. The CHE4 group (2% chitosan+rosemary essential oil nanoliposomes) was the most effective treatment for controlling the microbial flora, slowing down some physicochemical and sensory changes, and increasing the shelf life of chicken burgers ($p < 0.05$). As a result, chitosan coating with rosemary essential oil nanoliposome can guarantee microbiological safety and is recommended for long-term storage of chicken burgers during cold storage.

1. Introduction

Meat and its products are sources of protein, energy, B vitamins, minerals, and amino acids. They are very sensitive to microbial spoilage due to their nutrients and chemical (high water activity and natural pH) and physical characteristics. It is possible to grow different microorganisms, especially psychrotroph and pathogenic microorganisms [1]. Chicken meat and chicken burgers and industrial production of burgers and other ready-to-eat foods are important.

Due to the increase in food-borne diseases caused by various pathogenic microorganisms, new methods for meat preservation have been considered. Films and edible coating containing antimicrobial and antioxidant agents can increase the shelf life of meat products. Edible coating slows the release of antimicrobial and antioxidant agents into the meat [2]. The polysaccharide, protein, and fat origin coatings act as a barrier against the transfer of moisture, gases, and

soluble substances and therefore can increase the shelf life of meat products [3].

Chitosan ($C_6H_{11}NO_4$) is a natural polysaccharide that has been found in the exoskeleton of crustaceans, fungal cell walls, and other biological materials [4, 5]. It has antibacterial and antifungal properties that are suitable for food preservation and increases the quality and stability of the physical characteristics of the food by creating a semipermeable barrier to water vapor, oxygen, and carbon dioxide and increases the shelf life of the product [6].

Essential oils and extracts that are incorporated into the edible coating and films have antimicrobial and antioxidant activity. It has been claimed that essential oils are safe secondary metabolites (GRAS) and are recognized as alternatives to synthetic additives which can control pathogenic and spoilage bacteria and increase the shelf life of food [7].

Rosemary (*Rosmarinus officinalis* L.) which belongs to *Lamiaceae* family is native to South Asia and Europe. Several

studies have been conducted to evaluate the antimicrobial and antioxidant activity of rosemary essential oil and increase the shelf life of food [8–10]. Some studies on the use of chitosan [1, 11–14] and rosemary [15–17] in meat products were reported previously.

One of the most important challenges in using essential oils in food is improving and increasing their stability in processing and controlling their release. Therefore, it is better before using them in food and beverages in order to limit the aroma during processing and storage; they must be encapsulated [11]. During this process, very small particles or droplets are coated. The surrounding compound is called the core material, active compound, internal phase, and secondary compound; the material that covers the wall is known as the wall matrix, external phase, and membrane [18].

Due to the release of compounds with different hydrophilic properties, nanoliposomes can be a suitable system for the microencapsulation of fat-soluble compounds such as essential oils [19]. Liposomes, which usually have sizes between 3 and 6 nm, can create particles with a larger diameter by joining each other. Features such as low toxicity and biodegradability have caused liposomes to be considered as a very suitable carrier in modern systems [20].

The purpose of this study was to study the effect of chitosan and nanoliposomes of rosemary essential oil on the qualitative properties of chicken burgers at 4°C during 20 days.

2. Material and Methods

2.1. Materials. Freshly chicken meat was prepared from Sejzi slaughterhouse in Isfahan province, Iran. Rosemary (*R. officinalis* L.) was obtained from botanical herbarium (Isfahan, Iran). Chitosan with an acetylation degree of 75–80% and a molecular weight of 500,000 Da was purchased from Sigma-Aldrich Company (UK, Iran). All chemicals and microbial culture media were obtained from Merck (Germany).

2.2. Chitosan Solution Preparation. Briefly, 10 g of chitosan was mixed with 500 ml of acetic acid, and the solution was stirred with a magnetic stirrer for two days [12].

2.3. Essential Oil Extraction. The essential oil of rosemary was extracted by a Clevenger (Ashk-e-Shisheh, Iran) for 3–4 h. The collected essential oil was dehydrated by sodium sulfate and kept in the dark in the refrigerator (4°C) until the experiments [21].

2.4. Essential Oil Nanoliposome Preparation. In the thin layer coating method, 77.126 mg of soy phosphatidylcholine, 16.254 mg of cholesterol, 1.365 g of polyethylene glycol, and 7 ml of rosemary essential oil were mixed in 15 ml of chloroform and then deposited as a thin solid layer in a rotary evaporator at 40°C. Phosphate buffered saline solution (PBS) was used to hydrate the dried layer. The liposome particles were broken using an ultrasonic bath (Model 300, Pulse, Italy) and were passed through 0.45 and 0.25 µm filters, respectively. In order to separate the free essential oil, the solution was placed in a dialysis bag, in PBS at 4°C for 2 h [22].

2.5. Chicken Burger Preparation. For burger preparation, the chicken fillet was minced by a meat grinder. Then, all the ingredients (onion, 8% breadcrumbs, 2% spices, and 5% liquid oil) were added to the meat in a blender. After cutting burger pieces to 100 g (1 × 8 cm), the samples were placed between two films and were divided into 9 groups. The first group was the control (C) as without chitosan and rosemary essential oil. The second and third groups were wrapped in chitosan with a concentration of 1% and 2% (CH1 and CH2). The fourth and fifth groups were wrapped in 1% essential oil in free and nanoliposome forms (E1 and E2). The sixth and seventh groups were wrapped in 1% chitosan film containing 1% essential oil in free and nanoliposome forms (CHE1 and CHE2). The eighth and ninth groups were wrapped in 2% chitosan film containing 1% essential oil in free and nanoliposome forms (CHE3 and CHE4). The samples were stored in a refrigerator (4 ± 2.2°C) and tested for physicochemical, microbial, and sensory changes for 20 days (day 0, day1, day 10, and day 20).

2.6. Chemical Analysis. Briefly, 5 g of sample was homogenized with 45 ml of deionized distilled water for 1 min at 100 rpm at room temperature, and pH was measured with a pH meter (Metrohm, Switzerland). Total volatile nitrogen compounds (TVB-N) were determined according to the procedures previously characterized [2, 12, 23].

2.7. Oxidation Analysis. The peroxide value (PV) and thiobarbituric acid (TBARS) were determined according to the AOCS Cd 8-53 and Cd19-90 methods, respectively [24–26].

2.8. Color Analysis. For *L** evaluation, HunterLab (FMS Janzen GmbH & Co. KG, USA) was used at room temperature [27].

2.9. Microbial Analysis. 10 g of the chicken burger was homogenized with 90 ml of sterile normal saline, and after the preparation of serial dilutions, the pour-plate method was used for counting total mesophilic and psychophilic bacteria in plate count agar (PCA) and was incubated at 35°C for 72 hours and 7°C for 10 days, respectively. The LAB was enumerated in MRS agar and incubated at 35°C for 72 h. For the Enterobacteriaceae count, violet red bile dextrose agar (VRBA) was used after incubation at 35°C for 24 h. To count *Staphylococcus aureus*, the Baird-Parker Agar (BPA) was used after incubation at 35°C for 48 h. For counting mold and yeast, rose Bengal chloramphenicol agar was used after incubation at 25°C for 3–5 days. The colonies were reported as log CFU/g chicken burgers [28].

2.10. Sensory Evaluation. Sensory characteristics such as taste, aroma, color, texture, smell, and overall acceptance were evaluated based on a 5-point hedonic scale using 10 panelists on day 10 [23].

2.11. Statistical Analysis. The microbial and physicochemical parameters (parametric data) were analyzed using one-way variance and the Duncan test using SPSS software (version 20). The Kruskal-Wallis test was used to analyze

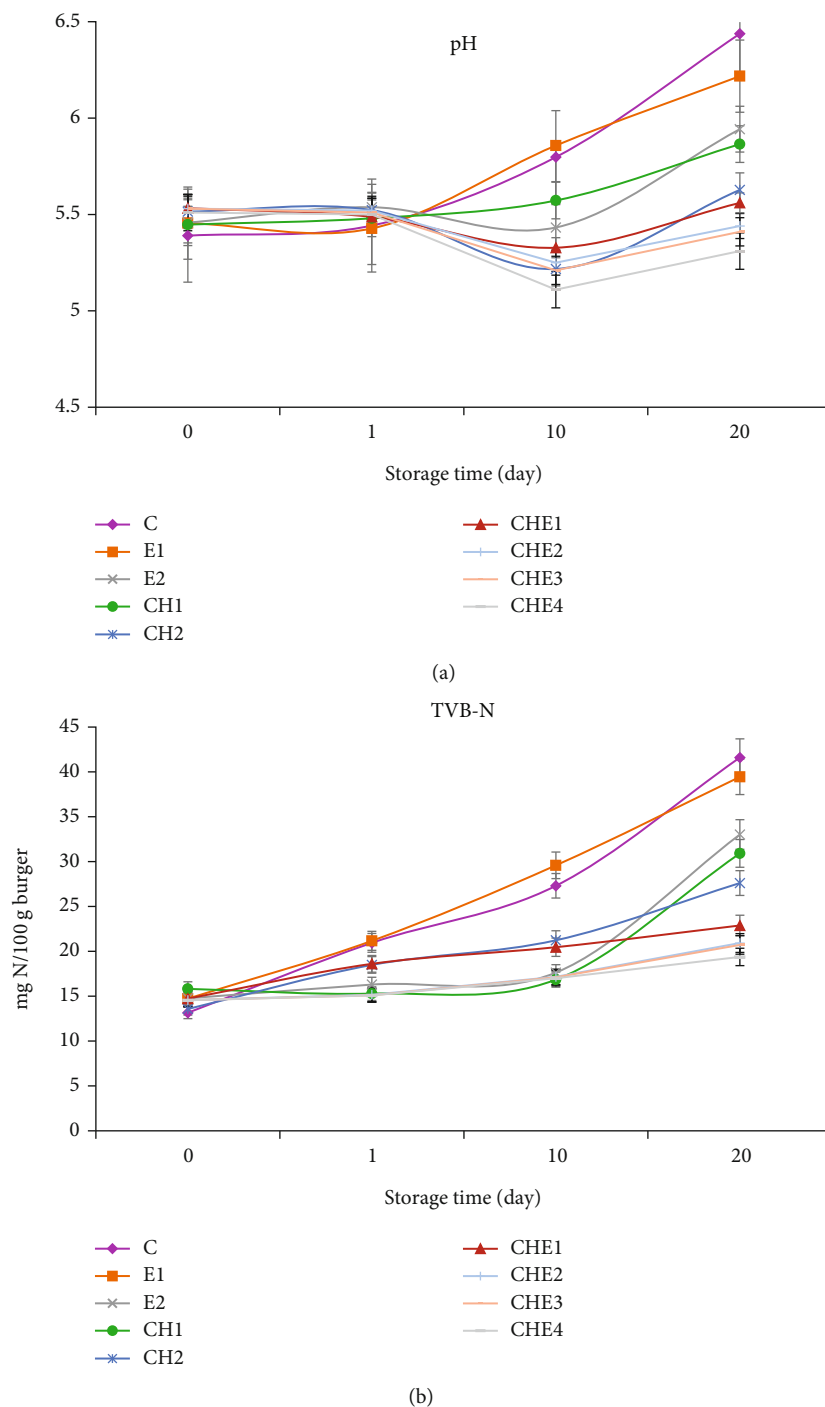


FIGURE 1: Chemical characteristics of chicken burgers during 20 days.

nonparametric data (sensory parameters). The analyses were conducted in triplicate.

3. Results and Discussion

3.1. Chemical Characteristics. At days 0 and 4, pH value was not significantly different in all groups ($p > 0.05$) (Figure 1(a)). pH value increased significantly during storage ($p < 0.05$). In the CHE1, CHE2, CHE3, and CHE4 groups, the changes in pH were not significantly different during

storage ($p > 0.05$). pH value of the control group was significantly higher than the treated groups at the end of storage ($p < 0.05$). pH increasing in different groups can be related to the activity of microorganisms and the accumulation of volatile bases [2].

At day 0, TVB-N was not significantly different ($p > 0.05$) in different groups (Figure 1(b)). The initial content of TVB-N in the studied groups was from 13.58 to 15.82 mg N/100 g burger, which indicates the high quality of the burger. TVB-N increased significantly during storage ($p < 0.05$),

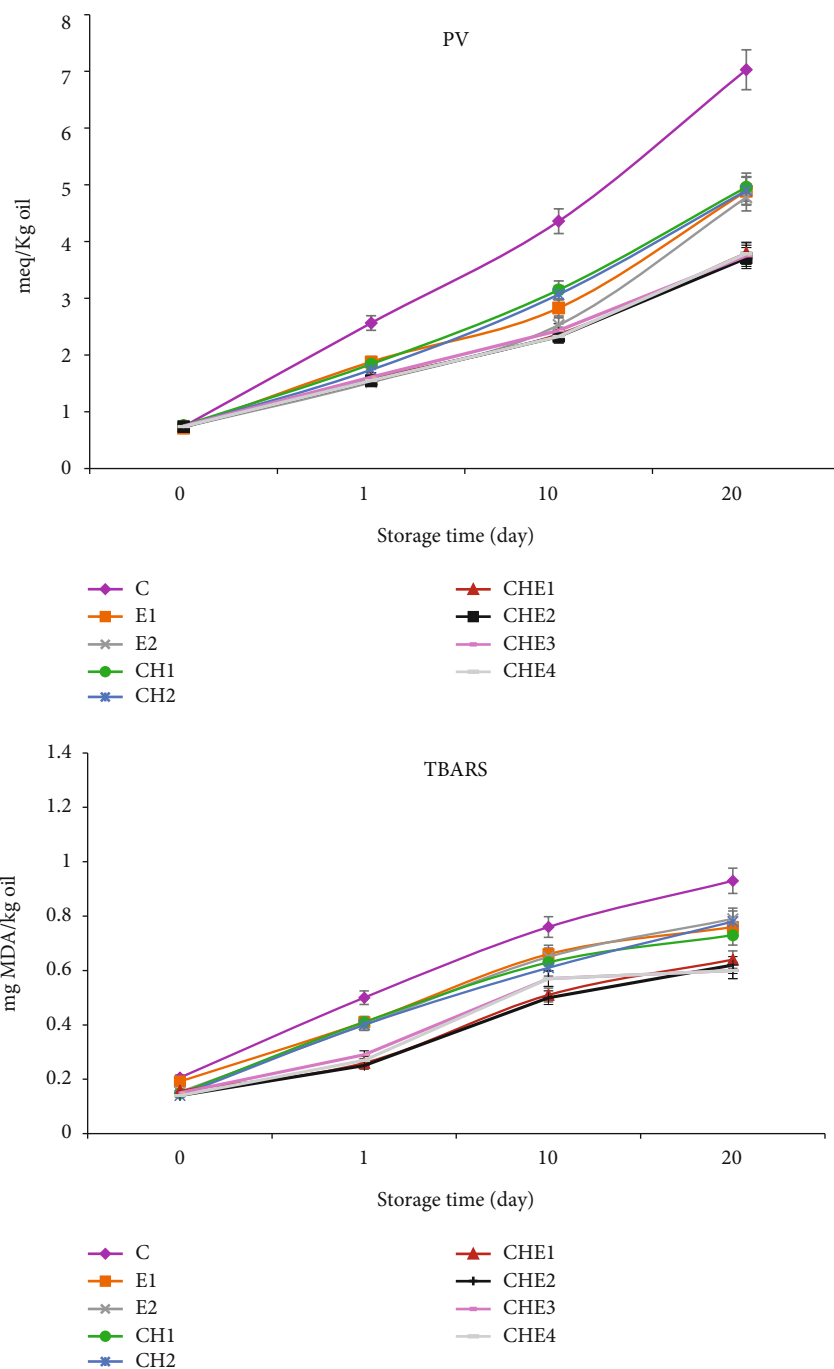


FIGURE 2: Oxidation stability of chicken burgers during 20 days.

but this increase was slower in the treatment groups than the control group.

The CHE3 and CHE4 groups were the most effective treatments for controlling pH and TVB-N changes in burger samples. At day 10, TVB-N in the control sample reached 92.30 mg N/100 g burger, which was higher than the standard limit (25 mg N/100 g burger). In all treated groups with chitosan and rosemary essential oil combination, TVB-N content did not exceed the limit during 20 days of storage [29]. Proteolytic enzymes of microorganisms affect protein

and nonprotein nitrogen compounds of meat and produce volatile nitrogen compounds [30]. Due to the inhibition of microbial activity, the TVB-N content in the treated groups was lower than the control group [2, 3, 28].

Sarmast et al. [30] reported that chitosan-gelatin coating containing lemon essential oil reduced the rate of pH increase in the salmon fillets during storage, thereby reducing microbial growth. Shankar et al. [31] showed that coating of fish fillets with alginate containing essential oils and citrus extracts reduced pH during storage. Abdeldae et al.

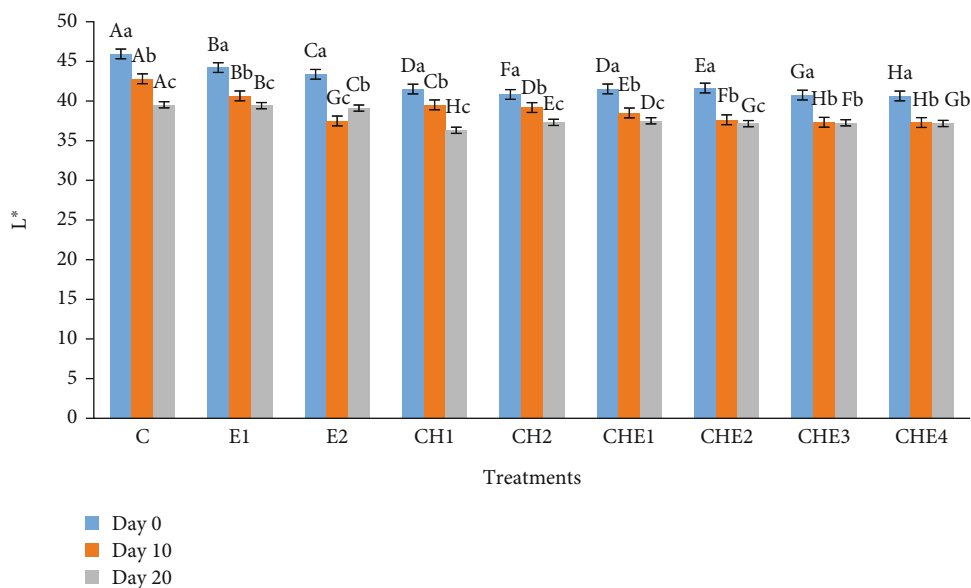


FIGURE 3: L^* analysis of chicken burgers during 20 days. The mean \pm SD (standard deviation) with different letters differs significantly ($p < 0.05$).

[32] found that calcium caseinate film containing rosemary essential oil reduces TVB-N in carp fillets during cold storage.

3.2. Oxidation Characteristics. As seen in Figure 2, at the beginning of the experiments (day 0), the peroxide value (PV) and TBARS do not show a significant difference ($p > 0.05$) but in all chicken burgers increased significantly during storage ($p < 0.05$).

PV represents the first oxidation products, and TBARS represents the secondary oxidation products. At the end of storage, PV and TBARS in the control sample were significantly higher than other treatments ($p < 0.05$) and reached to 7.03 meq/kg oil and 0.93 mg MDA/kg oil, respectively.

The results indicate that coating with chitosan and rosemary essential oil (free/nanoliposome) has been able to reduce oxidation in chicken burgers. Rosemary essential oil contains phenolic compounds and flavonoids that have strong antioxidant activity [15, 16]. The antioxidant activity of rosemary has been proven in many studies [17, 33, 34]. Also, chitosan creates a barrier that covers the surface of the chicken burger and therefore removes oxygen from it and reduces the oxidation of lipids in the chicken burger [12].

Hassanzadeh et al. [35] reported a decrease in TBARS levels in chitosan-coated chicken breasts containing grape seed extract. They showed a delay in increasing total volatile nitrogen and lipid oxidation in coated lamb.

3.3. L^* Analysis. Figure 3 shows the brightness of chicken burgers during 20 days. The control sample had the highest L^* (lightness). The brightness of all samples decreased significantly with time ($p < 0.05$).

Meat pigment is deoxymyoglobin (purple-red color). In the presence of O_2 , oxymyoglobin (MbO_2) is formed in a bright red color. Edible coatings reduce the oxygen level

and reduce color degradation and increase the shelf life of meat products [13].

Zhang et al. [36] showed that edible coating based on chitosan and bamboo vinegar increased L^* value in pork. The color of the coated nuggets with rosemary and licorice increased during storage [16].

3.4. Microbial Characteristics. The results of microbial analyses for chicken burger samples are shown in Figure 4. At the beginning of the experiment (day 0), no significant difference was observed between the number of mesophilic, psychrophilic bacteria, *Pseudomonas*, *Enterobacteriaceae*, *Staphylococcus aureus*, and mold and yeast in different groups ($p > 0.05$). During the storage period, the number of mesophilic and psychrophilic bacteria increased significantly in all groups ($p < 0.05$). At day 20, the number of mesophilic and psychrophilic bacteria was significantly higher in the control group than in the treated groups ($p < 0.05$). In relation to the number of psychrophilic bacteria, a similar trend was observed.

At day 10, the number of mesophilic, psychrophilic bacteria, *Pseudomonas*, *Enterobacteriaceae*, *Staphylococcus aureus*, and mold and yeast increased significantly ($p > 0.05$). The highest microbial count was observed in the control group, and the lowest count was observed in the CHE1, CHE2, CHE3, and CHE4 groups ($p < 0.05$).

The samples containing nanoliposomes of essential oil performed better than the samples containing free essential oil, which can be attributed to the gradual release of essential oil during storage. The best sample for controlling the bacteria in burger samples was the CHE4 group, because the lowest number of bacteria was found in this group. According to the national standard of Iran, the permissible limit of mesophilic bacteria in burger is 6 log CFU/g of burger [29], and in the control sample at day 10, the number of mesophilic bacteria was higher than the standard limit. Thus, the control

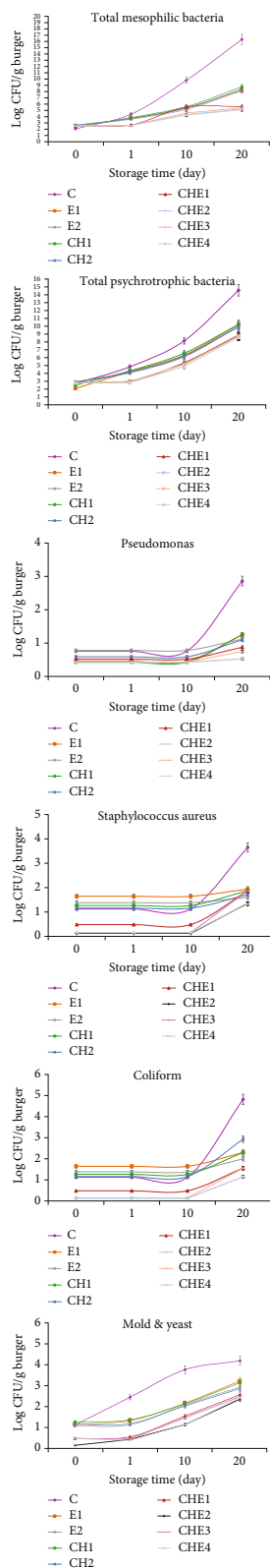


FIGURE 4: Microbial characteristics of chicken burgers during 20 days.

sample was acceptable for less than 10 days. In the CHE1, CHE2, CHE3, and CHE4 groups, the number of mesophilic bacteria did not exceed the standard limit during storage.

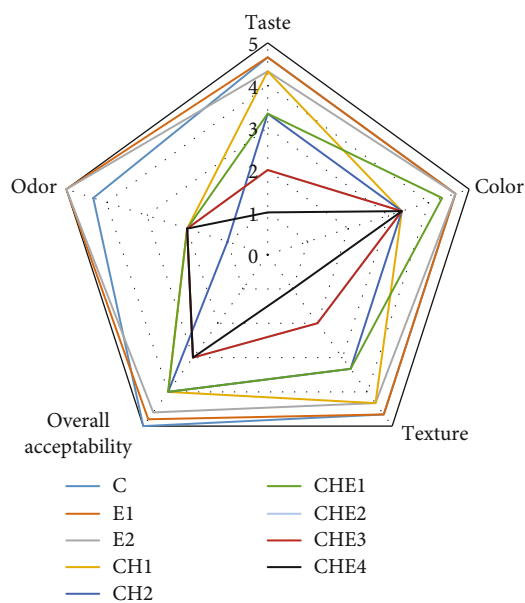


FIGURE 5: Sensory characteristics of chicken burgers.

In general, the microbial growth decreased with the addition of rosemary essential oil and chitosan, which is attributed to the antimicrobial properties of rosemary essential oil [15, 16] and chitosan [11, 30, 35, 36]. The essential oils destroy the cytoplasmic membrane of microorganisms and cause cell death [2]. The antimicrobial property of chitosan is attributed to its polycationic properties that break the cell membrane, but it may be due to its chelating behavior, water-binding properties, and inhibition of mRNA synthesis [37].

Hasani-Javanmardi et al. [28] showed that nanoemulsions of safflower oil and cumin essential oil reduced mesophilic and psychrophilic bacteria, Enterobacteriaceae, and lactic acid bacteria in lamb meat. Lacroix et al. [38] found that the edible coating based on whey protein isolate caseinate reduced the number of mesophilic bacteria in minced meat during refrigeration. Hassanzadeh et al. [35] reported similar results regarding chicken breast coated with chitosan and grape seed extract. Dini et al. [2] showed that composite films based on chitosan and cumin nanoemulsion essential oil reduced the mesophilic and psychrophile bacteria in beef. Abdeldaiem et al. [32] did not detect Enterobacteriaceae in carp fillets coated with calcium caseinate film containing rosemary essential oil.

Similar results have been reported by Fallah et al. [39], Pabast et al. [14], and Zhang et al. [40].

3.5. Sensory Characteristics. Figure 5 shows the sensory evaluation of chicken burgers on day 10. In terms of color, there is no significant difference between the groups ($p < 0.05$). In terms of taste and texture, the CHE4 group got the lowest score. From the point of view of smell, the E1 and E2 groups received the highest points. In the control samples, the E1, and E2 groups had the highest overall acceptance, and the lowest overall acceptance was related to the CHE4 group.

Rosemary essential oil contains many volatile compounds that have an important effect on aroma and taste. Paiva et al. [16] showed that all nugget treatments containing rosemary and licorice were similar and had good acceptability.

4. Conclusion

The coating of chicken burgers with chitosan and nanoliposome of rosemary essential oil decreased pH, TVB-N, PV, and TBARS. The coated samples had better color properties. Coating helped to prevent microbial growth in chicken burgers. Sensory evaluation showed that coated samples had a good score. Therefore, coating with chitosan and nanoliposome of rosemary essential oil is recommended to increase the shelf life of chicken burgers.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have no conflicts of interest to report.

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