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The effect of coating with chitosan containing cumin essential oil on the quality and shelf life of chicken fillets

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ABSTRACT

An edible coating of chitosan (CH) containing cumin essential oil (CEO) was used in this study to increase the shelf life of chicken fillets during refrigerated storage (4±1˚C). CEO was extracted by Clevenger apparatus, and the chemical compounds of CEO were determined. Four treatments were produced to investigate the shelf life of chicken fillets: T1: Control (C), T2: CH, T3: CH-CEO (0.75%), and T4: CH-CEO (1.5%). The chemical parameters (peroxide value, thiobarbituric acid, and total volatile basic nitrogen), microbial parameters (total bacterial counts, psychrotrophic bacteria, lactic acid bacteria), and sensory attributes (taste, odor, color, texture, and general acceptance) of the chicken fillets were periodically evaluated. Cumin aldehyde (25.79%), gamma-terpinene (21.95%), propanol (18.17%), p-menta-1,4-dien 7-al (11.73%), and beta-pinene (9.27%) were the chemical compounds of CEO. It was shown by the results that the microbial load and chemical oxidation of chicken fillets were inhibited by the active edible coating ($p<0.05$) and that better preservation effects have resulted from higher essential oil concentration ($p<0.05$). T4 achieved the best chemical, microbial and sensory results in most tests at the end of the storage period. The shelf life of chicken fillets during storage in the refrigerator was increased by the CH edible coating containing CEO, as demonstrated by this study.

1. Introduction

One of the world's most widely consumed meat products is poultry, which has many advantages over other types of meat, such as low-cost production, low-fat content, and high protein and nutrient value (1). However, poultry is also highly susceptible to microbial spoilage and fat oxidation because of its intrinsic characteristics, such as high protein, moisture content, and near-neutral pH, and extrinsic factors, such as aerobic conditions during storage. These factors lead to the deterioration of chicken's sensory, nutritional, and safety quality and reduce its shelf life to approximately 4 to 5 days (2). To extend the shelf life of poultry products and maintain their quality, natural compounds extracted from various natural sources, such as animals, plants, fruits, and others, have gained popularity among consumers and producers as alternatives to synthetic chemical preservatives. Natural extracts and essential oils (EOs) often have antimicrobial and antioxidant properties that can inhibit or delay microorganisms' growth and lipids' oxidation in food products. These properties are attributed to their structure's phenolic and flavonoid compounds, which can act as free radical scavengers and membrane disruptors (2-4). One example of such a natural compound with beneficial effects on food preservation is cumin (*Cumin cyminum*), a plant belonging to the Apiaceae family and widely used for medicinal purposes and as a spice and flavoring agent in various foods. The essential oil extracted from cumin seeds contains high concentrations of monoterpene compounds, such as cumin aldehyde, gamma-terpinene, propanol, p-menta-1,4-dien 7-al, and beta-pinene. These compounds have significant antioxidant and antimicrobial activities against various foodborne pathogens and spoilage microorganisms (5-7). Despite the high potential of EOs for food preservation, their direct application to food

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products is limited by some drawbacks, such as their strong flavor and volatility, which can affect the sensory acceptability of the food and reduce their effectiveness over time. To overcome these drawbacks, edible coatings can be used as carriers of these natural compounds. Edible coatings are thin layers of edible materials that can be applied to the surface of food products to form a protective barrier against moisture loss, gas exchange, microbial contamination, and lipid oxidation. Edible coatings can also improve the appearance and texture of food products by providing glossiness and crispiness (8). Chitosan (CH) is a polysaccharide derived from the deacetylation of chitin, one of the most abundant natural polymers in living organisms such as crustaceans, insects, and fungi (9). Chitosan has been successfully used in the food industry as an edible coating material due to its antimicrobial and antioxidant properties, which are related to its positive charge that can interact with negatively charged bacterial membranes and its hydroxyl and amino groups that can donate hydrogen atoms to free radicals. Chitosan has also been shown to have the potential for use in various meat products by enhancing their shelf life and quality parameters (7, 8, 10-14). The use of edible coatings and plant EOs can provide a synergistic effect on the preservation of meat products by reducing the population of pathogens and spoilage agents and controlling chemical changes during storage. However, to our knowledge, no studies have reported the application of chitosan coating incorporated with cumin essential oil (CEO) on chicken fillets. Therefore, the aim of this study was to investigate the antioxidant and antimicrobial effects of CH edible coating incorporated with CEO on chicken fillets during refrigerated storage. The hypothesis was that CH edible coating incorporated with CEO would increase the shelf life of chicken fillets by inhibiting microbial growth and lipid oxidation during refrigerated storage.

2. Materials and methods

Cumin plants were collected from Birjand, Khorasan, Iran, and their parts were washed and dried immediately. The dried parts were then pulverized by a shredder and stored at 25 °C until the experiment. All chemicals and reagents used were obtained from Merck Chemical Co. (Darmstadt, Germany) and had an analytical grade.

2.1. Preparation and analysis of CEO

100 g of cumin powder and 1000 ml of distilled water were placed in the Clevenger apparatus for 4 hours to extract the essential oil. The oily layer was separated from the water distillate and dried with Na2SO4. The essential oil was stored in the refrigerator until further analysis. 1 ml of essential oil was injected into the GC/MS (Thermo Scientific, Austin, TX, USA) using the water distillation method. The relative percentage of each constituent of the essential oil was determined by comparing the area under each peak of the chromatogram with the total area under the curve (15)

2.2. Measurement of total phenolic compounds

Total phenolic content was measured using Folin–Ciocalteu reagent. Gallic acid was used as a standard to draw calibration curves. Total phenolic content was reported as "mg gallic acid equivalent per gram of essential oil" (16).

2.3. Preparation of edible coating of CH/CEO

A CH coating was prepared by dissolving 2 g of CH in 100 ml of 1 wt. % acetic acid solution and stirring gently on a magnetic stirrer. Then, 0.75 ml of glycerol as a plasticizer and 0.2% Tween 80 as an emulsifier were added. The pH was adjusted to 5.7-6 using 1 M sodium hydroxide solution and stirred gently for 30 minutes. Finally, the solution was filtered through a Whatman paper filter and sterilized at 121°C. Different concentrations of CEO (0.75% and 1.5%) were mixed with the CH solution on a magnetic stirrer at 55 °C to obtain an active edible coating. The final homogeneous solution and coating were prepared with a homogenizer at 7000 rpm for 2 minutes (17).

2.4. Chicken fillets coating

The fresh chicken was purchased from a slaughterhouse in Tehran province and quickly transported to the food laboratory in boxes containing ice. Fillets (approximately 25×10 cm and weighing approximately 55 g) were prepared from the breast meat by hand. The fillets were washed with water and then placed on sterilized plastic trays for draining. The fillets were immersed in coating solutions according to the designed group for 30 seconds. The coated fillets were drained for 2 minutes and immersed in calcium chloride for 30 seconds for better bonding. The coated samples were exposed to ambient conditions for 1 h for drying. The samples were placed in sterile polyethylene bags and stored at 4°C until testing. Microbiological and chemical evaluation of fillets was performed periodically from the 3rd to the 12th day and compared with control samples prepared in water solutions without coating material. The present study included four treatments; (1): control (without coating), (2): CH 2% coating, (3): CH coating + CEO 0.75%, and (4): CH coating + CEO 1.5%.

2.5. Chemical evaluation

The peroxide value (PV) of chicken fillets was evaluated according to the method of Bagheri et al. (18). The thiobarbituric acid (TBA) content was determined by the colorimetric method described by Valipour Kootenaie et al. (19).

2.6. Microbiological analysis

For microbial experiments, 10 g of fillet meat sample was homogenized with 90 ml of 0.85% sterile saline for 60 seconds in a laboratory blender under sterile conditions. Each sample was analyzed in triplicate. Homogenized samples were used

for culture in the following microbiological media: Plate count agar for total viable count (TVC) and psychrotrophic bacteria (PTC), and de Man, Rogosa, and Sharpe (MRS) agar for lactic acid bacteria (LAB). The inoculated plates were incubated at 37 °C for 2 days for TVC, 7 °C for 10 days for PTC, and 30 °C for 2 days for LAB (20).

2.7. Sensory evaluation

Uncoated and coated fillets were examined after cooking at 185 °C for 60 minutes by a ten-member trained panel in terms of taste, odor, color, texture, and general acceptance characteristics. The results were expressed on a 5-point hedonic scale, and the fillet with a score of less than 3 was defined as unacceptable.

2.8. Statistical analysis

Data analysis was performed using analysis of variance (ANOVA) according to the normality of the data and the homogeneity of variance with SPSS software (version 22). Duncan's test at the 5% level was used to compare the means of the data. All data were reported as mean ± standard deviation, and evaluations were performed in three replicates. The parametric Friedman test was used for statistical analysis of the sensory test.

3. Results and discussion

3.1. Chemical composition of CEO

The chemical composition of CEO is shown in Table 1. A total of 12 compounds, accounting for 99.26% of the essential oil, were identified. The major compounds of CEO were cumin aldehyde (25.79%), gamma-terpinene (21.95%), propanol (18.17%), p-menta-1,4-dien 7-al (11.73%), and beta-pinene (9.27%).

Table 1. Percentage composition of the chemical compounds of cumin essential oil (CEO).

No	Compound	Percentage (%)
1	Cuminylal dehyde	25.79
2	γ-Terpinene	21.95
3	Propanal	18.17
4	p-mentha-1,4dien-7-al	11.73
5	β-pinene	9.27
6	P-cymene	7.85
7	Pulegone	2.45
8	α-pinene	0.75
9	Sabinene	0.58
10	α-phellandrene	0.42
11	α-Cedrene	0.21
12	Myrcene	0.09
	Total	**99.26**

Rana (21) reported that the main compounds of CEO in the West India region were cumin aldehyde (49.40%), p-cymene (17.40%), beta-pinene (6.30%), and gamma-terpinene (6.10%). Ekhtelat et al. (22) stated that the constituents of Kashan cumin seed essential oil were cumin aldehyde (28.24%), gamma-terpinene (21.39%), and p-cymene (13.78%). In most studies, cumin aldehyde was the predominant constituent of CEO.

3.2. Total phenolic content of CEO

The total phenolic content of CEO was 91.75± 1.75 mg gallic acid equivalent/g essential oil. Ladan Moghadam (23) reported a lower total phenolic content of CEO as 53.91 mg gallic acid equivalent/g essential oil. However, Haghiroalsadat et al. (24) reported a similar total phenolic content of CEO as 90.22 mg gallic acid equivalent/g essential oil. The variation in the total phenolic content of essential oils in different studies can be attributed to the geographical area of growth, harvest time, environmental and seasonal conditions, drying and extraction methods, and genetic difference of the plant (4, 25).

3.3. Chemical evaluation of coated fillets

Fat oxidation is the main cause of spoilage of meat products. Peroxide concentration, usually expressed as the peroxide value (PV), is a measure of oxidation or decay in its early stages (19). In the second stage, hydroperoxides are converted to aldehydes and ketones, which form malondialdehyde, a by-product of oxidation that causes an unpleasant taste and odor in the product. Thiobarbituric acid (TBA) is one of the oldest methods for measuring oxidation by-products in meat (26).

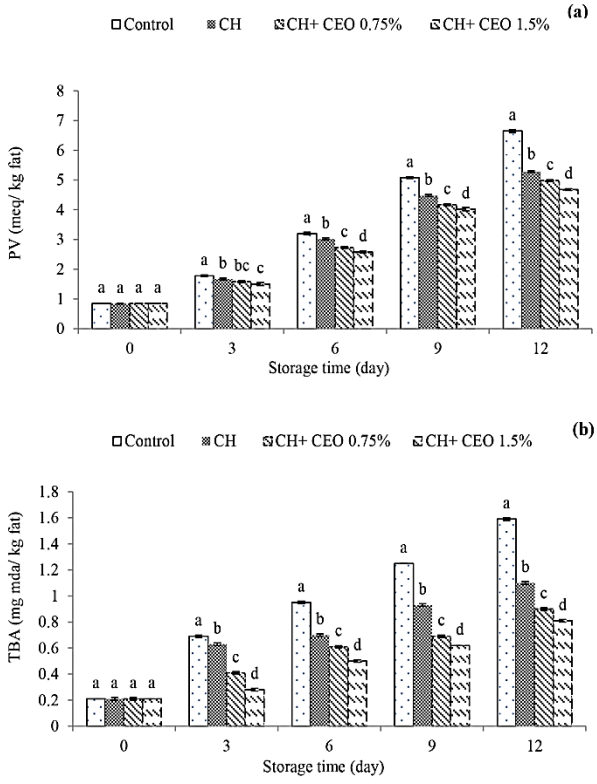

Fig. 1. Changes in peroxide value (a) and thiobarbituric acid (b) of chicken fillet coated by chitosan (CH) coating with Cumin essential oil. Values on the same day with different small letters significantly differ at $p<0.05$.

The results of PV (Fig. 1a) and TBA (Fig. 1b) were consistent, as they increased over time in all treatments. The comparison of these two parameters in the control sample with the rest of the treatments in different storage periods showed that the treatments containing preservatives slowed down their increase compared to the control treatments. The increasing trend of oxidation indices was slower in treatments containing edible coatings than in the control treatment. Bingöl et al. (27) stated that CH has antioxidant power to preserve lipid oxidation in food. Valipour Kootenaie et al. (19) also reported that silver carp fillet coating with CH slowed down the upward trend of oxidation indices compared to the control treatment. In general, the use of edible coatings and essential oils slowed down the increasing process of oxidation indices, and increasing the concentration of essential oils had a positive effect on this process. The lowest value of the oxidation index was observed in the treatment of CH+ CEO 1.5% (4.68 mEq/kg fat and 0.81 mg malondialdehyde/kg fat). The highest value was observed in the control treatment (6.65 mEq/kg fat and 1.59 mg malondialdehyde/kg fat). These results indicate that essential oil and chitosan may have synergistic antioxidant effects. The effect of essential oil on the oxidative stability of chicken fillet samples may be related to phenolic acids in essential oil; because phenolic compounds prevent oxidation by inactivating free radicals and peroxyl radicals, and therefore can increase the shelf life of chicken fillets (12, 28). This property increased with increasing the percentage of essential oil. Other researchers have reported that increasing essential oil concentration slows down the oxidation index of meat products (12, 28, 29). These results are consistent with those of Chen et al. (14), who reported that chitosan loaded with oregano or cinnamon essential oil was effective against lipid oxidation in roast duck stored at 4±1°C. They also confirm the results obtained by Taheri et al. (13), who reported that chitosan coating with cumin essential oil was effective in retarding lipid oxidation reactions in turkey breast fillets stored at 4±1°C.

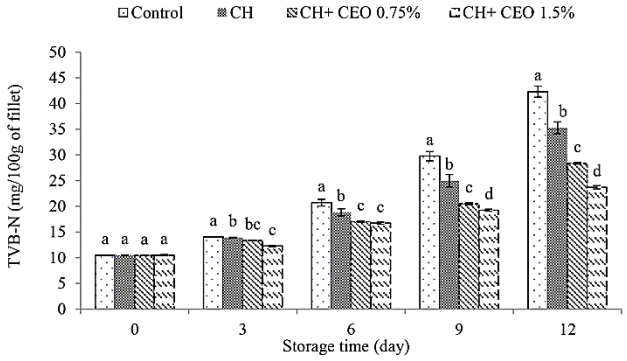

Fig 2. Changes in total volatile base nitrogen of chicken fillet coated by chitosan (CH) coating with Cumin essential oil. Values on the same day with different small letters significantly differ at p<0.05.

Total volatile basic nitrogen (TVB-N) (Fig. 2) is a quantitative factor for determining the amount of ammonia and amines of the first, second, and third types in meat. An increase in this number indicates an increase in the activity of spoilage bacteria and meat enzymes. In general, in all treatments, the amount of TVB-N increased with increasing time. The increase in TVB-N in chicken may be due to various enzymatic processes such as the deamination of free amino acids, the decomposition of nucleotides, and the oxidation of amines Valipour Kootenaie et al. (19). According to the results, the highest values were observed in the control treatment on most days. Since the presence of bacteria in meat leads to the autolysis and degradation of proteins, the breakdown of compounds such as trimethylamine oxides, peptides, amino acids, and others. The higher bacterial load observed in control samples can explain the increase in TVB-N in them (12). The use of edible coatings and essential oils slowed down the increasing process of TVB-N, and increasing the concentration of essential oils had a positive effect. The lowest value of TVB-N was observed in the treatment of CH+CEO 1.5% (23.70 mg/100 g fat) and the highest value was observed in the control treatment (42.31 mg/100 g fat). Edible coatings act as antimicrobial agents and affect the volatile base values. The addition of essential oil also slowed down the increasing process of TVB-N, and increasing the concentration also had a positive effect in this regard. The lower amount of TVB-N in this treatment compared to other treatments can be due to the reduced bacterial population of these treatments, the reduced oxidative ability of bacteria to separate amines from non-volatile nitrogen compounds, or both due to the effect of essential oil on bacteria in fillets. As the concentration of essential oil increased due to the increase of phenolic compounds, its antibacterial effect also increased (28). These results are consistent with those of Fattahian et al. (7), who reported that chitosan coating containing green cumin essential oil was effective against TVB-N in meat stored at 4±1°C.

3.4. Microbial evaluation of coated fillets

The total viable count (TVC) of bacteria (Fig. 3a) increased in all treatments over time, and these changes were more pronounced in the control treatment, which reached 10.05 log CFU/g at the end of the storage period. The use of edible coatings and essential oils slowed down the increasing process of TVC, and increasing the concentration of essential oils had a positive effect on this process. The lowest values of TVC were observed in the treatment of CH+ CEO 1.5% (6.91 log CFU/g). Similar results were observed for psychrotrophic bacteria (PTC) (Fig. 3b). At the end of the storage period, the control treatment reached 9.33 log CFU/g. The use of edible coatings and essential oils slowed down the increasing process of PTC, and increasing the concentration of essential oils had a positive effect on this process. Better results were observed in most of the storage times in the combined treatment of edible coatings and essential oils so that on the 12th day of storage, the lowest value of PTC was observed in the treatment of CH+ CEO 1.5% (6.99 log CFU/g). Several factors affect the antibacterial activity of CH. Although the exact mechanism is unclear, different hypotheses have been proposed. One hypothesis suggests that this effect of CH is due to the presence of positive charge amino groups that bind to large negative

charge molecules on the surface of the microbial cell, leading to rupture of the bacterial cell membrane, leakage of intracellular material, and eventually death of the cell (25).

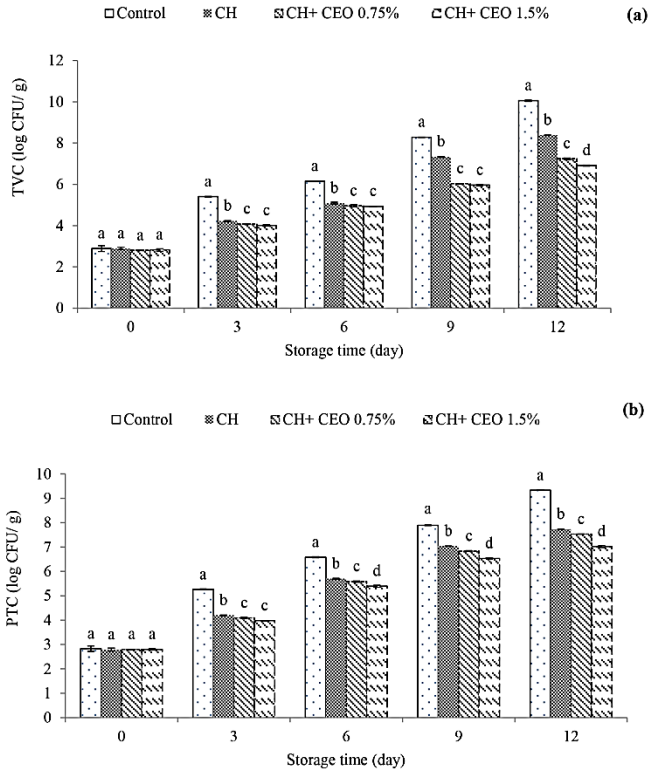

Fig. 3. Changes in total viable count (a) and psychrotrophic bacteria (b) of chicken fillet coated by chitosan (CH) coating with Cumin essential oil. Values on the same day with different small letters significantly differ at $p<0.05$.

The lower total burden of TVC and PTC in treatments containing essential oils can be attributed to phenolic compounds such as cumin aldehyde. Active ingredients in essential oils can destroy cell membrane structure, increase cell membrane permeability, damage various enzyme systems, and inhibit cell growth and conidia germination (14). The antimicrobial properties of natural preservatives depend on the concentration used and as the concentration increases, their antimicrobial properties increase (7, 12, 25, 28, 29). Fattahian et al. (7) investigated the effect of chitosan coating containing green cumin essential oil on the TVC of meat and observed a positive trend due to adding essential oil to the chitosan coating in reducing the overall microbial count. The acceptable TVC and PTC values for poultry meat have been suggested to be 7 log CFU/g (30). At the end of the storage period, only the treatment of CH+ CEO 1.5% had the recommended acceptable level of these bacteria. The same results were observed by Taheri et al. (13), who stated that turkey breast meat coated with chitosan coating enriched with cumin (*Cuminum cyminum* L.) essential oil had an acceptable level of TVC and PTC until the end of the storage period. The results of the present study showed that the number of lactic acid bacteria (LAB) (Fig. 4) increased in all treatments with increasing time, and these changes were more pronounced in the control treatment, which reached 8.38 log CFU/g at the end of the storage period. The use of edible coatings and essential oils slowed down the increasing process of LAB, and increasing the concentration of essential oil had a positive effect on this process. Better results were observed in most of the storage times in the combined treatment of edible coatings and essential oils so that on the 12th day of storage, the lowest value of LAB was observed in the treatment of CH+ CEO 1.5% (6.46 log CFU/g).

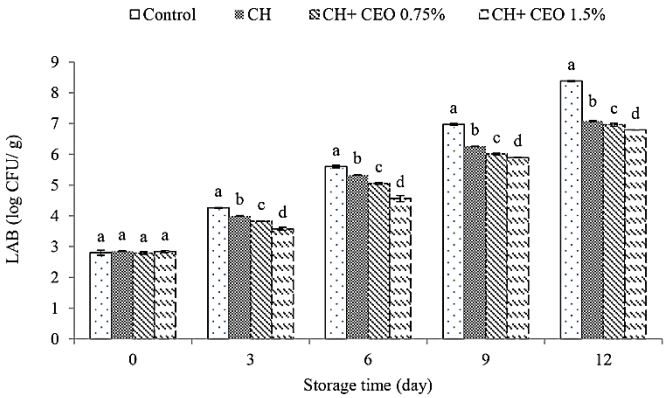

Fig. 4. Changes in chicken fillet lactic acid bacteria coated by chitosan (CH) coating with Cumin essential oil. Values on the same day with different small letters significantly differ at $p<0.05$.

CH coatings act as oxygen barriers and prevent the growth of LAB bacteria. The presence of cumin aldehyde and polyphenolic compounds of CEO, which have antimicrobial properties, is also effective in reducing LAB. Phenolic compounds in plant essential oils destroy the outer membrane of microorganisms, causing the release of lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP. ATP release leads to the depletion of cell energy storage and cell death (4, 31). Chen et al. (14) reported similar results, stating that roast duck packaged with chitosan coating incorporated with oregano or cinnamon essential oil had lower LAB than the control treatment.

3.5. Sensory evaluation of coated fillet

Sensory characteristics such as taste and texture are undoubtedly the most important factors in accepting the product from the consumer's point of view. Therefore, studying the sensory characteristics by considering the product's marketability is very important. The results of sensory properties (Fig. 5) in all treatments decreased significantly over time. Using composite coating and essential oil had a minor adverse effect on chicken fillet samples. Also, in the control group, unpleasant sensory properties were detected after 6 days of storage at refrigerator temperature, and the control samples were completely unacceptable after 6 days. Changes in sensory properties were consistent with chemical and microbial results, so on the 12th day of storage, the highest values of the sensory score were observed in CH+ CEO 1.5%. This can be explained by the fact that fat oxidation leads to degradation and loss of sensory quality, reduces the amount of

nutrients, and produces toxic oxidation products. Also, the correlation between changes in bacterial spoilage and sensory evaluation has already been proven to be related to the activity of microorganisms responsible for food spoilage (8).

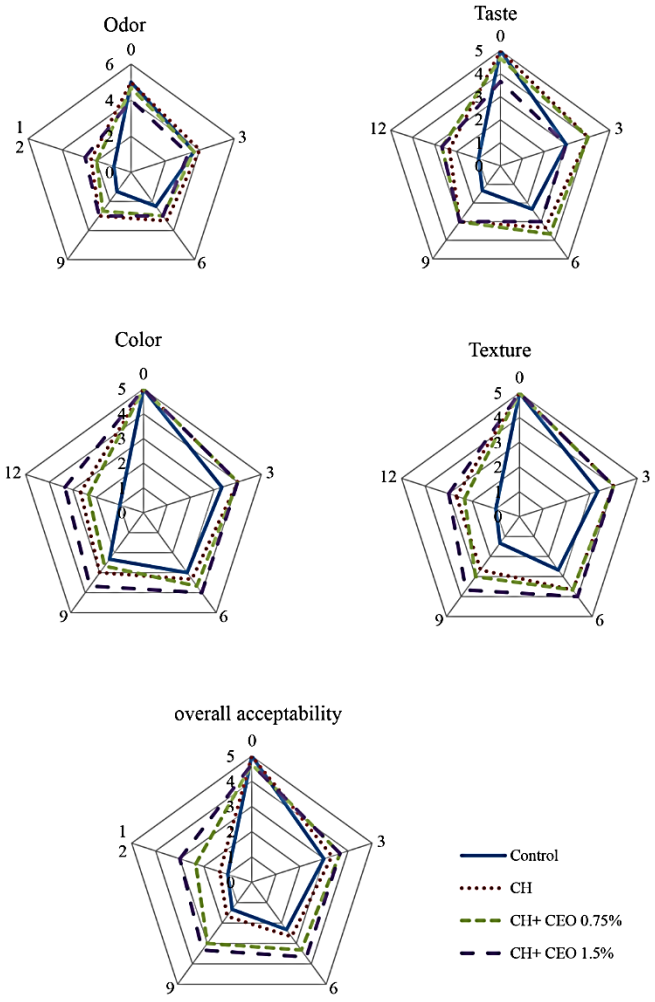

Fig. 5. Sensory evaluation of chicken fillet coated by chitosan (CH) coating with Cumin essential oil.

Improvement of sensory properties can be attributed to using an edible coating, which is a good barrier to oxygen entry, as well as CEO's antioxidant and antimicrobial properties. The combination of the two prevented adverse sensory effects. Shokri et al. (32) also stated that the use of CH coating with *Ferulago angulate* essential oil improved the sensory properties of rainbow trout; they also stated that the changes in sensory properties were consistent with chemical and microbial results.

4. Conclusion

The antioxidant and antimicrobial effects of chitosan (CH) edible coating incorporated with cumin essential oil (CEO) on chicken fillets during refrigerated storage were investigated in this study. The coated and uncoated fillets' chemical, microbial, and sensory parameters were measured periodically and compared with the control samples. Antioxidant and antimicrobial properties that could inhibit or delay the growth of microorganisms and the oxidation of lipids in chicken fillets were exhibited by the CH coating. These properties were enhanced by the addition of CEO to the CH coating, which provided phenolic and monoterpene compounds that could act as free radical scavengers and membrane disruptors. The most effective treatment in reducing the oxidation indices, total volatile basic nitrogen, and bacterial counts of chicken fillets was the CH+ CEO 1.5% coating. This coating also improved the sensory properties of chicken fillets by preventing unpleasant taste and odor formation. This coating can ensure the microbiological and chemical safety of chicken fillets and it can be a good solution for the long-term preservation of chicken fillets during refrigeration. This coating can also increase the consumer acceptance of chicken fillets by providing natural and edible materials that can improve their appearance and texture. The effect of the composite coating of CH and CEO with other preservation technologies, such as modified atmosphere packaging or high-pressure processing, on the quality of various meat products, especially ready meals, should be evaluated in future studies. These studies provide more insights into the synergistic or antagonistic effects of different preservation methods on meat quality and shelf life.

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