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Original Article

Effects of Green Coating on Quality Improvement of Chicken Fillets in Refrigeration Time

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ABSTRACT

Background and Objectives: Effects of edible coatings, including sodium alginate (Alg), pullulan (Pul) and carboxymethyl cellulose (CMC) with bitter orange peel extract (BOE), on the chemical, microbial and texture qualities of chicken fillets during storage in refrigerator was investigated.

Materials and Methods: Samples treated with biopolymer coatings with BOE included significantly lower free fatty acids, peroxide value, total volatile basic nitrogen content and microbial count during cold storage, compared to fillets treated with pure BOE, biopolymer composites (no BOE) and controls.

Results: Effectiveness of the coatings on bacterial population was as follows Alg/Pul/BOE > Pul/CMC/BOE > Alg/CMC/BOE > BOE > Alg/Pul > Alg/CMC > Pul/CMC > control. Coating fillet samples with pure BEO showed greater inhibitory activities against microbial spoilage, compared with composite coatings with no extracts.

Conclusions: Treatment efficiency as of antimicrobial agents and maintenance of physicochemical characteristics were as follows Alg/Pul/BOE > Pul/CMC/BOE > Alg/CMC/BOE. Based on the current findings, these composite coatings can be promising options to improve the quality of fresh foods during shelf-life.

Keywords: Biopolymer, Chicken fillet, Composite, Coating

Introduction

Chicken fillets play important roles in healthy diets due to the high contents of proteins and unsaturated fatty acids (omega 3). The high moisture contents, free amino acids and other non-nitrogen substances cause biochemical, physical and microbiological changes during the distribution and storage of the products and shorten food shelf life. Since chicken fillets are vulnerable to chemical and microbial spoilage, preserving their quality during the storage in refrigerator is important (1, 2). Chilling and freezing are common methods for storing meat products; however, these methods are not enough to delay product spoilage. Therefore, a combination of several preservation methods such as modified atmosphere packaging, vacuum packaging, smoking, marinating, adding essential oils (EOs) and plant extracts and packaging in edible (green) coatings is used to increase the shelf life of red meat products, poultries and fishes (3). In the last two decades, use of edible films and coatings to preserve food quality has become quite common. Edible coatings can be prepared from a variety of raw materials, including polysaccharides, proteins and lipids. These coatings act as barriers against the penetration of moisture, oxygen and other gases, decrease the rate of oxidation, decrease the loss of moisture, prevent surface microbial contamination, preserve sensory and textural characteristics and increase shelf life of the products (4, 5). Thus, incorporation of antioxidant and antimicrobial compounds in coatings can increase efficiency of the coating as well as improving its performance. Various natural polymers are used as biopolymers and green polymers (biodegradable) as well as natural preservative compounds in preparation of edible coatings for food preservation at laboratory and industrial scales (6).

Alginate, including units of β-di-mannuronic acid (mannuronan) and α-L-gluronic acid (gluronan), is composed of sodium or potassium salts of alginic acid

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which is extracted from brown seaweeds. Alginate includes unique colloidal characteristics and can produce strong gels (7, 8). It is used as coating on the surface of fruits, vegetables, meats, poultries and seafood to prevent microbial growth and chemical spoilage. Previous studies have shown the effects of alginate coating containing cinnamon and nisin on the quality of eel tissues (9) as well as effects of alginate coating on the quality of chicken fillets and inhibition of the oxidation progress during the storage (10). Pullulan (PUL) is an extracellular polysaccharide and one of the most important biodegradable polymers, which is produced by fungi such as *Aerobasidium pullulans* and is structurally composed of maltose units. Edible films of pullulan are semi-transparent homogeneous films with high thermal stability and tensile strength, compared to other polysaccharide edible films such as chitosan and polysaccharides extracted from seaweeds (11). Pullulan is used for producing edible coatings due to its favorable characteristics such as colorless, odorless, tasteless and low permeability against oxygen and moisture. Pullulan use in food packaging has widely been investigated (7, 12).

Carboxymethyl cellulose (CMC) is one of the most widely used commercial biodegradable biopolymers. Moreover, CMC is a cellulose derivative achieved by partial substitution of hydroxyl groups (-OH) with carboxymethyl groups (-CH2-COOH). Carboxymethyl cellulose is used as thickening agent, emulsifier, preservative and stabilizer. It preserves original taste and freshness of the food products and increases food shelf life. Carboxymethyl cellulose is used in a wide range of composites used in food packaging as well as production of edible coatings and films (13, 14). Antimicrobial and antioxidant potentials of edible coatings can be improved by incorporating antioxidant and antimicrobial compounds to biopolymers. Citrus peel is a by-product of the fruit product industries and previous studies have shown that these wastes are rich in phenolic compounds such as phenolic acids and flavonoids (15). Pulps and peels of bitter oranges include bioactive compounds such as terpenes and flavonoids with high antioxidant potentials and play important roles in health. Furthermore, antimicrobial effects of polymethoxylated flavones isolated from the peels of bitter oranges were identified (16-18). Based on the studies, effects of the composite biopolymer coatings containing bitter orange peel extracts (BOE) on the quality of food products during the storage have not been investigated. In this study, effects of edible coating consisting of sodium alginate, pullulan and carboxymethyl cellulose with BOEs on the quality of chicken fillets during the storage in refrigerator were investigated.

Materials and Methods

Chemicals and materials

All the chemicals and biopolymers were purchased from Scharlau Chemical, Barcelona, Spain. Bitter oranges (*Citrus aurantium*) were purchased from the local markets in Sari, Mazandaran Province, north of Iran. Peels were cut into pieces of 2–2.5 cm^2 and stored at -34 °C ±1 to inhibit oxidation of the bioactive compounds. Chicken fillets were purchased from a local supermarket in Sari, Iran.

Preparing peel extract

Extraction was carried out based on methods by Marzouk et al. (2013) and Azizkhani et al. (2023) (19, 20).

Total phenolic content

Total phenolic content (TPC) of the peel extract was assessed through Folin-Ciocalteu method (21). Briefly, 0.2 ml of the peel extract was mixed with 1.5 ml of Folin-Ciocalteu reagent and set for 5 min at ambient temperature. Then, 1.5 ml of 60 g/l sodium carbonate solution were added to the mixture and incubated for 90 min at ambient temperature and then the absorbance was measured at 765 nm using UV/VIS spectrophotometer (T-80 model, PG Instrument, Australia). Results were reported as mg gallic acid equivalents (GAE) per gram (g) of the peel.

Preparation of the composite coatings containing bitter orange peel extract

Sodium alginate (Alg) solution (1% w/v), pullulan (Pul) solution (1% w/v) and carboxymethyl cellulose (CMC) solution (1% w/v) were prepared by dissolving 1 g of each polymer in 100 ml sterile distilled water (DW). All solutions were separately homogenized at 25 ºC ±1 for 1 h using magnetic stirrer (Hei-PLATE Mix 20 I, Heidolph Instruments, Germany). Then, a 50:50 ratio of the solutions was mixed and homogenized at 10000 *g* for 10 min using homogenizer (model HC-2A, Hoacheng, China). Then, 1% (v/v) of glycerol as the plasticizer and 5% (v/v) BOE were added to the solutions to prepare the coating formula as follows SA and PUL, SA and CMC, PUL and CMC, SA and PUL and BOE, SA and CMC and BOE, PUL and CMC and BOE. Prepared coating mixtures were stirred for 1 h using magnetic stirrer (22).

Coating the chicken fillets with composite biopolymer solutions

Fillets were cut into 50 g ±5 pieces under hygienic conditions and divided into eight groups of control, F1 (BOE), F2 (Alg/Pul), F3 (Alg/CMC), F4 (Pul/CMC), F5 (Alg/Pul/BOE), F6 (Alg/CMC/BOE) and F7 (Pul/CMC/BOE). Each group samples were soaked twice in the coating solution for 30 s and then transferred on a sterile sieve (for 2 min) to drain. Samples were allowed to drain for 5 min using biological safety cabinet, packed in polyethylene zip-locker bags, wrapped in aluminum foils to

avoid photo-induced oxidation and stored in refrigerator (4 ºC ±1). Analyses were carried out with 3-d intervals during 12 days of storage.

Chemical **analysis**

pH assessment

Ten grams of the fillet samples were added to 25 ml of neutral DW, homogenized, set for 10 min at room temperature (RT) and filtered using Whatman papers no. 1 (Whatman, USA). The pH was assessed using pH meter (23).

Assessment of drip loss

Drip loss is water escaping from raw poultry meat during storage. Drip loss was assessed according to Rahma et al. (2015) and Azizkhani et al. (2023) (20, 24).

Free **fatty acids content**

Free fatty acid (FFA) content was assessed based on a method of Rahman et al. (2015). Five grams of fillet samples were homogenized in 30 ml of chloroform at 11000 *g* for 1 min and then filtered using Whatman filter paper no.1 (Whatman, USA) to remove fillet particles from the filtrates. After adding 4–5 drops of ethanolic phenolphthalein (1%) as indicator, filtrates were titrated with ethanolic potassium hydroxide solution and FFA content was calculated based on oleic acid as follows (24).

$$FFA\ (\%) = (V \times N \times 28.2) / W$$

Where, "V" was the volume of titration (ml) with KOH, "N" was the normality of the KOH solution and "W" was the sample weight (g).

Total volatile base nitrogen

Total volatile base nitrogen (TVB-N) compounds are the sum of primary, secondary and tertiary amines in the forms of volatile amines and toxic nitrogen compounds, which are used as the biomarkers of protein and amine degradation and spoilage. These toxic compounds include significant adverse effects on the organoleptic characteristics and acceptability of meat products and increase during storage of the products (25, 26). To assess TVB-N, 10 g of the fillet samples, 2 g of magnesium oxide (MgO) and 500 ml of DW were transferred into a balloon and volatile nitrogen compounds were accumulated in a solution of boric acid (2%) and methyl red (indicator). Titration of the solution was carried out using sulfuric acid and results were reported as mg TVB-N /100 g of chicken fillets based on the following equation (27).

TVN = Sulfuric acid × 14

Peroxide value

Peroxide value (PV) was assessed based on a method explained by Rahman et al. (2015). Ten grams of the samples were weighed in a 250-ml Erlenmeyer flask and heated at 60 °C for 3 min to melt the fats using water bath. Then, 30 ml of acetic acid:chloroform solution (3:2 v/v) were added and the flask was thoroughly agitated for 2 min to dissolve the fats. To separate tissue particles from the liquid, suspension was filtered using Whatman filter papers no. 1 (Whatman, USA); then, 0.5 ml of saturated potassium iodide solution and 4–5 drops of starch solution (indicator) were added to the filtrate. Solution was titrated against the standard solution of sodium thiosulfate. The PV was expressed as milliequivalent peroxide per kilogram of the sample and calculated by the following equation.

$$PV\ (meq/kg) = (V \times N) / W \times 100$$

Where, "V" was the volume of titration (ml), "N" was the normality of sodium thiosulfate solution and "W" was the sample weight (g) (24).

Microbial analysis

For preparing the decimal dilutions, 10 g of each of the fillets were mixed with 90 ml of 0.1% peptone water and inoculated onto the plate count agar, violet red bile glucose agar, de Man Rogosa and Sharpe (MRS) agar and *Pseudomonas* agar for total mesophilic and psychrotrophic bacteria, *Enterobacteriaceae*, lactic acid bacteria (LAB) and for *Pseudomonas* spp., respectively. Counting results were expressed as $\log^{10}$ CFU/g and carried out in triplicate (28).

Texture analysis

Texture analyzer instrument (TA.XTplusC, Stable Microsystems, Surrey, UK) was used to analyze softness/firmness of the fillet samples (29).

Statistical analysis

Two-way analysis of variance (ANOVA), Duncan's test and SPSS software v.22.00 (IBM, USA) were used for data analysis (confidence levels of 99 and 95%).

Results

In the current study, TPC of the bitter orange peels was 145.25 mg GAE/g. Results of pH measurements (Fig. 1) indicated that the initial pH of chicken fillets was similar to the pH reported by previous studies (30, 31). Significant differences were detected between the treatments containing pure peel extract (F1) and treatments of extract-included biopolymers (F2, F4, F5, F6 and F7) and the control ($p < 0.01$) (Fig. 1). Moreover, F3 (Alg and CMC) showed a pH value similar to that of the control on Days 6 and 12 of storage as shown in Fig. 1 ($p > 0.01$). Significant increases were seen in pH of F2, F3, F4 and F5 on Days 3 and 6 followed by decreases on other days to the end of storage, while decreases in pH were observed in control fillets (without coating), F1 (BOE), F6 (Alg and CMC and BOE) and F7 (Pul and CMC and BOE). The lowest pH belonged to F1 (BOE) and F7 (Pul and CMC and BOE) and the highest to the control, F2 (Alg and Pul) and F3 (Alg and CMC) ($p < 0.01$). Based on the results, samples including Alg showed higher pH during the storage ($p > 0.01$).

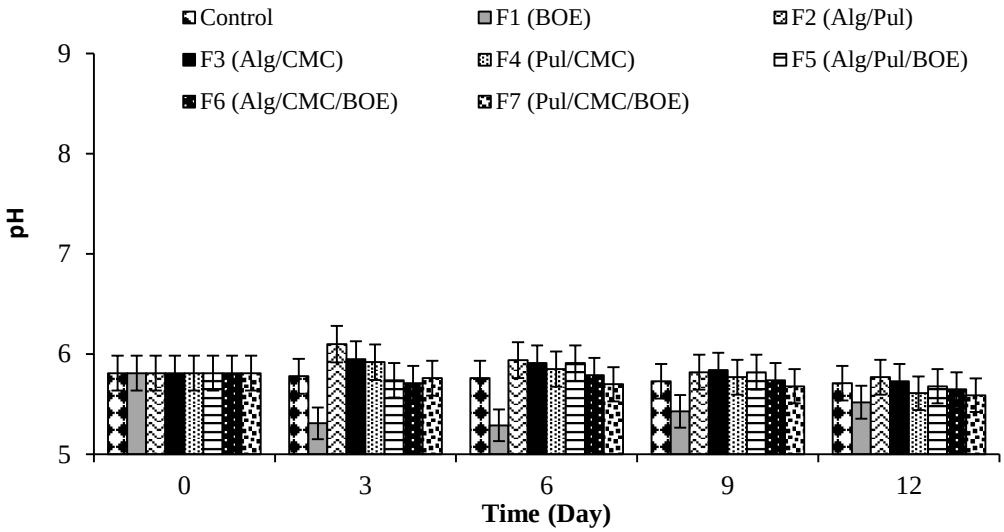


Figure 1. The pH values of the chicken fillet samples coated with biopolymers and bitter orange peel extract during storage in refrigerator

Based on the results (Fig. 2), significant differences were reported in samples coated with BOE, biopolymers, biopolymers containing BOE and control ($p < 0.05$). The FFA contents increased slowly in treated samples during storage. Chicken fillets coated with biocomposites/BOE showed lower FFA contents (0.1–0.13%), compared to samples coated with biocomposites only (0.19–0.21%) ($p < 0.05$). Composite coatings of Alg/Pul caused lower FFA productions in the samples ($p < 0.01$) followed by Pul/CMC ($p < 0.01$).

Total volatile base nitrogen

Data of TVB-N for fillet samples (Fig. 3) demonstrated that the value of the control increased to 39.45 mg/100g on Day 12 of storage. In F3 (Alg/CMC), this value reached 37.3 on Day 12 followed by F4 (Pul/CMC), F2 (Alg/Pul) and F1 (BOE) ($p < 0.05$). The lowest TVB-N value belonged to F5 (Alg/Pul/BOE) followed by F7 (Pul/CMC/BOE) and F6 (Alg/CMC/BOE) as 16.73, 18.11 and 20.05 mg/100g at the end of the cold storage ($p < 0.05$). In the biopolymer coated groups with peel extract, TVB-N was significantly lower than that in control and groups of biopolymers without extract or extract without biopolymers ($p < 0.05$) through the storage and treatments containing Pul included the lowest TVB-N, compared to other samples ($p < 0.05$).

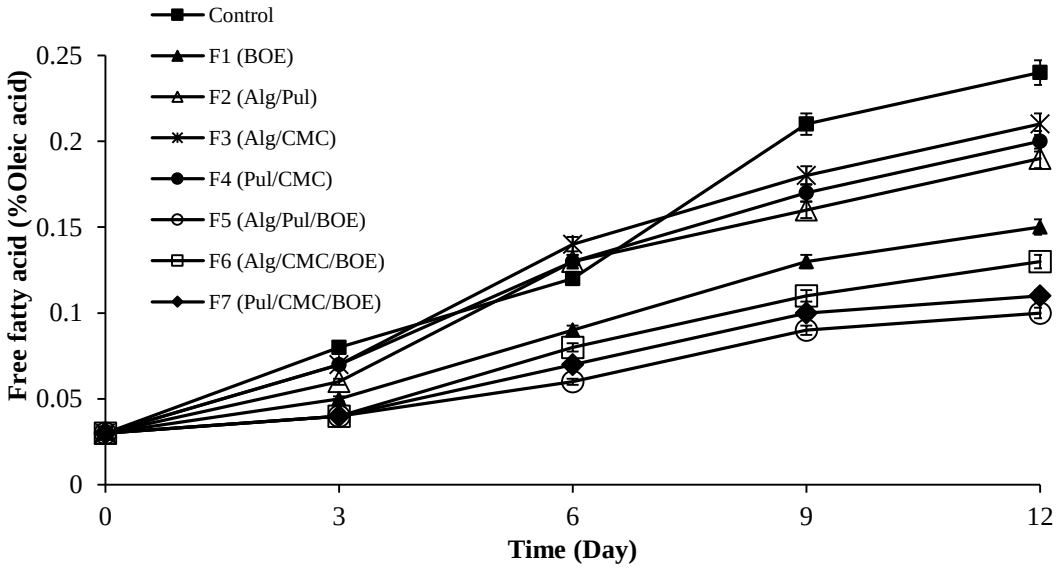


Figure 2. Free fatty acid contents of the chicken fillet samples coated with biopolymers and bitter orange peel extract during storage in refrigerator

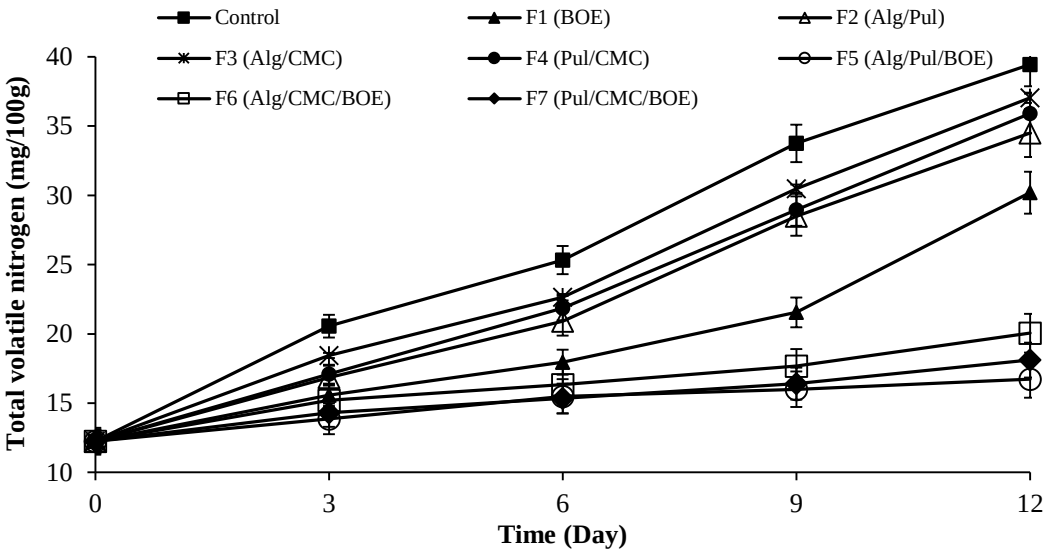


Figure 3. The TVB-N contents of the chicken fillet samples coated with biopolymers and bitter orange peel extract during storage in refrigerator

Oxidative deterioration progress in samples treated with BOE (F1) and BOE/biocomposites (F5, F6 and F7) was not significant during the first three days (Fig. 4). Moreover, PV of all the treated samples was lower than that of control ($p < 0.05$) and coatings with BOE showed a lower PV than that a similar composite without BOE did ($p < 0.05$). From the samples, F5 (Alg/Pul/BOE) and F7 (Pul/CMC/BOE) included the lowest PV during storage ($p < 0.05$) and composites with Pul caused a slower rate of peroxide formation in the fillets. Through the storage time, samples treated with BOE expressed a lower PV (0.45 meq/kg) than that samples coated with composites without BOE did ($p < 0.05$).

Number of total viable bacterial count (TVC) in the control enhanced during storage and its population was 8.44 on Day 12 (Table 1). The TVC of fillets with biopolymers and BOE (F5, F6 and F7) was lower than that of fillets within other groups ($p < 0.05$). In F5 (Alg/Pul/BOE) group, the count was 4.15 log on Day 12, which indicated significant differences compared to other treatments ($p < 0.05$). Totally, TVC was as follows control > F4 > F3 > F2 > F1 > F6 > F7 > F5. Treating fillets with pure BOE included higher inhibitory potentials against the bacterial activity, compared to coatings without extract (F2, F3 and F4) ($p < 0.05$). Lower counts (1–2 log on Days 9 and 12 of storage) were observed in samples treated with biopolymers with BOE, compared to coatings without BOE ($p < 0.05$).

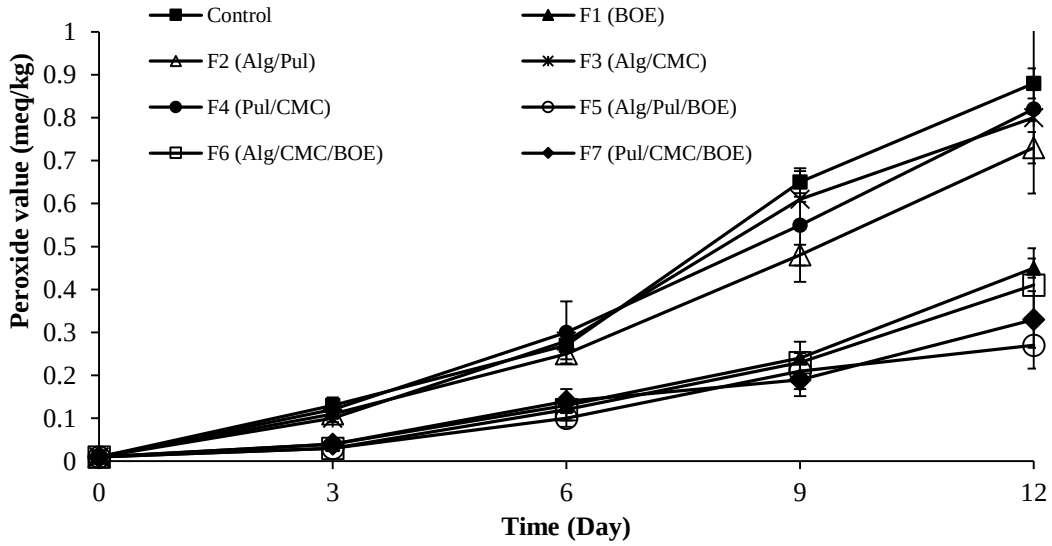


Figure 4. Peroxide values of the chicken fillet samples coated with biopolymers and bitter orange peel extract during storage in refrigerator

Table 1. Changes in total viable bacterial count (log CFU/g) of the fillets coated with bitter orange peel extract and biopolymer coatings during storage at 4 ºC ±1

	Time (day)				
	0	3	6	9	12
Control	2.15±0.09^{Aa*}	3.81±0.25^{Ba}	5.35±0.36^{Ca}	6.58±0.44^{Da}	8.44±0.95^{Ea}
F1 (BOE)	2.15±0.09 ^{Aa}	2.77±0.85^{Be}	3.56±0.19^{Cf}	4.81±0.35^{De}	5.49±0.14^{Ee}
F2 (Alg/Pul)	2.15±0.09 ^{Aa}	2.90±0.33^{Be}	3.90±0.66^{Ce}	5.03±0.29^{Dd}	5.78±0.65^{Ed}
F3 (Alg/CMC)	2.15±0.09 ^{Aa}	3.07±0.49^{Bd}	4.87±0.79^{Cb}	5.44±0.30^{Dc}	6.13±0.30^{Ec}
F4 (Pul/CMC)	2.15±0.09 ^{Aa}	3.55±0.23^{Bb}	4.96±0.51^{Cb}	5.81±0.40^{Db}	6.37±0.12^{Eb}
F5 (Alg/Pul/BOE)	2.15±0.09 ^{Aa}	2.58±0.10^{Bf}	3.01±0.47^{Cg}	3.94±0.20 ^{Dg}	4.15±0.09^{Eh}
F6 (Alg/CMC/BOE)	2.15±0.09 ^{Aa}	3.37±0.69^{Bc}	4.45±0.34^{Cc}	4.98±0.51^{De}	5.37±0.21^{Ef}
F7 (Pul/CMC/BOE)	2.15±0.09 ^{Aa}	3.05±0.91^{Bd}	4.11±0.16^{Cd}	4.47±0.13^{Df}	4.90±0.10^{Eg}

*Data are presented as mean ± SD.
[a]represents a statistically significant difference ($p < 0.05$) between means of the treatments at the same day (in each column).
[A]represents a statistically significant difference of means for the same treatment during the storage (in each row).

On Days 9 and 12, psychrotrophic bacterial count of the fillets treated with biopolymers with BOE was 1–1.5 log CFU/g lower than that of the fillets treated with biopolymers and 2.8–3 log CFU/g lower than that of the control, indicating that F5, F6 and F7 were effective against these group of bacteria ($p < 0.05$) (Table 2). In addition, samples treated with Alg/Pul/BOE included the lowest psychrotrophic bacterial count during the storage, followed by Pul/CMC/BOE with 0.32 log CFU/g difference ($p < 0.05$). During the storage, the psychrotrophic bacterial count of the samples treated with pure BOE was lower than that of the samples coated with biopolymer composite (without BOE) ($p < 0.05$). Coatings composed of Pul as a part of the composite included higher antibacterial effects on the psychrotrophic bacterial population ($p < 0.05$).

The current results (Table 3) demonstrated that composite coatings (without BOE) did not act efficiently in inhibiting *Pseudomonas* growth. Samples treated with BOE and biopolymers incorporated with BOE included significantly lower *Pseudomonas* counts (nearly 1 log CFU/g), compared to other samples during the storage ($p < 0.05$). The most effective coatings in limiting growth of *Pseudomonas* spp. were F5 (Alg/Pul/BOE) and F7 (Pul/CMC/BOE) ($p < 0.05$).

Based on the data reported in Table 4, control samples included the highest population of *Enterobacteriaceae* during cold storage ($p < 0.05$). All the coatings included inhibitory effects on the growth of *Enterobacteriaceae* in the following order of F5 > F7 > F6 > F1 > F4 > F3 > F2, which showed significant differences compared with the control ($p < 0.05$). Composite treatments with Pul included higher antibacterial activities, as seen for F5 and F7 ($p < 0.05$).

Table 2. Changes in psychrotrophic bacteria count (log CFU/g) of the chicken fillets treated with bitter orange peel extract and biocomposite coatings during storage at 4±1 ºC

	Time (day)				
	0	3	6	9	12
Control	3.18±0.25^{Aa*}	4.25±0.50^{Ba}	5.69±0.15^{Ca}	6.97±0.90^{Da}	7.51±0.44^{Ea}
F1 (BOE)	3.18±0.25^{Aa}	2.95±0.22^{Be}	3.70±0.41^{Cd}	4.65±0.18^{Dc}	5.10±0.26^{Ee}
F2 (Alg/Pul)	3.18±0.25^{Aa}	3.30±0.25 ^{Bc}	4.76±0.19^{Cb}	5.02±0.11^{Db}	5.39±0.45^{Ed}
F3 (Alg/CMC)	3.18±0.25^{Aa}	3.92±0.28^{Bb}	4.71±0.30^{Cb}	5.04±0.20^{Db}	5.67±0.16^{Ec}
F4 (Pul/CMC)	3.18±0.25^{Aa}	3.08±0.43^{Be}	4.55±0.71^{Cc}	5.15±0.38^{Db}	5.90±0.60^{Eb}
F5 (Alg/Pul/BOE)	3.18±0.25^{Aa}	2.98±0.26^{Be}	3.40±0.08^{Ce}	3.75±0.18^{Df}	4.03±0.10^{Eh}
F6 (Alg/CMC/BOE)	3.18±0.25^{Aa}	3.40±0.10^{Bc}	3.68±0.16^{Cd}	4.20±0.31^{Dd}	4.89±0.19^{Ef}
F7 (Pul/CMC/BOE)	3.18±0.25^{Aa}	3.15±0.07^{Bd}	3.61±0.38^{Cd}	4.00±0.10^{De}	4.35±0.25^{Eg}

*Data are presented as mean ± SD.
[a]represents a statistically significant difference ($p < 0.05$) between means of the treatments at the same day (in each column).
[A]represents a statistically significant difference of means for the same treatment during the storage period (in each row).

Table 3. Changes in Pseudomonas Spp. count (log CFU/g) of the chicken fillets treated with bitter orange peel extract and biocomposite coatings during storage at 4±1 ºC

	Time (day)				
	0	3	6	9	12
Control	2.05±0.14^{Aa*}	3.05±0.25^{Ba}	3.76±0.21^{Ca}	4.38±0.00^{Da}	4.89±0.30^{Ea}
F1 (BOE)	2.05±0.14^{Aa}	2.92±0.31^{Ba}	3.13±0.10^{Cc}	3.70±0.10^{Dc}	3.95±0.21^{Ed}
F2 (Alg/Pul)	2.05±0.14^{Aa}	2.51±0.11 ^{Bc}	3.22±0.35^{Cc}	3.83±0.25^{Db}	4.10±0.51^{Ec}
F3 (Alg/CMC)	2.05±0.14^{Aa}	2.85±0.05 ^{Bb}	3.61±0.20^{Cb}	4.10±0.30^{Db}	4.39±0.37^{Eb}
F4 (Pul/CMC)	2.05±0.14^{Aa}	2.75±0.07^{Bb}	3.55±0.41^{Cb}	3.90±0.12^{Db}	4.25±0.20^{Eb}
F5 (Alg/Pul/BOE)	2.05±0.14^{Aa}	2.33±0.18^{Bd}	2.50±0.00^{Cf}	2.75±0.26^{Df}	3.00±0.23^{Eg}
F6 (Alg/CMC/BOE)	2.05±0.14^{Aa}	2.65±0.13^{Bc}	3.07±0.29^{Cd}	3.39±0.41^{Dd}	3.61±0.11^{Ee}
F7 (Pul/CMC/BOE)	2.05±0.14^{Aa}	2.56±0.08^{Bc}	2.81±0.17^{Ce}	3.01±0.05^{De}	3.28±0.09^{Ef}

*Data are presented as mean ± SD.
[a]represents a statistically significant difference ($p < 0.05$) between means of the treatments at the same day (in each column).
[A]represents a statistically significant difference of means for the same treatment during the storage period (in each row).

Table 4. Changes in Enterobacteriaceae count (log CFU/g) of the chicken fillets treated with bitter orange peel extract and biocomposite coatings during storage at 4±1 ºC

	Time (day)				
	0	3	6	9	12
Control	1.45±0.05^{Aa*}	2.81±0.11^{Ba}	3.64±0.25^{Ca}	4.75±0.18^{Da}	5.87±0.40^{Ea}
F1 (BOE)	1.45±0.05 ^{Aa}	2.10±0.15^{Bc}	2.78±0.31^{Cd}	3.51±0.03^{Dd}	3.79±0.15^{Ed}
F2 (Alg/Pul)	1.45±0.05 ^{Aa}	2.25±0.35^{Bc}	2.88±0.17^{Cd}	4.01±0.00^{Dc}	4.33±0.29^{Ec}
F3 (Alg/CMC)	1.45±0.05 ^{Aa}	2.47±0.10^{Bb}	3.31±0.55^{Cb}	4.33±0.20^{Db}	4.85±0.10^{Eb}
F4 (Pul/CMC)	1.45±0.05^{Aa}	2.35±0.13^{Bb}	3.01±0.21^{Cc}	4.45±0.27^{Db}	4.95±0.47^{Eb}
F5 (Alg/Pul/BOE)	1.45±0.05^{Aa}	1.55±0.00^{Bf}	1.68±0.16^{Cg}	1.96±0.11 ^{Dg}	2.05±0.08^{Eg}
F6 (Alg/CMC/BOE)	1.45±0.05^{Aa}	1.95±0.01^{Bd}	2.32±0.17^{Ce}	2.81±0.06^{De}	3.00±0.0.25^{Ee}
F7 (Pul/CMC/BOE)	1.45±0.05^{Aa}	1.75±0.11^{Be}	1.90±0.01^{Cf}	2.08±0.20^{Df}	2.55±0.07^{Ef}

*Data are presented as mean ± SD.
[a]represents a statistically significant difference ($p < 0.05$) between means of the treatments at the same day (in each column).
[A]represents a statistically significant difference of means for the same treatment during the storage period (in each row).

Changes in LAB population in chicken fillets treated with BOE and biocomposite coatings during storage at 4 ºC ±1 are demonstrated in Table 5. The initial count of LAB was nearly 4.15 log CFU/g that increased progressively during storage until reaching 5–6 and 7 log CFU/g on Day 12 for coated samples and control, respectively. Coatings of pure BOE inhibited growth of LAB and use of composites of biopolymers and BOE resulted in decreased LAB counts ($p < 0.05$). Growth of LAB in fillets coated with Alg/Pul/BOE was significantly lower than that of the other samples through storage, followed by Pul/CMC/BOE and Alg/CMC/BOE.

The initial drip loss of the fresh fillet sample was approximately 6.16% (Fig. 5). Drip loss decreased in all the treated samples, except increases in control and F1 (BOE treated) during the storage ($p < 0.01$). Samples coated with CMC/Pul and CMC/Pul/BOE included the lowest drip loss during the storage ($p < 0.01$). Drip loss proportions of the fillet samples coated with biopolymers were significantly lower than those of the control (without coating) and F1 (BOE coated without biopolymers) ($p < 0.05$). Drip loss on Day 12 of biopolymer-coated fillets was significantly lower than those on Day 3, 6 and 9 of refrigerated storage ($p < 0.05$).

Texture firmness of the fillets on Day 0 was 4.07 N ±0.15, which slowly decreased in all fillets during storage ($p < 0.05$) (Fig. 6). Samples treated with F1 (BOE) and biopolymers (F2–F7) included a lower firmness on Days 6, 9 and 12 of storage, compared to the control ($p < 0.05$). As seen in Fig. 6, composites with BOE (F5, F6 and F7) caused a higher softness in the fillets, compared to that coatings without BOE did (F2, F3 and F4) ($p < 0.05$). Findings showed that BOE, as the free extract or included in composites, decreased the firmness. Fillets coated with a combination of Pul and CMC included a lower firmness, followed by Alg/CMC and Alg/Pul ($p < 0.05$).

Table 5. Changes in Lactic acid bacteria count (log CFU/g) of the chicken fillets treated with bitter orange peel extract and biocomposite coatings during storage at 4±1 ºC

	Time (day)				
	0	3	6	9	12
Control	4.15±0.23^{Aa*}	5.50±0.45^{Ba}	6.05±0.30^{Ca}	6.91±0.23^{Da}	7.55±0.30^{Ea}
F1 (BOE)	4.15±0.23^{Aa}	4.93±0.11^{Be}	5.47±0.25^{Cd}	5.65±0.11^{Dd}	5.87±0.45^{Ee}
F2 (Alg/Pul)	4.15±0.23^{Aa}	5.11±0.26^{Bd}	5.58±0.10^{Cc}	5.81±0.22^{Dc}	6.35±0.51^{Ed}
F3 (Alg/CMC)	4.15±0.23^{Aa}	5.40±0.32^{Bb}	5.83±0.34^{Cb}	6.03±0.50^{Db}	6.49±0.29^{Eb}
F4 (Pul/CMC)	4.15±0.23^{Aa}	5.25±0.10^{Bc}	5.49±0.37^{Cc}	5.91±0.33^{Dc}	6.28±0.35^{Ec}
F5 (Alg/Pul/BOE)	4.15±0.23^{Aa}	4.40±0.20^{Bf}	4.75±0.12^{Cg}	4.93±0.41^{Dg}	5.10±0.11^{Eh}
F6 (Alg/CMC/BOE)	4.15±0.23^{Aa}	4.85±0.00^{Be}	5.22±0.20^{Ce}	5.36±0.10^{De}	5.50±0.17^{Ef}
F7 (Pul/CMC/BOE)	4.15±0.23^{Aa}	4.61±0.35^{Bf}	4.98±0.09^{Cf}	5.15±0.27^{Df}	5.33±0.58^{Eg}

*Data are presented as mean ± SD.
[a]represents a statistically significant difference ($p < 0.05$) between means of the treatments at the same day (in each column).
[A]represents a statistically significant difference of means for the same treatment during the storage period (in each row).

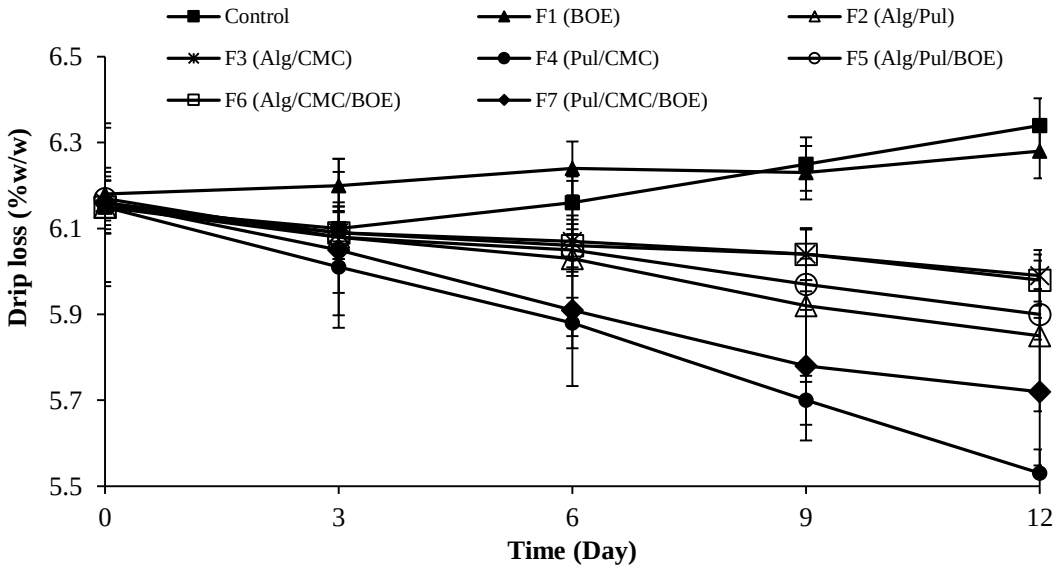


Fig. 5. Drip loss percentage of chicken fillet samples coated with biopolymers and bitter orange peel extract during storage in the refrigerator

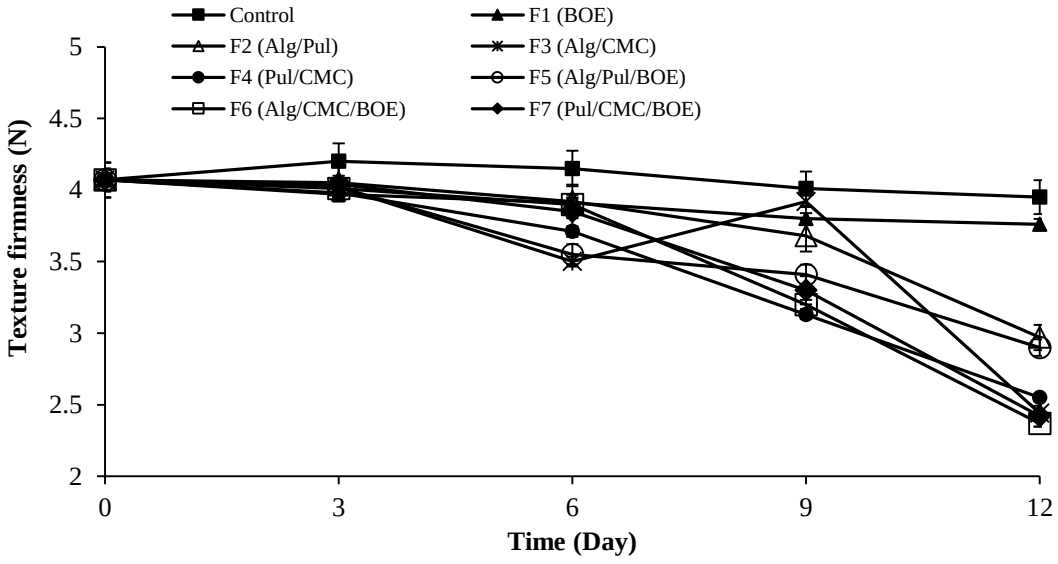


Fig. 6. Texture firmness of chicken fillet samples coated with biopolymers and bitter orange peel extract during storage in the refrigerator

Discussion

Citrus peel is addressed as a valuable by-product of the fruit industries with a high quantity of phenolic components. In this study, TPC of bitter orange peels was 145.28 mg GAE/g, which was significantly higher than those reported by Ersus and Can (2012) and Olowu and Firincioglu (2021) as 48.7 and 69.05, respectively (32, 33). Differences in TPC might be due to the effects of climatic, soil and agricultural conditions on fruit quality and phenolic contents (34). Results of pH measurements indicated that the initial pH of chicken fillets was similar to the pH reported by previous studies (30, 31). Presence of organic acids and the fermentation of muscular glycogen to lactic acid caused decreases of pH. It is noteworthy that formation of carbonic acid from CO_2 resulting from the metabolism of spoilage-causing microorganisms during storage, made mild decreases in pH (31). Samples coated with Alg included higher pH values at the end of the storage ($p > 0.01$). Similar results were reported by Baek et al. (2021) that shrimps coated by nanoparticles of Alg-based edible films showed higher pH than other treatments. They claimed that Alg might be responsible for maintaining pH of the shrimps during storage (35). In the present study, pH of the chicken fillets decreased mildly during cold storage while Khare et al. (2016) and Giteru et al. (2017) reported mild increases in pH of chicken fillets coated with carrageenan containing citric acid and cinnamon oil and citral and quercetin, respectively (36, 37). Increases in pH during the storage might be due to the degradation of the proteins and other nitrogenous compounds by endogenous enzymes and microorganisms and production of volatile bases such as amines (38), which seemed to occur at lower rates in the current samples.

Formation of FFA is resulted from the microbial or enzymatic spoilage of triglycerides or phospholipids. As the molecular size of FFAs is smaller than that of triglycerides, they are affected by oxidative agents faster than that large molecules are (39). It was detected that addition of the peel extract to composite coatings caused synergistic antioxidant effects. Furthermore, it was revealed that Pul was an effective agent in inhibiting increases of FFAs. In this study, FFA content in the samples during storage was as follows control > Alg/CMC > Pul/CMC > Alg/Pul > BOE > Alg/CMC/BOE > Pul/CMC/BOE > Alg/Pul/BOE. Shahosseini et al. (2021) assessed the antioxidant effects of Pul edible coating with watercress extract on the chemical changes of fresh beluga sturgeon fillets during cold storage. They reported that the lowest FFA content was observed in treatment of Pul coating containing 1000 ppm of the extract (40).

The TVB-N value in meat products is addressed as a determining parameter of freshness and its increase is due to the activities of spoilage bacteria and endogenous enzymes. In this study, TVB-N was significantly lower in control and groups of biopolymers without extract or extract without biopolymers in all biopolymer composite coating groups containing peel extract through the storage. It is noteworthy that treatments containing Pul included a lower TVB-N, compared to that the other samples did. In a study by Shahosseini et al. (2021) on Pul coating with watercress extract for fish fillets, the lowest TVB-N was observed in the treatment of Pul coating with the extract due to the presence of a protective layer of Pul acted as a barrier and decreased bacterial population, compared to other treatments. Saeid Asr et al. (2020) used CMC coating incorporated with rosemary essential oil (REO) and sodium acetate to improve the quality of rainbow trout fillets during shelf-life. The initial TVB-N value of trout fillets significantly increased through a 16-d storage at 4 °C. They reported that while TVB-N reached 36.46 mg/100 g in the control at the end of the storage, samples coated with CMC and CMC/REO included significantly lower TVB-N values (5). Jalali et al. (2016) reported the great effects of composite coating of Alg/CMC containing clove EO to inhibit increase of TVB-N in fish fillets during storage (41). Effects of Alg coating layer containing *Citrus wilsonii* extract on the shelf-life quality of shrimps were investigated by Liu et al. (2016). The lowest increase rate of TVB-N was observed in shrimps treated with Alg/extract, compared to the control (42). The current results are similar to those of the highlighted studies.

Technically, PV shows formation of the primary products of lipids such as hydroperoxides. The current results showed the antioxidant activity of phenols and flavonoids in the BOE against formation and propagation of radicals and peroxides. The present study demonstrated that biopolymers with BOE significantly delayed the primary oxidation of fillets, while this parameter increased sharply in controls over the time. Joneidi Jafari et al. (2017) investigated effects of chitosan film incorporated with propolis on the characteristics of chicken fillets stored in refrigerator. They reported that the oxidative spoilage in the samples was not significantly different within the first three days. However, on Days 6 and 9, PV of the samples containing chitosan/propolis extract was lower than that of the control and pure chitosan groups (27). Effects of Alg coating enriched with *Mentha longifolia* EO on the quality of refrigerated bighead carp fillets were assessed by Heydari et al. (2015). The PV of the fillets increased gradually in all treatments during refrigeration storage and significant differences were observed between the samples with Alg/EO pure EO and the control or pure Alg. They attributed these results to the antioxidant activity of EO and its polyphenol contents (43). It is well-established that phenolic antioxidants inhibit formation of FFA radicals, which react with oxygen or absorb it in auto-oxidation

processes; therefore, they delay onsets of the auto-oxidation in fats (44).

Chemical composition of the chicken meat makes it appropriate for microbial growth and occurring spoilage during storage. Number of the TVC in the control group increased during storage. Similarly, significant decreases were reported by Heydari et al. (2015) in TVC of bighead carp fillets coated with sodium alginate and enriched with horsemint (*Mentha longifolia*) EO during 12 days of storage at 4 °C (43). Results of a study by Saeid Asr et al. (2020) showed that growth of mesophilic bacteria in trout fillets treated with CMC/REO was significantly slower than that in controls (5). Moreover, an edible coating of Alg/Pul incorporated with capsaicin inhibited the growth of mesophilic aerobic bacteria (45). Langroodi et al. (2018) concluded that use of an edible coating supplemented with EO and extracts could possibly induce the structural destruction of mitochondrial and cellular membrane lipids in the bacteria and inhibit microbial proliferation (26). These effects were attributed to the presence of the phenolic compounds and terpenoids that possess antimicrobial activities. As declared by the previous studies, biopolymers act as barriers against oxygen transfer, resulting in the growth inhibition of aerobic bacteria (46).

At refrigerated temperatures, the major group of bacteria responsible for the spoilage of fresh chicken fillets includes Gram-negative psychrotrophics (47). Saeid Asr et al. (2020) demonstrated that the psychrotrophic count of fish fillets treated with Alg was nearly 1 log CFU/g lower than that of the control, expressing that SA was effective against these bacteria and samples treated with CMC/SA/REO included the lowest count during the storage. It is indicated that Pul/chitosan-based active coating incorporated with polyphenols from lemon peel decreased psychrotrophic bacterial populations in raw poultry meats (48). Results are similar to those of the highlighted studies that composing biopolymers with EOs or extracts leading to enhancement of the antimicrobial effects of the two of them.

It is well known that *Pseudomonas* spp. play important roles in the spoilage of fresh chicken fillets during cold storage. Data by Saied Asr et al. (2020) showed that Alg coating was not efficient in decreasing population of *Pseudomonas* spp. of fish fillets; however, CMC, Alg/CMC and Alg/CMC/REO included higher antimicrobial effects. It suggested synergistic effects of Alg, CMC, Pul and BOE in decreasing *Pseudomonas* population in the samples. Antimicrobial activity of the bitter orange extract was due to the phenolic compounds, tannins, saponins and flavonoids that were biologically active. It has been shown that tannin in citrus peel extracts forms irreversible bonds with proline-rich proteins, leading to the inhibition of protein synthesis in the cells (49).

All the coatings showed inhibitory effects on the growth of *Enterobacteriaceae* in the following order of F5 > F7 > F6 > F1 > F4 > F3 > F2. Antibacterial effects of the Pul and chitosan-based coatings by incorporating polyphenols of lemon peel extract in raw poultry meats were assessed by Maru et al. (2021). Samples coated with pullulan in combination with lemon peel extract showed increased bacterial lag phases and growth inhibition (48). Moreover, it was reported that Alg coating incorporated with REO included antibacterial effects against *Enterobacteriaceae* (Saied Asr et al., 2020). One of the secondary metabolite compounds in the ethanolic extract of citrus peels is an alkaloid that includes toxicity against foreign cells and organisms. Previous studies reported that flavonoids of citrus peel extract included a wide range of biological activities such as antimicrobial, cytostatic and antioxidant activities. In addition, terpenoids in ethanolic extracts are involved in cell membrane disruption by the lipophilic compounds (50). The current results described that BOE and biopolymer composite/BOE included great potentials to inhibit growth of *Enterobacteriaceae*.

Based on the current findings, growth of LAB in fillets coated with Alg/Pul/BOE was significantly lower than that in other samples through storage, followed by Pul/CMC/BOE and Alg/CMC/BOE. Saied Asr et al. (2020) showed that Alg/REO actively decreased population of LAB by nearly 2 log CFU/g which was lower than that of the control (5). Choulitoudi et al. (2017) reported the lowest LAB count in eel fillets treated with CMC incorporated with herbal EO (51). According to Shetty et al. (2016), peels of *Citrus* fruits generally treated as wastes can be used as effective economical antimicrobial agents as they are available for no costs with no side effects (52).

Drip loss decreased in all the coated samples, except in control and F1 (BOE coated), during the storage. It seemed that Pul and CMC showed higher potentials of inhibiting moisture loss from the texture, compared to that the Alg did as sodium alginate included great effects on decreasing drip loss. As shown by Khan et al. (2022), drip loss of the broiler breast samples coated with pullulan-mediated silver nanoparticles was significantly lower than that of the control (without packaging) (29). In the present study, chicken fillets treated with CMC, Pul and Alg included significantly lowest drip losses, compared to that F1 and control did, indicating better muscle integrity and water stability of the texture (53). Degradation of collagens and myofibrillar proteins due to the microbial activity on poultry meat surfaces resulted in the release of intercellular fluids during the storage (54). The low drip loss revealed a lower microbial spoilage in biopolymer-treated samples. It has been established by previous studies that Pul decreases drip loss due to the electrostatic interactions between the OH group of pullulan or CMC and phenolic hydroxyl

derivative of herbal extracts or other sources. These electrostatic interactions form a thicker water barrier between the product surface and the environment and protect it against spoilage and limit high drop losses during the storage (55, 56). In a study by Khare et al. (2016), chicken fillets were coated with sodium alginate, citric acid, calcium chloride and cinnamon EO solutions via three methods of spraying, brushing and dipping. Based on the results, drip loss of alginate/cinnamon-treated fillets was lower than that of other samples (36).

Results of texture analysis showed that BOE, free or incorporated in the biopolymers, decreased the firmness. Fillets coated with a combination of Pul and CMC included a lower firmness, followed by Alg/CMC and Alg/Pul. In a study by Khan et al. (2022) on the use of Pul active packaging incorporated with curcumin in broiler meats during cold storage, meat samples treated with Pul/curcumin coating included a higher tenderness in all the treated groups on Days 7 and 14 of refrigerated storage (29). Garavito et al. (2020) reported that firmness of the fresh chicken breast fillets coated with edible biopolymers containing oregano EO decreased through the storage and this value was lower for the coated fillets than that for the control fillets (30). This could be due to a higher rate of degradation of muscle fibers in the coated fillets as a result of the greater volume of moisture preserved by the coatings, enhancing developments of collagen hydrolysis and protein degradation (57).

Conclusions

In the present study, combined activities of carboxymethyl cellulose, sodium alginate, pullulan coating and BOE on the physical, chemical and microbiological quality of refrigerated chicken fillets were assessed. The BOE showed significant preservative effects on the samples and by combination with biopolymers enhanced its activity and included corrective effects on drip loss and texture. Samples treated with biopolymers/BOE included significantly lower PV, FFA, TVB-N and microbial count during cold storage, compared to the fillets treated with pure extract, composites (without BOE) and controls. Treatment efficiency as of antimicrobial agents and preserve of physicochemical characteristics were as follows of Alg/Pul/BOE > Pul/CMC/BOE > Alg/CMC/BOE. Based on the current findings, these composite coatings can be promising options to improve the quality of fresh foods during shelf-life.

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