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Active packaging films as a carrier of black cumin essential oil: Development and effect on quality and shelf-life of chicken breast meat

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ABSTRACT

Fabrication of active PET films assembled with antimicrobial chitosan and alginate coatings incorporating black cumin oil(BCO) was performed by layer-by-layer(LbL) technique and effect of active packaging film on quality and shelf-life of chicken breast meats stored at 4 °C for 5 days was investigated. Multilayer films were characterized in terms of surface morphology, color, thickness, and antimicrobial activity. Incorporation of BCO into film demonstrated antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and spherical particles on surface profile. Changes in weight loss, color, pH, total aerobic mesophilic(TAMC), and psychrotrophic bacteria counts(PBC) of chicken meat, packaged in trays containing antimicrobial films, were observed. Increase in pH values was higher in control samples than samples packaged with antimicrobial film during storage. Samples stored in active packaging had slightly lower TAMC and PBC compared to control samples. Results indicated that active film containing BCO has the potential to maintain safety and quality of chicken meat.

1. Introduction

Chicken meat is very susceptible to microbial spoilage, thus resulting a very short shelf-life. It provides an excellent substrate for the growth of microorganisms due to high moisture and protein contents. Beside microbial spoilage, lipid content of the meat leads to oxidation reactions which affect adversely meat quality in the presence of oxygen (Vaithiyanathan, Naveena, Muthukumar, Girish, & Kondaiah, 2011). The short shelf-life of chicken meat represents a high risk for consumers as well as economic losses for producers. Nowadays, applications of active packaging where active compound/s directly or indirectly interact with the packaged food product have been shown for preserving the quality and extending the shelf-life of meat and meat products (Mulla et al., 2017).

Antimicrobial food packaging is one form of active packaging, based on the incorporation of natural antimicrobial substances into packaging materials, for the purpose of eliminating undesirable changes in the quality of foods (Sung et al., 2013). Additionally, an active packaging incorporating naturally derived antimicrobial compounds such as essential oils, plant extracts becomes an alternative to use of chemical preservatives for the consumers who demand natural preservatives (Bazargani-Gilani, Aliakbarlu, & Tajik, 2015). Recently, a number of essential oils have been employed as promising natural preservatives in packaging of chicken meat. Essential oils from ginger (Noori, Zeynali, & Almasi, 2018), clove (Mulla et al., 2017), rosemary (Sirocchi et al., 2017), basil, and thyme (Sharma et al., 2017) have indicated antimicrobial effects in the shelf-life of chicken meat. Antimicrobial and antioxidant activities are primarily considered for selection of an essential oil in food packaging. However, the aromatic compounds in the essential oil are another consideration in terms of sensory quality and these compounds limit the amount of essential oil which is used in active packaging due to the effect on sensory properties of food product. As an example, researchers found 9 essential oils with high antimicrobial activity, but four of all namely, clove oil, holy basil oil, cassia oil, and thyme oil have limited utilizable/applicable concentration from sensory point of view (Sharma et al., 2017). In contrast, black cumin oil, with a mild flavour, is widely used for flavouring of foods as a spice (Bourgou, Pichette, Marzouk, & Legault, 2010).

Black cumin is known for its nutritional value, functional and pharmaceutical properties such as antimicrobial, anti-inflammatory, anticancer, antihypertensive, and antidiabetic (Ghosheh, Houdi, & Crooks, 1999). Black cumin seeds and oil have been commonly utilized in nutritional and pharmaceutical applications as a natural remedy (Bourgou et al., 2010). The seeds have been used for many diseases such as asthma, bronchitis, influenza, cough, headache and rheumatism (Ramadan, 2016). Traditionally, it is consumed as an ingredient in

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foods such as bread, bakery products, and salads. The crude black cumin oil showed strong radical scavenging activity due to its content of polyunsaturated fatty acids. Major unsaturated fatty acids in black cumin oil are oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids. (Mariod, Saeed Mirghani, & Hussein, 2017). The main active compounds of black cumin are described as carvone, D-limonene, α-pinene and *p*-cymene (Ramadan, 2007). Nair, Vasudevan, and Venkitanarayanan, (2005) indicated that black cumin oil has a significant antimicrobial activity against *Listeria monocytogenes*. Moreover, incorporation of black cumin oil into soft cheese at 0.1 and 0.2% (w/w) concentration significantly reduced the growth of foodborne pathogens including *Staphylococcus aureus, Salmonella enteritidis*, *Escherichia coli, Listeria monocytogenes* (Hassanien, Mahgoub, & El-Zahar, 2014). Thus, the use of black cumin oil as natural antimicrobial can provide antimicrobial protection in foods and increase their shelf-life. In addition, black cumin oil includes plenty of essential fatty acids and tocopherols, so it has been used in functional and dietary supplemental products (Ramadan & Wahdan, 2012).

Multilayer films of synthetic polymer joined with edible layers incorporating active compounds have gained attention in food packaging industry. In an attempt to produce multi-layer films, layer-by-layer (LbL) self-assembly technique is an effective method by deposition of oppositely charged biopolymers (Jiang et al., 2015). However, non-polar properties of various polymers such as polyethylene (PE), linear low-density polyethylene (LLDPE), and polyester (PET) restrict the layer formation. Several approaches such as corona discharge, plasma treatment, and etching with chemicals have been applied to improve adhesion property of polymers (Shin et al., 2002). Surface modification of polymers with corona discharge results in the formation of polar groups on film surface and makes available for production of multilayer films (Sadeghnejad, Aroujalian, Raisi, & Fazel, 2014). The layer-by-layer self-assembly technique offers many advantages compared to other methods that form multilayer thin films. Firstly, main advantage of this technique is an inexpensive process. The mechanism is simple and only needs immersion of the film into positive and negative charged solutions to form multilayers. Other advantage is non-specific to electrostatic forces and can be held by different types of interactions such as hydrogen bonds and hydrophobic interactions (Cheng & Swaminathan, 2018). LbL self-assembly is also widely used to produce controlled drug release in multilayer films for biomedical applications. The method enables drugs-loaded platform with desired functions and structures (Choi & Hong, 2014). In order to form multilayers, a positively charged polysaccharide or protein comes into contact with a negatively charged polysaccharide or protein. In this study, alginate and chitosan were used as biopolymers which are oppositely charged. Alginate is an anionic polysaccharide composed of (1→4) linked α-L-guluronic and β-D-mannuronic acid residues at different proportions. In contrast, chitosan is a cationic polysaccharide consisting of (1 → 4)-linked 2-amino-2-deoxy-β-D-glucan in molecular structure (Wang et al., 2014). Chitosan is commonly used in the applications of antimicrobial food packaging due to its good film-forming ability and antimicrobial activity. Previous studies have indicated that surface modified polypropylene films assembled with chitosan and pectin layers (Elsabee, Abdou, Nagy, & Eweis, 2008), chitosan and various preservatives layers (Lei et al., 2014) can be used for antimicrobial food packaging. The objectives of this work were to fabricate a novel multilayer film based on corona discharge treated PET films combined with chitosan and alginate layers incorporating black cumin oil, to determine the characteristics of the multilayer film in terms of surface morphology, color, thickness and antimicrobial activity as well as antioxidant activity (ABTS radical scavenging activity) to observe the deposition of BCO in the active film, and to investigate the effect of film on quality and shelf-life of chicken breast meat as an active packaging.

2. Materials and methods

2.1. Materials

Corona discharge treated polyethylene (PET) films (PEF) were provided from Polinas Packaging Company (Manisa, Turkey). The films were corona discharged with chemicals on one side by the company and tensile strength, oxygen transmission rate and water vapor transmission rate of PEF were given as 24 kg/mm^2, < 55 cm^3/m^2.day and < 25 g/m^2.day, respectively. Chitosan (degree of deacetylation min 85%) and alginic acid sodium salt from brown algae (medium viscosity) were supplied by Sigma-Aldrich (St. Louis, USA). Glycerol and acetic acid were purchased from Merck (Darmstadt, Germany). Cold-pressed black cumin (*Nigella sativa* L. seeds) essential oil was supplied from Gürtim Company (İzmir, Turkey).

2.2. Preparation of chitosan/alginate multilayer films

Corona discharge treated PEF were cut into 7 cm x 9 cm. Multilayer formation on PEF were formed by using the layer-by-layer (LbL) self-assembly technique with positively and negatively charged biopolymers. Alginate and chitosan, negatively and positively charged biopolymers, were selected for deposition of bilayers.

Alginate (ALG) coating solution (1% w/v) were prepared by dissolving alginate in distilled water and stirred at 500 rpm for 16 h. Then, 1% (w/v) black cumin oil (BCO) was added into alginate solution and homogenized at 9000 rpm for 15 min by using homogenizator (IKA Ultraturrax T25, Germany). Similarly, chitosan (CHI) coating solution (1% w/v) were prepared by dissolving chitosan in acetic acid solution (1% (v/v)) and stirred at 500 rpm for 24 h.

The corona treated PEF were immersed into CHI solution for 10 min and then the films were rinsed with distilled water. Then, the films were allowed to dry in air and followed by immersion into ALG solution incorporating BCO for 10 min. The films were rinsed with distilled water. This immersion process was repeated until obtaining 10 layers on the films (PEF-CHI-ALG-CHI-ALG-CHI-ALG-CHI-ALG-CHI-ALG). The outer layer of coated PEF was ALG solution incorporating BCO which contacted the food product. Number of layers on the film was decided based on the amount of loaded BCO evaluated by antioxidant activity as a percentage inhibition in multilayer films having 5, 10 and 15 layers. All the prepared films were dried at room temperature (25 ± 2 °C) prior to application.

2.3. Antioxidant activity

The antioxidant activity of coated PEF was evaluated using ABTS radical scavenging activity assay (Re et al., 1999). Film samples (0.20 g) were weighed and transferred into 10 ml of ethanol (95%). The solution was mixed at 600 rpm for 3 h and then, filtration was carried out through a filter paper to remove the insoluble film particles. For ABTS radical scavenging activity, equal amount of 7.4 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed and kept under dark for 16 h at room temperature. The absorbance of solution was arranged to 0.70 ± 0.02 at 734 nm using a spectrophotometer (Shimadzu UV-2450, Japan). Test solution was prepared by mixing 10 µl of sample and 1 ml of $ABTS^+$ solution. The solution was kept under dark for 5 min at room temperature. Then, absorbance of the solution was measured at 734 nm. The antioxidant activity of films was expressed as a percentage inhibition of absorbance calculated by following Eq. (1).

$$inhibition\% = \frac{(A_{ABTS+} - A_{sample})}{A_{ABTS+}} \times 100 \quad (1)$$

where A_{ABTS+} was the absorbance of $ABTS^+$ solution at 734 nm and A_{sample} was the absorbance of $ABTS^+$ solution mixed with sample after at 734 nm.

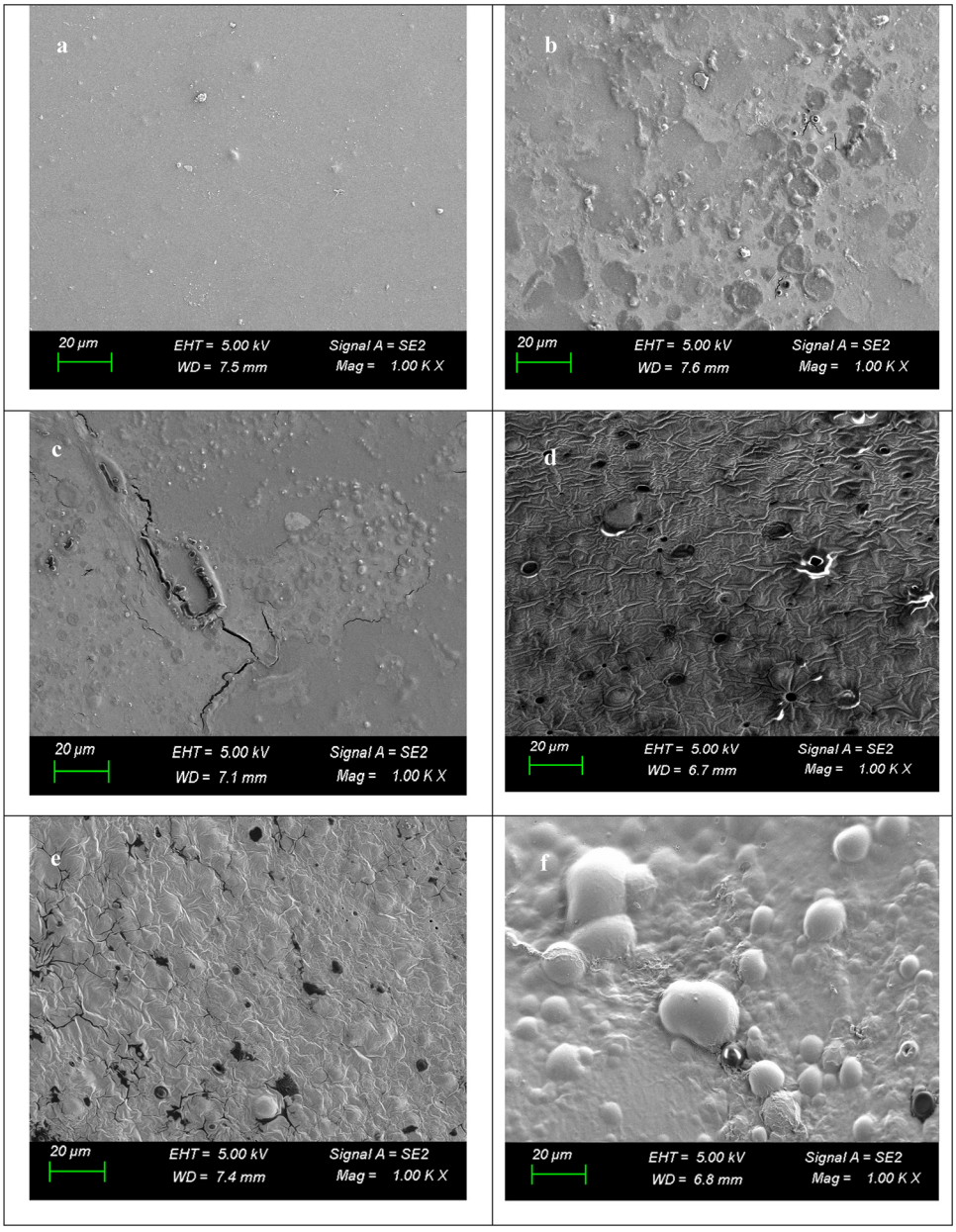


Fig. 1. SEM surface images of corona discharge treated PEF without coating (a), coated with 2 layers (b), coated with 4 layers (c), coated with 6 layers (d), coated with 8 layers (e), and coated with 10 layers (f).

2.4. Film thickness and color

The thickness of films was measured at six different points using a digital micrometer (Comecta Electronic Digital Micrometer, Cod. 5900602, Spain) with a precision of 0.001 mm. Color values of films were measured using a Minolta Colorimeter (Konica Minolta Sensing Inc., Japan) and CIE L*, a* and b* color values were measured. Six readings were performed at different positions on the films (7 cm × 9 cm). Measurements were performed in triplicate.

2.5. Scanning electron microscopy (SEM)

The morphology of the surface of the multilayer films were observed using a scanning electron microscopy (SEM, Carl Zeiss, 300 VP, Germany) operating at a voltage of 5 kV. A small piece of the film sample was coated with gold (8 nm) using QUORUM Q150 RES coater prior to the examination. Coated films were placed on the SEM, and the images were captured at the magnification of 1000×.

2.6. Antimicrobial activity of films

Antimicrobial activity of films was carried out against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) according to agar diffusion method (Siripatrawan & Vitchayakitti, 2016) with slight modifications. *S. aureus* (RSKK 95047) or *E. coli* (NRRL B-3008) cultures were obtained from the culture collection of Food Engineering Department of İzmir Institute of Technology (IZTECH). The cultures were grown in Nutrient Broth (Merck, Germany), incubated for 24 h at 37 °C, and the cell concentrations were adjusted to 0.5 McFarland unit containing 1.5×10^8 cfu/ml. The active films were aseptically cut into square shapes (1 cm × 1 cm) and three samples were placed on Petri dish containing Nutrient Agar (Merck, Germany) inoculated with 0.1 ml of inoculum having 10^8 cfu/ml of *S. aureus* and *E. coli* microorganisms. Then, the Petri dishes were incubated for 24 h at 37 °C, and the inhibition zone surrounding the film was measured in mm by using the digital micrometer.

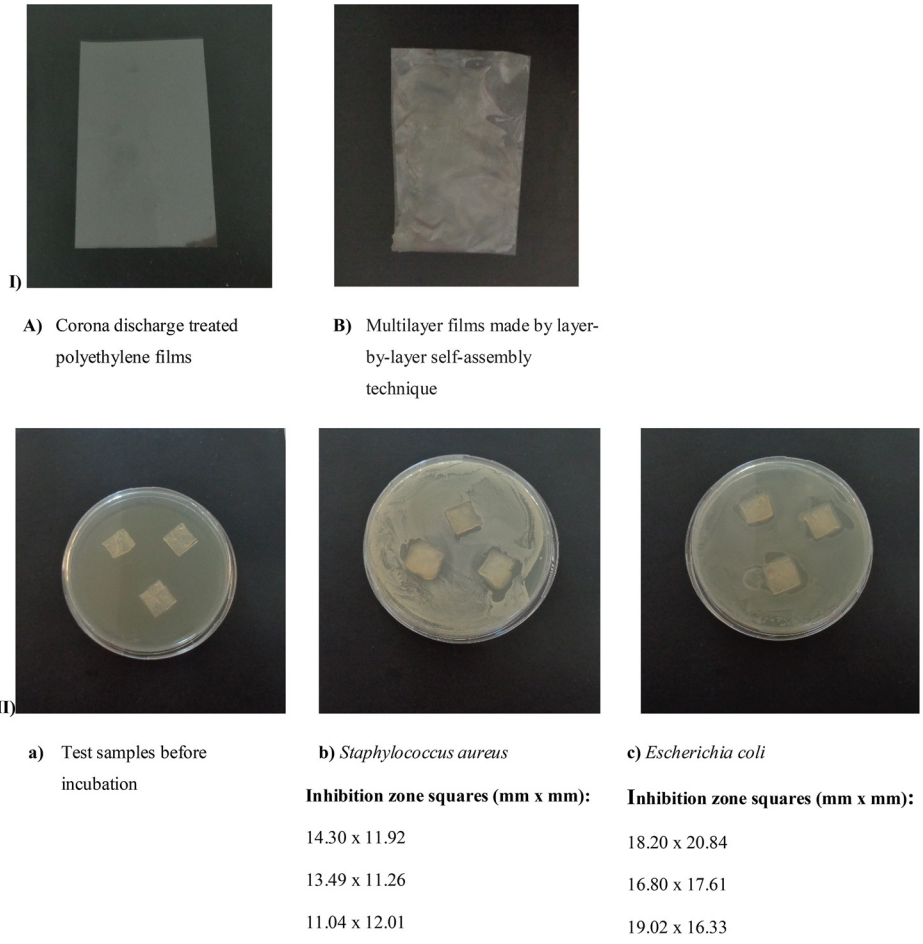


Fig. 2. Picture of the film before (A) and after (B) coating (I), antimicrobial activity of active films by agar diffusion method against *Staphylococcus aureus* and *Escherichia coli* and inhibition zone squares of film samples against test microorganisms (II).

2.7. Packaging of chicken meat samples using active films

Chicken breast meat was purchased freshly from local market in İzmir, Turkey and was aseptically cut into half (20–25 g). Corona treated PEF coated with antimicrobial coatings were placed on the inner surface of foam trays. Chicken samples were put on to the active films and aerobically packaged with cling film. Samples were in contact with the outer layer, alginate coating incorporating BCO, inside the package. Control samples were packed with the same procedure without the active film. All samples were stored under cold storage conditions of 4 °C up to 5 days. Overall quality of samples was evaluated by microbiological and physicochemical analyses at 0, 1, 3, and 5 days of storage.

2.8. Microbiological analyses of chicken meat

Chicken breast meat samples (10 g) were homogenized in 90 ml of sterile 0.1% peptone water (Merck, Darmstadt, Germany) using a stomacher (Bagmixer 400P, Interscience, France) for 1 min. Serial dilutions (1:10) were prepared in 0.1% peptone water solution and appropriate dilutions of homogenates were transferred on plates. Total aerobic mesophilic counts (TAMC) and psychrotrophic bacteria counts (PBC) were determined using plate count agar (PCA, Merck, Darmstadt, Germany) incubated at 30 °C/48 h and 4 °C/7 days, respectively. Microbiological counts were expressed as logarithms of the number of colony forming units per g sample (log cfu/g). Microbiological analyses were carried out in duplicate during storage.

2.9. Physicochemical analyses of chicken meat

2.9.1. Determination of weight loss

Weight loss was measured as a percentage loss of initial weight by recording weight changes of chicken samples during storage. All physicochemical measurements were performed in triplicate.

2.9.2. Determination of pH

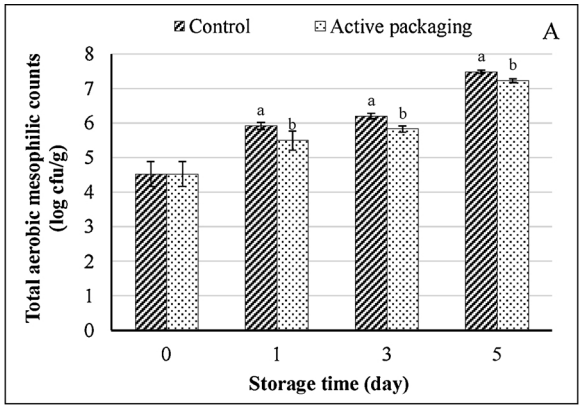
Chicken sample (10 g) was homogenized with 100 ml of distilled water for 1 min and pH value of homogenate was measured using a pH meter (Seven Compact, Mettler Toledo, USA).

2.9.3. Determination of color

Color values (CIE L*, a* and b*) of chicken breast meats were determined by using a Minolta reflective colorimeter (CR-400, Konica Minolta Sensing Inc., Japan) at 0, 1, 3 and 5 days of storage. Measurements were performed in the same chicken sample for each measurement. Hue angle (h°) and chroma (C*) were also determined and the total color change (ΔE) was calculated by the following equation:

$$\Delta E = \sqrt{(L^* - L_0^*) + (a^* - a_0^*)^2 + (b^* - b_0^*)} \quad (1)$$

where L_0^*, a_0^*, and b_0^* are lightness, redness, and yellowness values of chicken breast meats at day 0.



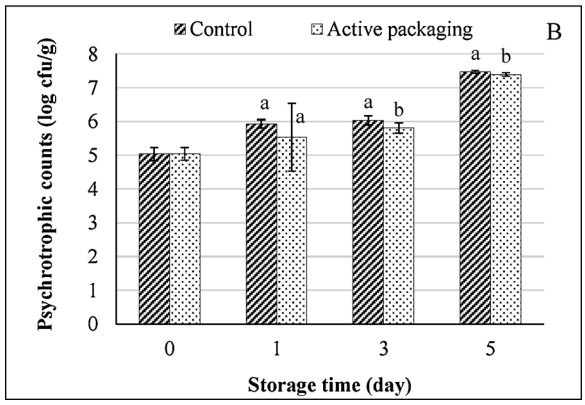
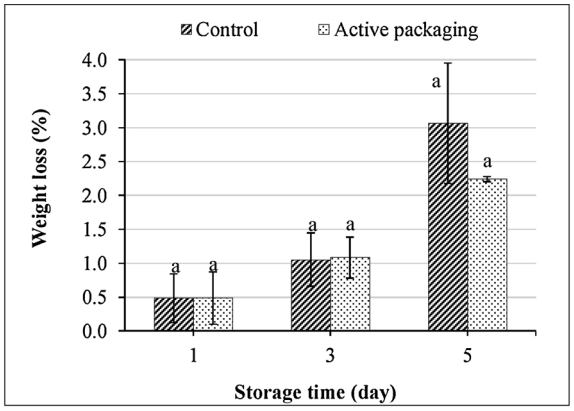


Fig. 3. Effects of active packaging on total aerobic mesophilic counts (A) and psychrotrophic counts (B) of refrigerated chicken breast meat stored at 4 °C for 5 days.



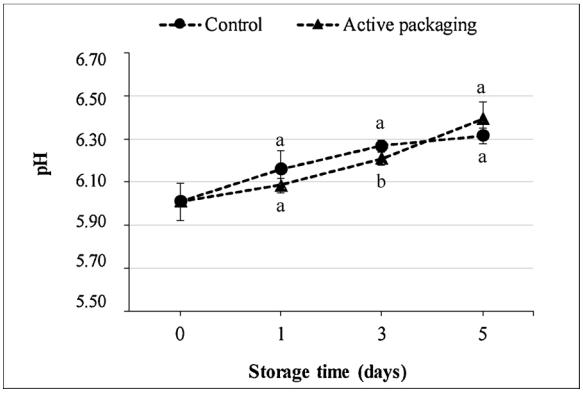


Fig. 4. Effects of active packaging on weight loss and pH values of refrigerated chicken breast meat stored at 4 °C for 5 days.

2.10. Statistical analysis

All experiments were conducted in two replications for each treatment. Results were presented as mean values with standard deviation (SD). Statistical analysis was performed by analysis of variance (ANOVA) with Minitab Software (Minitab Inc., State College, PA, USA) using Tukey's test and significant difference was considered at $p < 0.05$.

3. Results and discussions

3.1. Antioxidant activity

Number of layers in multilayer films is an important issue for the film performance and cost. Deposition of antimicrobial compounds applied to film surfaces that come in contact with food material is expected to increase by the increase in the number of layers. As mentioned above, BCO has also strong antioxidant activity derived from polyunsaturated fatty acid content (Mariod et al., 2017). Thus, deposition of BCO as active compound was examined by its antioxidant activity in the study. Antioxidant activities of multilayer films having 5, 10 and 15 layers were evaluated as percentage inhibition (%) to observe the effect of number of layers on the amount of loaded BCO. Control films (PEF) did not indicate antioxidant activity. Multilayer films incorporated with black cumin oil by means of coated layers showed an ABTS radical scavenging activity which is 21.36, 24.21, and 26.79% for the films having 5, 10 and 15 layers, respectively. The ABTS radical scavenging activity of films increased with an increase in the layer. However, no significant ($p > 0.05$) difference was observed between the ABTS radical scavenging activity of films with 10 and 15 layers. Regulation of layer number to deposit more layer than 15 layers was limited due to the difficulties such as time consuming and high cost. In this study, 10 layers were selected as the proper number of layer in terms of feasible production and loading of essential oil. Additionally, the results demonstrated that ABTS radical scavenging activity of films was limited. In order to retard the oxidation reactions in food by active packaging, the concentration of BCO in the active film could be increased.

Table 1
Effects of active packaging on CIE L*, a*, b*, C* and hº values of refrigerated chicken breast meats stored at 4 °C for 5 days.

		Days			
Color values	Treatment	0	1	3	5
L*	Control	54.11^{a}	53.62^{a}	52.11^{a}	48.21^{a}
	Active Packaging	53.51^{a}	51.85^{b}	51.65^{a}	48.77^{a}
a*	Control	0.73^{a}	0.58^{a}	0.69^{a}	1.76^{a}
	Active Packaging	1.11^{a}	1.19^{b}	1.14^{b}	1.37^{a}
b*	Control	4.84^{a}	5.50^{a}	5.85^{a}	6.57^{a}
	Active Packaging	4.79^{a}	5.38^{a}	5.53^{a}	6.66^{a}
C*	Control	4.92^{a}	5.55^{a}	5.91^{a}	6.91^{a}
	Active Packaging	4.96^{a}	5.52^{a}	5.66^{a}	6.81^{a}
h°	Control	81.49^{a}	83.55^{a}	82.99^{a}	74.96^{a}
	Active Packaging	76.60^{a}	77.26^{b}	70.94^{a}	69.83^{a}

Means of control and active packaging treatment in same column with different lowercase letters are significantly different ($p < 0.05$) for each color parameter.

3.2. Film thickness and color

Multilayer films made by layer-by-layer self-assembly technique had a thickness of 108 μm. Film thickness demonstrated the formation of multilayers on the corona discharge treated PEF having the thickness of

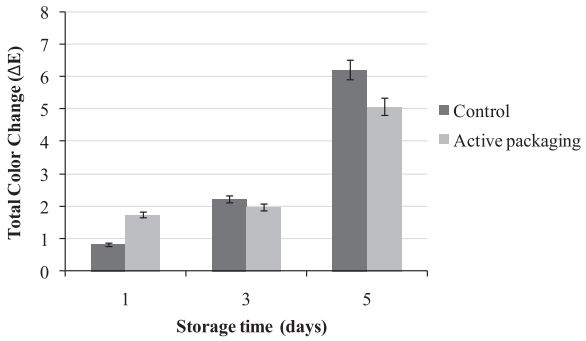


Fig. 5. Effects of active packaging on total color change (ΔE) values of refrigerated chicken meat stored at 4 °C for 5 days.

23 μm. Picture of the corona discharge treated PEF before coating and multilayer films made by layer-by-layer self-assembly technique were presented in Fig. 2. Color values were measured to observe difference in optical properties of films. Color parameters of L* (lightness), a* (redness) and b* (yellowness) for PEF without coating were 96.62 ± 0.08, 0.20 ± 0.05, and 1.98 ± 0.02, respectively. Multilayer films with 10 layers of alginate and chitosan had a L* (lightness), a* (redness) and b* (yellowness) values of 95.87 ± 0.48, -0.37 ± 0.02, 6.74 ± 0.47. The results represented that the addition of chitosan and alginate layer induced a slight decrease in lightness (L*) and redness (a*) values, and a considerable increase in yellowness (b*) value as compared to PEF. The increase in yellowness (b*) value could be mainly related to BCO in alginate layer.

3.3. Scanning electron microscopy (SEM)

Formation of LbL self-assembled multilayer films are simlpy based on electrostatic interactions, hydrogen bonds, covalent bonds, or charge-shift interactions between the deposited thin films. Film characteristics such as surface structure, roughness, and morphology depend on the used materials and their interactions (Choi & Hong, 2014). SEM surface images of corona discharge treated PEF without coating and PEF coated with 2 layers, 4 layers, 6 layers, 8 layers, and 10 layers are given in Fig. 1. The images of the PEF without coating indicated a smooth and homogeneous matrix without pores (Fig. 1a) and increase of layers on the PEF resulted in heterogeneity associated with the presence of two oppositely charged biopolymers: alginate and chitosan. The homogeneous film surface started to disrupt, and different surface morphology was observed after coating of PEF by using the layer-by-layer (LbL) self-assembly technique. Formation of 2 layers on PEF represented the interaction of positively charged chitosan and negatively charged alginate on the surface of the film (Fig. 1b). There was an increase in irregularities on the film surface related with the dispersing of more chitosan and alginate layers (Fig. 1c and d) and spherical droplets on the surface appeared in the SEM images of PEF coated with 8 layers (Fig. 1e). The significant increase of spherical and wrinkled surface was observed in PEF coated with 10 layers (Fig. 1f), which might be due to the hydrophobicity of black cumin oil incorporated into alginate layer. Similar findings were also observed by Azadbakht, Maghsoudlou, Khomiri, and Kashiri, (2018) for chitosan film containing Eucalyptus globulus essential oil. It was mentioned that increasing concentration of oil in the film led to form the droplets on surface of film. The oil droplets on the film surface proved the loading of BCO in film layers which is essential for antimicrobial activity. Homogeneous spreading of oil droplets is another consideration for development of antimicrobial film. The irregularity of the oil droplets in film surface can result in different antimicrobial activity when come in contact with food material. In a future study, nanoemulsions of film solutions could be applied to disperse the oil droplets regularly and effectively, which can also increase the antimicrobial activity of active film.

3.4. Antimicrobial activity of films

The packaging films enriched with active compounds such as antioxidants or antimicrobials may ensure the quality and safety of food products. BCO as an active compound was loaded in packaging film for antimicrobial protection of chicken meat. The antimicrobial activity of corona treated PEF coated with chitosan and alginate layers containing BCO was determined against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) which are common foodborne pathogenic bacteria (Kim, Kim, Lee, Hwang, & Rhee, 2014). Inhibition zone squares against test microorganisms are presented in Fig. 2. Active films showed a considerable inhibitory effect against *E. coli*, but there was slight inhibitory zone surrounding the film for *S. aureus*. Chitosan is well known with its antimicrobial property; however, the limited antimicrobial activity of chitosan films was reported by Tripathi, Mehrotra, and Dutta (2008). While the chitosan film containing propolis extract showed an inhibitory effect against all bacteria, the chitosan film without propolis did not showed any inhibitory effect and this was explained by the decrease in antimicrobial activity of chitosan when it is in the form of an insoluble film (Siripatrawan & Vitchayakitti, 2016). In this study, the results suggested that the developed films based on chitosan and alginate coating incorporating BCO had an antimicrobial activity against Gram-negative bacteria, *E. coli*, and Gram-positive bacteria, *S. aureus*. The results showed that *S. aureus* was more resistant than *E. coli* against BCO incorporated at 1% (w/v) ratio into the film. However, *E. coli* growth was effectively inhibited by active films. This was probably related to different structure of Gram-negative and Gram-positive bacteria. Therefore, application of the active films incorporated with BCO could improve the safety of meat and meat products.

3.5. Microbiological analyses of chicken meat

Changes in TAMC and PBC of samples are shown in Fig. 3A and Fig. 3B, respectively. TAMC and PBC values of chicken breast meats in both treatments increased during storage at 4 °C. The increase was higher in control samples than the samples in active packaging. The initial TAMC of chicken breast meat was 4.53 log cfu/g and TAMC exceeded the value of 7.00 log cfu/g which is the limit of acceptability on day 5. The results for the initial TAMC were in agreement with the study of Bazargani-Gilani et al. (2015) which was found as 4.85 log cfu/g for TAMC of fresh chicken breasts. Mexis, Chouliara, and Kontominas, (2012) reported also an increase in total viable counts of chicken meat packaged with an O_2 absorber from initial value of 5.9 log cfu/g to 8.7 log cfu/g after 10 days of storage at 4 °C.

Effect of active packaging for TAMC was suppressing slightly the growth of microorganisms during storage at 4 °C. Moreover, PBC values were not significantly differentiate between control samples and samples in active packaging until day 3, then the control samples had significantly higher PBC than samples in active packaging. As presented in Fig. 3B, at day 3 and day 5, chicken breast meat in active packaging indicated lower psychrotrophic counts. The antimicrobial activity mechanism of BCO is unclear and it can be dissimilar against different kinds of microorganisms. It was concluded that antimicrobial packaging incorporating BCO was more effective against total aerobic mesophilic bacteria compared to psychrotrophic bacteria for chicken breast meat.

3.6. Physicochemical analyses

3.6.1. Changes in weight loss and pH values

Changes in weight loss with respect the storage time are presented in Fig. 4. The weight loss of all samples increased during storage. No significant differences ($p > 0.05$) were observed in weight loss between samples packaged with active films and control during storage. Thus, the active packaging application had no significant ($p > 0.05$) impact on weight loss of chicken breast meats.

The effect of active packaging application on the pH values of chicken breast meats are shown in Fig. 4. The initial pH of fresh chicken breast was 6.01 and pH values of the samples slightly increased during 5 days of storage in both control and active packaged samples. During storage, the pH values of samples were lower in the active packaged samples than in control samples. This lower pH in samples in active packaging could be associated with the presence of chitosan in the multilayer film. A similar manner was observed by the study which performed chitosan active coatings application for fresh chicken meat refrigerated at 4 °C (Hassanzadeh et al., 2017). However, on day 5, pH values of chicken breast meat samples in active packaging and control samples were 6.40 and 6.32, respectively. Although, the mild increase occurred in pH of active packaged chicken breast meats until 5 days of storage, pH of samples in active packaging was higher than that in control samples at the end of storage. A major reason for increase in pH of chicken breast meats may be the accumulation of metabolites from microbial growth such as amines and ammonia produced by psychrotrophic bacteria (Cortez-Vega, Pizato, & Prentice, 2012).

3.6.2. Changes in color values

Consumer decisions on purchase of fresh meat significantly based on color which is influenced by packaging, type and aging of meat (Suman, Hunt, Nair, & Rentfrow, 2014). Changes in surface CIE L*, a*, b*, C^*, and h° values of chicken breast meat samples throughout storage are shown in Table 1. L* values of samples diminished from 53.51 and 54.11 at day 0 to 48.77 and 48.21 at day 5 for the chicken breast meats packaged with active film and control. The decrease in lightness indicates that the chicken breast meats became darker during storage. The results for lightness were not in agreement with some studies which mentioned increase in lightness of meat during refrigerated storage (Bingol & Ergun, 2012). In contrast, decrease in lightness of chicken breast meats during refrigerated storage was reported by Cortez-Vega et al. (2012) and our results were compatible with their findings. CIE a* values, in other words redness values, indicated fluctuation during storage. At the beginning of storage, *a** values of control samples slightly decreased and that of active packaged samples maintained almost stable. However, all samples showed a dramatic increase in redness between 3 and 5 days of storage. CIE b* values, represents yellowness of the chicken breast meats, increased during storage. The results obtained for b* values were in accordance with the study which also exhibited an increase in b* value in vacuum packaging based on polybutylene succinate (PBS) and polybutylene succinate-co-adipate (PBSA) for chicken meat stored at 4 °C for 5 days (Vytejčková et al., 2017). The chroma value of samples did not show significant difference during storage. Hue angles showed significant difference at day 1 and were higher in control samples. Furthermore, total color changes (ΔE) of chicken breast meat samples increased during storage (Fig. 5). During storage, the change in ΔE values was higher in control samples compared to active packaged samples.

4. Conclusion

The utility of active packaging applications for food packaging depends mainly on its functional properties such as antimicrobial and antioxidant activities. Novel multilayer films based on alginate and chitosan layers were developed and characterized as a potential antimicrobial carrier for meat packaging. Corona treated PEF coated with chitosan and alginate coatings incorporating black cumin oil (BCO) indicated antimicrobial activity against *E. coli* (Gram-negative) bacteria which is an important foodborne pathogen especially in raw meat products. Active packaging based on the fabricated antimicrobial films provided lower microbial growth, less variation in pH, lower color changes for chicken breast meats during 5 days of storage at 4 °C. In compliance with the obtained results, antimicrobial active packaging incorporating BCO can be used as a promising system to maintain quality and safety of fresh meat products. In order to increase effectiveness of the antimicrobial packaging, higher BCO concentrations could also be investigated in further studies.

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