



Original research

## Effect of vacuum packaging and edible coating containing black pepper seeds and turmeric extracts on shelf life extension of chicken breast fillets

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### ABSTRACT

Poultry meat is a susceptible product for growth of spoilage and pathogenic microorganisms. The shelf life extension of raw poultry meat is a challenge in food industry. In this research, the combined effect of dipping in a carboxymethyl cellulose (CMC) solution containing black pepper seed (*Piper Nigrum*) extract (BPE) and turmeric (*Curcuma Longa* L.) extract (TE) (2% w/w) and vacuum packaging (VP) on shelf life extension of refrigerated chicken breast fillets was investigated. The parameters that were studied were microbiological (total aerobic mesophilic bacteria (TAMB) and total aerobic psychrotrophic bacteria (TAPB)), chemical (FFA, PV, TBARS and TVB-N) and sensory (odor, appearance and total acceptability) attributes. The coating containing TE showed a strong antimicrobial activity against TAMB and TAPB, but the effect increased synergistically in the presence of VP. The lowest FFA, PV and TBARS levels found in the vacuum packaged fillets coated with 2% TE in CMC solution. Color and appearance, odor and overall acceptability of VP-CMC-TE sample were the best during storage. Generally, the TE was more effective than BPE in extending the durability of chicken breast fillets.

Keywords: Chicken breast, Vacuum packaging, Edible coating, Antimicrobial extracts, Shelf life extension

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## 1. Introduction

Poultry meat is a very popular food commodity around the world and its consumption has increased over the last decades in many countries. Some of the reasons for the popularity are the relatively low cost of production as compared to meat products as beef or lamb, low fat content, high nutritional value and distinct flavor of poultry meat (Latou et al., 2014). However, because of its composition (high moisture and protein content) and high pH, poultry meat is ideal for growth of spoilage and pathogenic microorganisms (Sharma et al., 2017). Shelf life extension of raw poultry meat comprises a challenge for the poultry industry around the world.

One of the traditional ways of controlling microbial growth in these products is application of antimicrobial or antioxidant dips or sprays on the surface of the product (Hygreeva et al., 2014). Consumers' growing concern over the safety of foods containing

synthetic chemical preservatives has led to the investigation of alternative "natural" preservation technologies (Ahmad et al., 2015). Several natural antimicrobial and antioxidant agents have been used to direct addition to meat products (Noori et al., 2018). However, in this form of application, efficiency of the antimicrobial substances is restricted due to uncontrolled migration into the food and partial inactivation of the active compounds because of the interaction with food components.

One new approach to overcome these limitations is the use of antimicrobial edible films and coatings that are able to increase the shelf life of food products upon direct contact (Umaraw & Verma, 2017). The efficiency of various edible coatings incorporated by different herbal extracts such as mustard extract (Olaïmat & Holley, 2016), green tea extract (Özvural et al., 2016), sumac extract (Mojaddar Langroodi et al., 2018) and henna extract (Jridi et al., 2018) on the shelf life extension of chicken and meat products has been investigated.

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According to literature review, the preservative effect of black pepper seeds (*Piper Nigrum*) and Turmeric (*Curcuma Longa* L.) extracts on real food systems has not been reported. Turmeric belonging to the family of *Zingiberaceae*, is a perennial rhizomatous shrub native to Southern Asia (Gupta et al., 2015). The main chemical compounds of turmeric are curcumin, desmethoxycurcumin and bisdesmethoxycurcumin, three bioactive substances commonly used as spices and coloring agents in food. Turmeric has also anti-inflammatory, antifungal, antimicrobial, antioxidant and anti-proliferative properties (Zamarioli et al., 2015). Pepper is a natural spice. It is widely cultivated throughout the world and its usage as a spice is well known. *P. nigrum* (black pepper) finds extensive use in Iranian folk medicine (Haghju & Almasi, 2015). Black pepper finds an extensive use in traditional antibacterial preparations. A number of piperidine and pyrrolidine alkaloids are known to occur in *P. nigrum*. The piperine as the most important bioactive compound of black pepper is known to possess antibacterial properties (Venkat Reddy et al., 2004).

Another approach to increase the shelf life of meat and poultry is using of vacuum packaging (VP). The preservative effect of VP is due to the creation of an oxygen-deficient environment resulting in a severe or total inhibition of potential spoilage organisms. Spoilage of chicken generally occurs when the aerobic plate count reaches  $10^7$ – $10^8$  CFU.g<sup>-1</sup>, which is usually after 4 days. By using VP, the shelf life of chicken can be extended up to 7 days (almost double) when stored at 4–8°C (Narasimha Rao & Sachindra, 2002). Several research groups have been investigated the effect of VP alone or in combination with other procedures to increase the shelf life of chicken (Pavelková et al., 2014) or other meat products (Redondo-Solano et al., 2013; Brenesselová et al., 2015).

To our knowledge, no definite data exist on the application of carboxymethyl cellulose (CMC) based edible coating in chicken meat products. Also, the black pepper seeds and turmeric extracts have not been used in the formulation of active edible coatings. Therefore, the objective of the present work was to study the combined effect of CMC based antimicrobial active coating containing black pepper seeds and turmeric extracts and VP to extend the shelf life of fresh chicken breast fillets stored at 4°C.

## 2. Material and Methods

### 2.1. Materials

Black pepper seeds (*Piper Nigrum*) and turmeric (*Curcuma Longa* L.) water extracts (95%) were purchased from Magnolia flavor & fragrance Co. (Saveh, Iran). Food-grade CMC (99.9%) with an average molecular weight of 41,000 g.mol<sup>-1</sup> (obtained from Caragum Parsian, Tehran, Iran) was used for the preparation of coating formulations. Glycerol, and other reagents used were of analytical grade and were purchased from Merck (Darmstadt, Germany).

### 2.2. Preparation of coating solution

The 1.5 g CMC were dissolved in 100 mL distilled water and stirred at a controlled temperature of 75°C until the mixture became clear. 0.03 g (2% wt. of CMC) glycerol was added as plasticizer. Then, solutions were cooled to 50°C and antimicrobial agents (black pepper seeds and turmeric extracts) was added with constant stirring. The amount of antimicrobial agent incorporated into the coating solutions was 2% wt. of CMC. This concentration was

selected according to the previously conducted taste panel experiments.

### 2.3. Preparation and treatment of chicken meat

Fresh chicken breast fillets were provided by a local poultry processing plant (Atameh Pars Co., Tehran, Iran) within one hour after slaughter in insulated polystyrene boxes in ice. Chicken meat fillets were aseptically portioned into three smaller pieces weighing 200–250 g. The prepared chicken fillets were coated by dipping them into the prepared coating solutions for 120 s at room temperature, then drying for 60 s. The coated and dried fillets were divided into two groups, for vacuum and aerobic packaging. Coated chicken breast fillets (about 1000 g) were stored in low-O<sub>2</sub>-permeable (8–12 mL/m<sup>2</sup>/24 h at standard temperature and pressure-STP) polystyrene/ethylvinylalcohol (EVOH)/polyethylene (PE) trays and then, all samples were placed in low density polyethylene/polyamide/low density polyethylene (LDPE/PA/LDPE) barrier pouches. For aerobic packaging, pouches were heat-sealed using a BOSS model N48 sealer (BOSS, Bad Homburg, Germany). For preparing vacuum packaged fillets, each sample was packaged using a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic).

The following lots of samples were prepared: Air-packaged control samples (without any coating) (AP), Vacuum-packaged control samples (VP), AP with neat CMC coating (AP-CMC), VP with neat CMC coating (VP-CMC), AP with CMC coating containing 2% of black pepper seeds extract (AP-CMC-BPE), VP with CMC coating containing 2% of black pepper seeds extract (VP-CMC-BPE), AP with CMC coating containing 2% of turmeric extract (AP-CMC-TE) and VP with CMC coating containing 2% of turmeric extract (VP-CMC-TE). All samples were kept refrigerated at 4°C for up to 16 days. Sampling was carried out on days 0, 4, 8, 12 and 16 of storage.

### 2.4. Meat quality testing

#### 2.4.1. Microbiological analyses

Chicken fillet samples (25 g) were transferred aseptically into individual stomacher bags (Seward Medical, UK), containing 225 mL of sterile buffered peptone water (BPW) solution (0.1 g.100<sup>-1</sup> mL) and homogenized in a stomacher (Lab Blender 400, Seward Medical, UK) for 60 s. Serial decimal dilutions were prepared in BPW (0.1 g.100<sup>-1</sup> mL) and 0.1 mL aliquots were spread on the surface of agar plates. Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrotrophic bacteria (TAPB) were determined on Plate Count Agar (PCA, Merck code 1.05463, Darmstadt, Germany), after incubation for 48 h at 37°C and 10 days at 4°C, respectively. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU.g<sup>-1</sup>).

#### 2.4.2. Total lipid extraction

Total lipid content of fillets was determined according to the method of Bligh and Dyer (1959). One gram of each sample was homogenized in 10 mL methanol in an Ultra Turrax for 2 min at 1200 rpm; 20 mL chloroform was added and the mixture was homogenized for a further 2 min, followed by vacuum-filtration through Whatman No. 1 filter paper. The extraction procedure for the residue was repeated and the supernatants combined. The

solvents were evaporated under vacuum at 40°C and total lipid was achieved. Extracted lipid was used for free fatty acids (FFA) and peroxide value (PV) measurements.

#### 2.4.3. Free fatty acids (FFA) measurements

FFA content of chicken breast's lipid was determined according to AOCS method (AOCS, 1955). For determination of acid value, two grams of the extracted lipid was dissolved in 50 mL of 1:1 mixed diethyl ether and ethanol carefully neutralized with 0.1 M NaOH using 1% phenolphthalein solution. The mixture was titrated with 0.1 M NaOH aqueous solution with constant shaking to faint pink color. Acid value was calculated by equation 1:

$$\text{Acid value (mgKOH.g}^{-1}\text{)} = \frac{\text{Titre value} \times 5 \times 61 \times 0.00282}{\text{Weight of sample (g)}} \quad (1)$$

The amount of FFA was calculated as being equivalent to half the value of acid value and reported as g.100 g<sup>-1</sup> or percent of lipid based on oleic acid.

#### 2.4.4. Peroxide value (PV) measurements

PV, expressed as meqO<sub>2</sub>.kg<sup>-1</sup> lipid, was determined in the lipid extract of samples according to method AOAC 965.33 (AOAC, 1990). Approximately 2 g of extracted lipid from each sample was weighed into a 250 mL Erlenmeyer flask and 25 mL of a mixture of acetic acid/chloroform (3:2 v/v) added; the contents were stirred continuously in order to dissolve the fat completely. A saturated solution of potassium iodide (1 mL) was added and the flask was allowed to stand for 1 min with occasional agitation. Distilled water (30 mL) was added and titrated against 0.01 N sodium thiosulfate, with 0.5 mL of 1% starch as an indicator. The blank was determined by titration of the samples not containing fat. The PV was calculated as follows:

$$\text{PV (meqO}_2\text{.kg oil}^{-1}\text{)} = \frac{(S - B) \times N \times 1000}{\text{sample weight}} \quad (2)$$

where S is the volume consumed during titration of the sample (mL); B is the volume consumed in the evaluation of the blank (mL); N is the normality of the sodium thiosulfate solution; and sample weight is the weight (g) of fat.

#### 2.4.5. Determination of thiobarbituric acid (TBA) value

Thiobarbituric acid reactive substances (TBARS) were determined as described by Song et al. (2011). Chicken fillet (5 g) was dispersed in 20 mL of thiobarbituric acid solution (0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 mol/l HCl). The mixture was heated in boiling water for 10 min, cooled with water and centrifuged at 3600 g for 20 min at room temperature. The absorbance of the supernatant was measured at 532 nm (Unico, S 2100 SUV, Dayton, NJ, USA). The standard curve was prepared using malondialdehyde (MDA). TBARS content was expressed as mg of MDA.kg<sup>-1</sup> chicken meat.

#### 2.4.6. Determination of total volatile basic nitrogen (TVB-N)

The micro-titration method was employed to analyze TVB-N. 10 g sample of chicken meat was dispersed in 100 mL of distilled water and stirred for 30 min, and then the mixture was filtered. After the addition of 5 mL MgO (10 g.l<sup>-1</sup>) to 5 mL filtrate, the mixture was to distill through Kjeldahl Apparatus (KDY-9820, Beijing, China). The distillate was absorbed by 20 mL aqueous solution of boric acid (2%) containing a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.1 g of methylene blue to 100 mL of ethanol. Afterward, the boric acid solution was titrated with a 0.01 mol.l<sup>-1</sup> hydrochloric acid (HCl) solution. TVB-N value was determined according to the consumption of hydrochloric acid (Noori et al., 2018):

$$\text{TVB} - \text{N} = \frac{\text{Consumed acid} \times 100}{\text{Weight of sample}} \times \frac{1}{4} \quad (3)$$

#### 2.4.7. Sensory evaluation

An experienced 10-member team at department of food science of Tabriz University evaluated the samples for the attributes of appearance and color, odor and total acceptance using a five-point descriptive hedonic scale, where 5=like extremely and 1=dislike extremely.

#### 2.5. Statistical analysis

All assays were performed in triplicate. Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure in SPSS (Version 21, SPSS Inc., Chicago, IL, USA) software. Duncan's multiple range test (p < 0.05) was used to detect differences among mean values of chicken fillets' properties in all test intervals.

### 3. Results and Discussion

#### 3.1. Microbial analysis

Changes in aerobic mesophilic flora of the chicken fillets stored both in air and VP conditions and subjected to the various antimicrobial coatings are presented in Fig. 1A. The initial number of TAMB of chicken breasts was 3.56 log CFU.g<sup>-1</sup> indicates acceptable quality and it increased during storage in all treatments. Total microbes of chicken breasts packaged under VP were significantly lower than that of control AP sample during all storage period (p < 0.05). The 1.03 of decimal reduction on TAMB for the VP samples is due to the displacement of the oxygen available for bacterial metabolism in the headspace of the packages. These results are in agreement with the findings reported by Ntzimani et al. (2010). Similar results were reported by Pavelková et al. (2014), who investigated the effect of VP on the bacterial flora of chicken breast. Lower population of total viable counts (TVC) was also observed in vacuum-packaged ostrich meat (Brenesselová et al., 2015) and ham (Redondo-Solano et al., 2013) in comparison to the air-packaged samples.

Chicken breast coated with pure CMC had a significantly lower number of total aerobic bacteria throughout the storage period (p < 0.05) and those of the AP-CMC samples were significantly lower than those of AP and VP after 16 days of storage (p < 0.05). Fernández-Saiz et al. (2013) found that the using of chitosan film ensured a decrease of 1–3 logarithmic units in pathogen number. The antimicrobial activity of chitosan is well documented against a

number of food spoilage and pathogenic microorganisms. However, Fernández-Pan et al. (2014), reported that the presence of the WPI coatings (without essential oils) did not have a significant antimicrobial effect against spoilage and pathogenic bacteria. It was attributed to an increased availability of protein (WPI) or carbohydrate (glycerol) sources for microbial growth. In this study, it was observed that the antimicrobial action of CMC was similar to that of chitosan. CMC has not any distinct antimicrobial activity and these observations may be attributed to the reducing O<sub>2</sub> availability and water activity at the surface of chicken fillets that causes to inhibit the microbial growth. When the vacuum packaging was combined with pure CMC coating, there was a significant effect on controlling TAMB ( $p < 0.05$ ). Combination of VP and CMC coating showed a 2 decimal reduction on total aerobic bacteria presented in chicken breast at day 16.

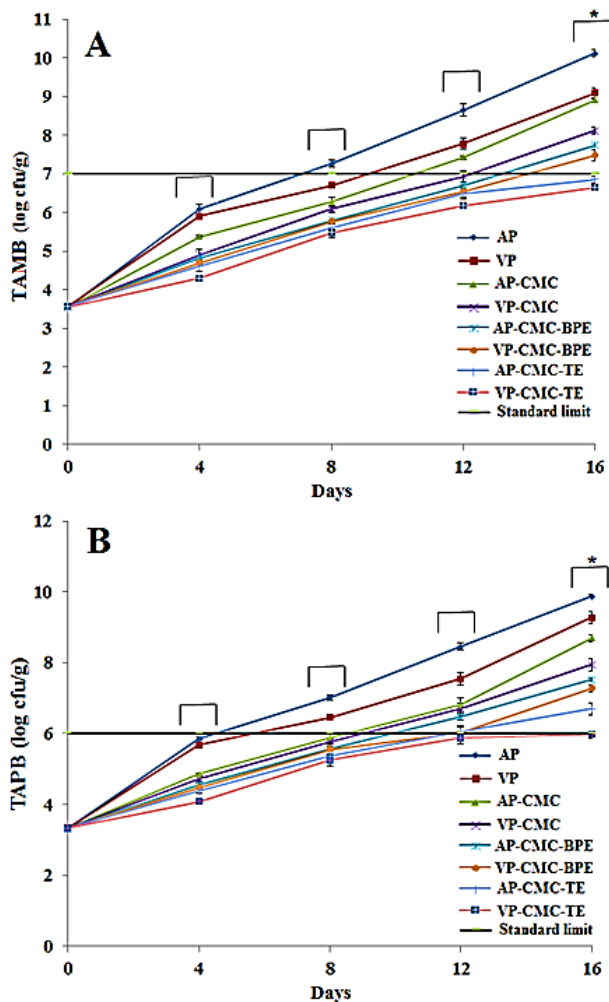


Fig. 1. Number of total aerobic mesophilic bacteria (TAMB) (A) and total aerobic psychrotrophic bacteria (TAPB) (B) (log CFU/g) in the air packaged (AP) and vacuum packaged (VP) chicken breasts coated with CMC based coatings containing antimicrobial agents (black pepper seeds extract (BPE) and turmeric extract (TE)) during storage at 4°C. There is significant difference ( $p < 0.05$ ) between treatments within groups identified by star.

It was observed that for the same air-packaged or vacuum-packaged samples, the edible coatings containing TE showed a higher effectiveness against the aerobic mesophilic bacteria, compared with edible coatings containing BPE. These observations were confirmed by the study of Gupta et al. (2015) who evaluated antimicrobial activity of extracts of *C. longa* tubers against six bacterial strains. The simultaneous application of the antimicrobial edible coating and VP was more lethal than either treatment used alone. The population of TAMB reached the value of 7 log CFU.g<sup>-1</sup>, which is considered as the upper microbiological acceptability limit, as defined by the International Commission on Microbiological Specifications for Foods (ICMSF, 1998), on day 7 for the air-packaged samples, day 9 for the vacuum-packaged samples, day 12 for the VP-CMC samples, and day 14 for VP-CMC-BPE samples, whereas this level was never reached in air-packaged and vacuum-packaged samples coated with 2% TE-contained edible coating (AP-CMC-TE and VP-CMC-TE) during 16 days of storage.

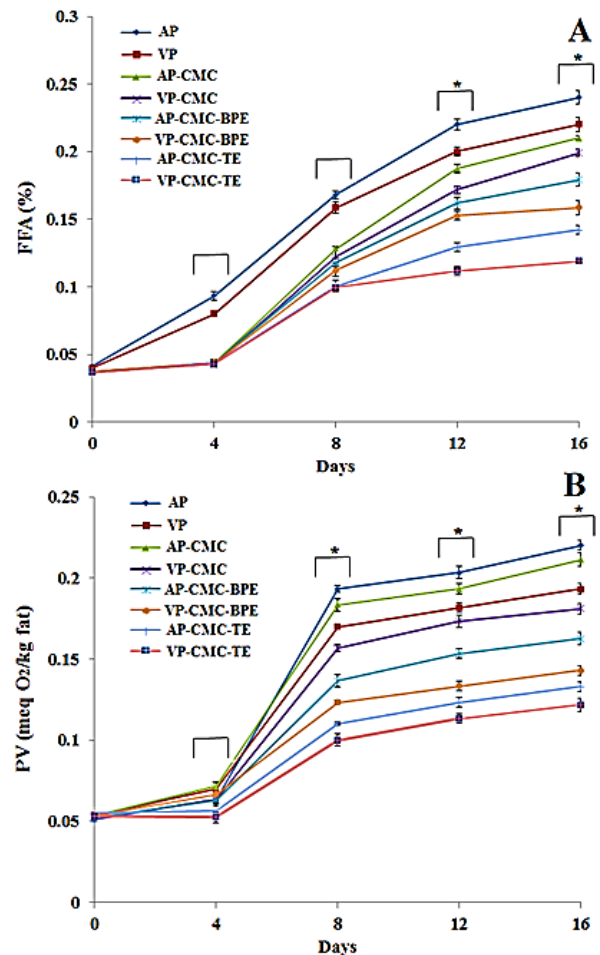


Fig. 2. Free fatty acids (FFA) (A) and peroxide value (PV) (B) in the extracted lipid of air packaged (AP) and vacuum packaged (VP) chicken breasts coated with CMC based coatings containing antimicrobial agents (black pepper seeds extract (BPE) and turmeric extract (TE)) during storage at 4°C. There is significant difference ( $p < 0.05$ ) between treatments within groups identified by star.

Psychrotrophic microorganisms are the primary organisms responsible for meat spoilage at the refrigerated storage conditions. The initial number of total aerobic psychrotrophic bacteria (TAPB) of chicken breasts increased during storage in all treatments and the number of them in control AP sample was over than  $6 \log \text{CFU.g}^{-1}$  at day 4 (Fig. 1B). In VP samples the final bacterial levels decreased by about 0.8 logs compared with the control AP. Total psychrotrophic microbes of chicken breasts coated with different formulations had no significant difference with each other throughout 12 days of storage ( $p > 0.05$ ). TAPB reached a value of  $6 \log \text{CFU.g}^{-1}$ , on day 8-12 for all the coated air-packaged and vacuum-packaged samples, except of VP-CMC-TE sample that this level was never reached in the mentioned sample during the 16 days of storage at  $4^\circ\text{C}$ . Murali (2012) reported that the solution of 10% TE is able to decrease more than  $5 \log \text{CFU.mL}^{-1}$  of the psychrotrophic bacteria population after 24 h. Turmeric owes its characteristic yellow color to three major pigments; curcumin (50-60%), demethoxycurcumin (20-30%) and bisdemethoxycurcumin (7-20%). All these curcuminoids are known to have antioxidant and antimicrobial activities (Abdou et al., 2018). Egan et al. (2004) reported that curcumin is an important natural colorant used in food, and it has antimicrobial effects against many microorganisms, especially against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. Furthermore, Liang et al. (2007) found that the curcumin can inhibit the growth of *Bacillus typhi*, and *Bacillus dysenteriae* and it has a good preservation effect on cooked mutton, bread and bean curd.

The decimal reduction of TAPB after 16 days of storage at  $4^\circ\text{C}$  was 0.8, 1.93, 2.6 and 3.9 for VP, VP-CMC, VP-CMC-BPE and VP-CMC-TE samples, respectively. However, the decimal reduction of TAMB after 16 days of storage at  $37^\circ\text{C}$  was 1.03, 2, 2.64 and 4.1 for these samples, respectively. These results revealed that the inhibitory effect of vacuum packaging, antimicrobial coating and the combination of them against aerobic mesophilic bacteria is more effective than aerobic psychrotrophic bacteria. These results on the effect of antimicrobial edible coatings on the mesophilic and psychrotrophic bacteria are in good agreement with those reported by Jonaidi Jafari et al. (2018) for chicken meat.

### 3.2. Free fatty acids (FFA) contents and peroxide value (PV)

The FFA percentage is a value to show the degree of lipid hydrolysis. Although the FFA content has not direct effect on quality attributes of poultry, however, the hydrolyzed lipids are more susceptible to oxidation. The FFA content increased progressively in all the samples (Fig. 2A) by increasing storage time. But the rate of this increment was lower in the coated samples. The FFA content of vacuum-packaged chicken fillets was significantly ( $p < 0.05$ ) lower than air packaged ones. The levels of FFA content were held steady for the first 4 days for coated samples and there were no significant differences ( $p > 0.05$ ) between the various coated treatments until 8 days. However, at day 16, a significant decrease ( $p < 0.05$ ) in FFA content was recorded in the vacuum-packaged and coated samples. The sample of VP-CMC-TE resulted the minimum FFA content. Decreasing in percentage of FFA by applying the vacuum packaging and active coatings could be attributed to the inhibition of microbial activity during storage of chicken breasts. By increasing of microbial inactivation rates in treated samples, the microbial lipase induced lipolysis of triglycerides and thus, the FFA contents were decreased significantly ( $p < 0.05$ ).

Hydroperoxides are the primary products of auto-oxidation of lipids. PV was used to monitor the effect of the coating type and packaging conditions on the oxidation of chicken breast's lipid. Fig. 2B plots the changes of PV for the different samples over 16 days at  $4^\circ\text{C}$ . Coating type and packaging conditions did not result in lipid oxidation as measured by the PV during 4 days of storage. However, a sharp increase was observed in PV levels in day 8 indicating the start of induction period of auto-oxidation process in chicken breast's lipid. At day 16, a significant decrease ( $p < 0.05$ ) in PV was recorded in the vacuum-packaged samples coated with the TE, and this finding was consistent with the observed decrease in the FFA contents (Fig. 2A). Degree of hydrolysis, presence of antioxidants and oxygen availability are three major factors affecting the auto-oxidation rate of lipids. Therefore, it could be concluded that the decreasing of FFA contents as a result of microbial growth prevention, antioxidant activity of natural extracts (specially TE (Zamarioli et al., 2015)) and decreased oxygen availability due to the use of VP are the reasons of decreased PV in treated samples. As can be seen in Fig. 2B, combination of VP and active coating has synergistic effect on the increasing of oxidative stability of chicken breast's lipid.

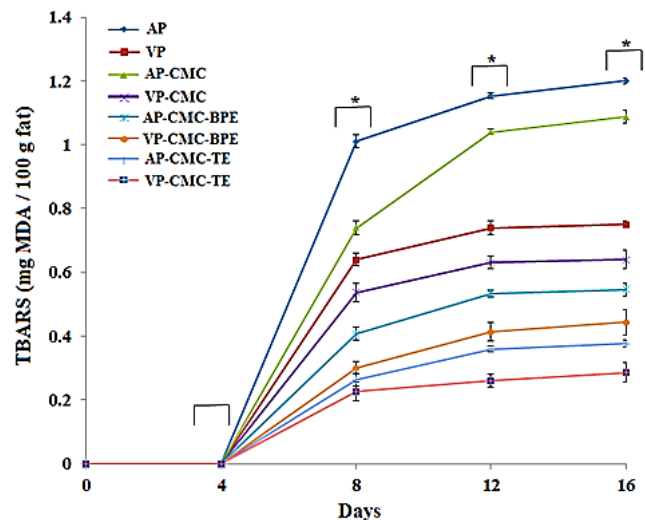


Fig. 3. TBARS index of air packaged (AP) and vacuum packaged (VP) chicken breasts coated with CMC based coatings containing antimicrobial agents (black pepper seeds extract (BPE) and turmeric extract (TE)) during storage at  $4^\circ\text{C}$ . There is significant difference ( $p < 0.05$ ) between treatments within groups identified by star.

### 3.3. TBARS index

Hydroperoxides are the primary products of auto-oxidation which in themselves are odorless. However, their decomposition to secondary products leads to the formation of a broad range of carbonyl compounds, hydrocarbons, furans and other products that contribute to the rancid taste of decaying food. TBARS is widely used as an indicator of the degree of lipid oxidation in foods. The increase in TBARS during storage is due to the increased oxidation of unsaturated fatty acids. The bitterness of meat increases as the TBARS values increase. It may also result in a change of the color. Moreover, the sensorial characteristics may be affected negatively (Sharma et al., 2017). Secondary products of lipid oxidation, as determined by TBARS measurements, are shown in Fig. 3. After 4

days of storage, the TBARS value of fresh chicken fillets ( $0.02 \text{ mg.kg}^{-1}$ ) increased significantly ( $p < 0.05$ ) because of higher availability of substrate and instability of peroxide molecules over time. The pure CMC coating on the surface of fillets, prevented the interaction between air and the surface of meat, and therefore the oxidation was decreased (PV results). As a result, the TBARS values were lowered. Moreover, the TBARS values of chicken breasts in VP were lower than those in AP throughout the storage period.

At day 16, TBARS values for all chicken breast treatments varied between  $0.29$  and  $1.21 \text{ mg MDA.kg}^{-1}$  meat (Fig. 3). These values are well below the  $2 \text{ mg.kg}^{-1}$  threshold at which rancid off-flavors become noticeable (Sharma et al., 2017). The vacuum-packaged fillets coated with CMC coating solution containing TE showed lower TBARS value than the other treatments. This finding was in consistent with the observed decrease in the FFA (Fig. 2A) and PV (Fig. 2B) contents. In addition to its inherent ability to attenuate the reactivity of oxygen free radical species, curcumin has shown to enhance the detoxifying enzyme activities such as glutathione- S-transferase in *in-vivo* conditions. Moreover, the reduced oxygen permeability of coating after addition of TE could be another reason for decreasing the oxidation of chicken meat after treatment by TE activated coating. Also, Chattopadhyay et al. (2004) found that in the *in-vitro* conditions, curcumin significantly inhibited the generation of reactive oxygen species (ROS) like superoxide anions,  $\text{H}_2\text{O}_2$  and nitrite radical generation by activated macrophages. It also has decreased lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates. Use of natural plant extracts (Sampaio et al., 2012; Radha Krishnan et al., 2014), active edible coatings (Song et al., 2011; Noori et al., 2018) and vacuum or modified atmosphere packaging (Hasapidou & Savvaidis, 2011; Latou et al., 2014; Brennesselová et al., 2015) to prevent lipid oxidation in meat, fish and poultry have been described in the literature.

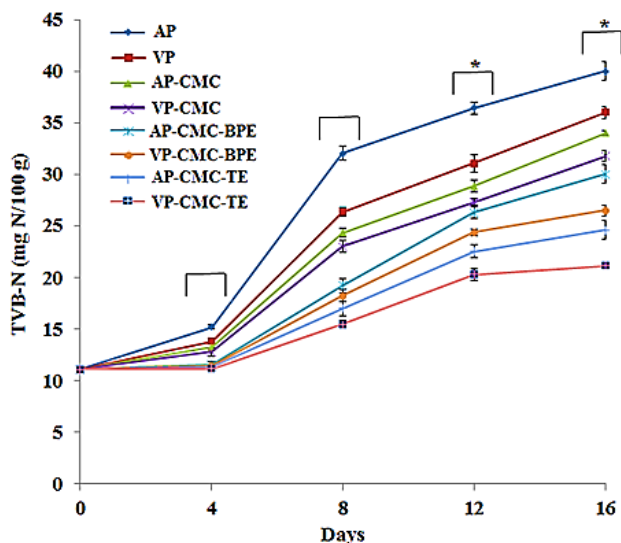


Fig. 4. Total volatile basic nitrogen (TVB-N) of air packaged (AP) and vacuum packaged (VP) chicken breasts coated with CMC based coatings containing antimicrobial agents (black pepper seeds extract (BPE) and turmeric extract (TE)) during storage at  $4^\circ\text{C}$ . There is significant difference ( $p < 0.05$ ) between treatments within groups identified by star.

### 3.4. Total volatile basic nitrogen (TVB-N)

In the stored-meat products, the TVB-N values indicate the existence of nitrogenous materials because of the action of proteolytic bacteria and endogenous enzymes. The production of nitrogenous materials resulted from the degradation of proteins and non-protein nitrogenous compounds, is the reason of producing a bad smell in the meat. High TVB-N values are not desirable, and the TVB-N value is widely used as an indicator for meat deterioration (Umaraw & Verma, 2017). The European Union (European Commission, 1995) has set an upper limit of between 25 and 35 mg TVB-N per 100 g of meat, poultry and fish. Meat products with TVB-N values more than  $35 \text{ mg.100g}^{-1}$  have not the capability of human use. Amount of  $25 \text{ mg.100g}^{-1}$ , is the highest level of acceptable TVB-N values in meat and poultry products.

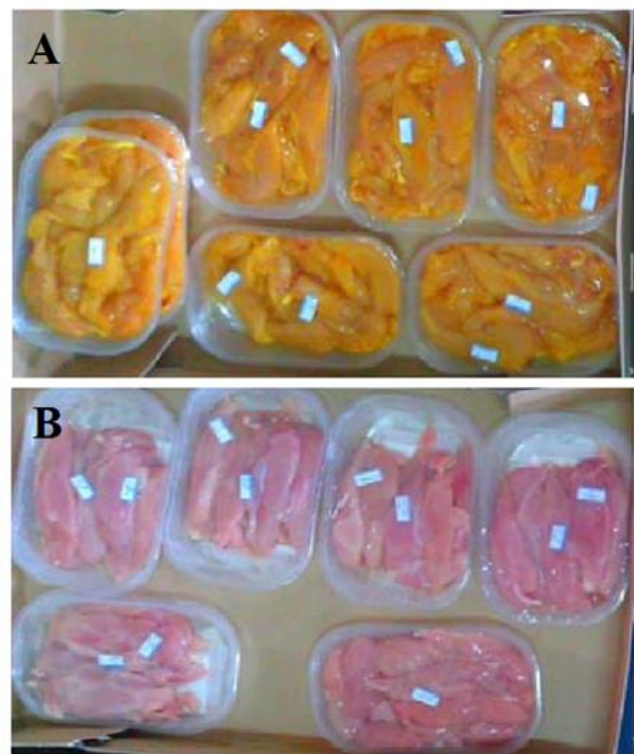


Fig. 5. Appearance of the air packaged chicken breast fillets coated with turmeric extract (A) and black pepper seed extract (B) contained CMC coating solutions.

Fig. 4 shows the effects of different treatments on TVB-N production in the chicken fillets stored at  $4^\circ\text{C}$ . The initial amount of TVB-N for fresh chicken breast fillets was  $11.1 \text{ mg.100g}^{-1}$  and there were no significant differences ( $p > 0.05$ ) between the TVB-N values of treated samples at the first 4 days, but a marked increment was observed after day 4. TVB-N value was found to increase in all samples during storage but the rate of this increasing in air-packaged sample was more than others. This increase is related to the activity of spoilage bacteria and endogenous enzymes (Sharma et al., 2017). Mean TVB-N values of the vacuum-packaged samples were lower than those of the air-packaged groups. Also, edible coating has significant effect ( $p < 0.05$ ) on the production of

nitrogenous compounds. [Abdeldaiem \(2013\)](#) reported a level of  $12.9 \text{ mg} \cdot 100\text{g}^{-1}$  of TVB-N for fresh chicken fillets that reached to  $28.64 \text{ mg} \cdot 100\text{g}^{-1}$  after 6 days of storage at  $4^\circ\text{C}$ . However, in the present study, the TVB-N values of AP-CMC and VP-CMC samples after 12 days of storage at  $4^\circ\text{C}$ , were  $28.83$  and  $27.30 \text{ mg} \cdot 100\text{g}^{-1}$ , respectively, that indicates the potential of pure CMC coating in preserving of chicken breast's quality.

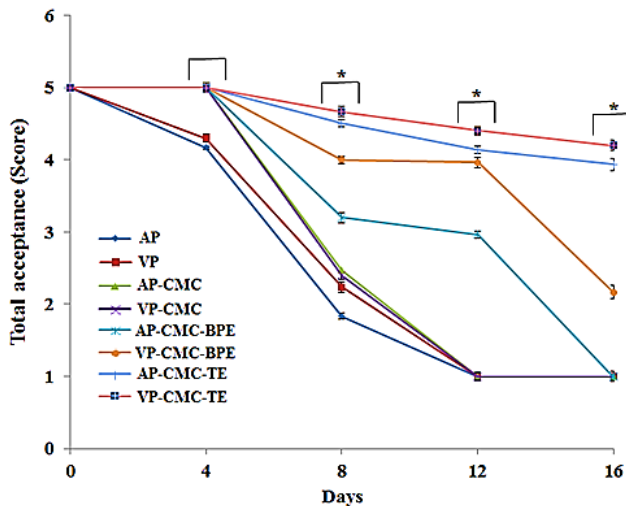


Fig. 6. Total acceptance in sensorial analysis of air packaged (AP) and vacuum packaged (VP) chicken breasts coated with CMC based coatings containing antimicrobial agents (black pepper seeds extract (BPE) and turmeric extract (TE)) during storage at  $4^\circ\text{C}$ . There is significant difference ( $p < 0.05$ ) between treatments within groups identified by star.

The TVB-N value of AP-CMC-BPE, VP-CMC-BPE, AP-CMC-TE and VP-CMC-TE were lower than that of just vacuum-packaged and/or pure CMC coated samples during the storage period and the differences became marked in the latest stage of the experiment (days 12-16) ( $p < 0.05$ ). The application of antimicrobial CMC coating could extend the shelf life, and the active CMC coating with VP was more effective compared to edible coatings alone. This can be attributed to either a more rapidly reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both ([Song et al., 2011](#)). Similar to previous properties, the greater antimicrobial potential of TE in comparison with BPE could be concluded. Vacuum-packaged and TE coated samples showed the lowest TVB-N values. According to the freshness standard ( $25 \text{ mg TVB-N per } 100 \text{ g of chicken meat}$ ), AP-CMC-TE and VP-CMC-TE samples could maintain their freshness for about 16 days. AP-CMC-BPE, and VP-CMC-BPE samples could only maintain their quality up to 12 days and 14 days, respectively. [Abdeldaiem \(2013\)](#) reported that the TVB-N in chicken breast fillets treated with direct addition of 3% water-soluble extract of turmeric powder increased to  $27.53 \text{ mg} \cdot 100\text{g}^{-1}$  after 9 days of storage. However, in the current research, by using of an edible coating containing 2% of TE, the TVB-N value of coated chicken fillets was less than  $25 \text{ mg} \cdot 100\text{g}^{-1}$  throughout 12 days of storage. This obvious difference, proves that the use of antimicrobial edible coatings is a much more effective method to control the microbial growth rate than the use of the direct addition of antimicrobial extracts. With edible coatings, the microbial

quality was under control in most cases during a period of time. The direct addition of extracts or essential oils (EOs) without the use of a structural matrix, did not in general improve the microbial quality and in some cases it even had a detrimental effect. We think that the edible coatings developed as insoluble, homogeneous and continuous matrices, were able to control the release of the antimicrobial agents on the food surface in such a way that resulted in an improvement of the microbiological quality of chicken breast. [Fernández-Pan et al. \(2014\)](#) observed that, while the edible coatings containing  $20 \text{ g} \cdot \text{kg}^{-1}$  of oregano EO showed effectiveness against the five microbial groups during the 13 days of storage of chicken breast fillets, the same  $20 \text{ g} \cdot \text{kg}^{-1}$  of EO without structural matrix did not show efficacy. Similar results were reported on the effect of antimicrobial and antioxidant edible films and coatings on the shelf life of fish species ([Song et al., 2011](#); [Alparslan & Baygar, 2017](#)).

### 3.5. Sensory evaluation

Odor and appearance or color scores of chicken breasts are given in [Tables 1 and 2](#). The lower acceptability score of 1 was reached for odor ([Table 1](#)) after 12 days for air-packaged sample, whereas this score was reached for the VP and CMC-treated samples after 16 days. The same pattern was shown for appearance ([Table 2](#)). As shown in [Table 1](#), the lower acceptability score of about 4 for odor was reached after 4-6 days for AP-CMC-BPE samples, 6-8 days for VP-CMC-BPE samples and 16 days for AP-CMC-TE samples. Acceptability score of vacuum-packaged and TE treated samples were not decreased to 4 until storage time and it was higher than that of the other groups in the all experiment periods. The presence of TE (2% w/w) in the surface of the coated samples produced a distinct but acceptable pleasant odor, well received by the panelists.

The effects of all coating and packaging combinations were significant ( $p < 0.05$ ) for the mean values of general appearance suggested by the panelists ([Table 2](#)). In the non-coated vacuum-packaged samples (VP sample), no huge difference was found, but the pure CMC coatings appeared an appealing surface because they formed a more homogeneous solution; and therefore, a shiny surface on the fillets was observed. With regards to [Table 2](#), panelists preferred the appearance of coated samples with the addition of TE. The mean value of appearance of the vacuum-packaged samples coated with TE was the highest (4.22) at the end of storage. The application of TE provided a deeper golden-yellow color due to the presence of carotene that increased the affinity of the panelists. However, a distinct pink color was created by BPE that was not more acceptable by panelists. [Fig. 5](#) shows the visual appearance of coated chicken breast fillets by both of TE and BPE. A lower BPE concentration, i.e. 0.5% v/w would perhaps be a more suitable level to apply in the chicken samples, although this was not investigated in the present study.

[Fig. 6](#) shows the total acceptance scores of treated chicken breast fillets. The total acceptance scores of the all samples decreased with storage time. The observed shelf life of chicken fillets, as determined by panelists was 12 days for AP, VP, AP-CMC and VP-CMC samples and 16 days for AP-CMC-BPE sample. The VP-CMC-TE sample obtained the highest score for total acceptance at the end of storage time.

The results of sensorial analyses were similar to the scores in literature. In the coated fillets, desired surfaces were observed with blisters and no cracks. The smell, taste, and flavor of the fillets were improved and the desired color was formed. The results of this study were similar to the earlier findings reported in literature

(Ntzimani et al., 2010; Latou et al., 2014; Noori et al., 2018). Based on sensory scores it can be stated that CMC coatings containing 2% of TE had the highest positive effect on shelf life extension of fresh chicken meat.

Table 1. Odor scores of air packaged (AP) and vacuum packaged (VP) chicken breasts coated with CMC based coatings containing antimicrobial agents (black pepper seeds extract (BPE) and turmeric extract (TE)) during storage at 4°C.

Sample	Storage time (days)				
	0	4	8	12	16
AP	5.0±0.0 <sup>Aa</sup>	4.16±0.12 <sup>Ba</sup>	1.05±0.04 <sup>Ba</sup>	1.0±0.0 <sup>Ba</sup>	1.0±0.0 <sup>Ba</sup>
VP	5.0±0.0 <sup>Aa</sup>	4.38±0.21 <sup>Bb</sup>	2.65±0.03 <sup>Cb</sup>	1.0±0.0 <sup>Da</sup>	1.0±0.0 <sup>Da</sup>
AP-CMC	5.0±0.0 <sup>Aa</sup>	4.74±0.01 <sup>Ac</sup>	3.43±0.23 <sup>Bc</sup>	1.0±0.0 <sup>Ca</sup>	1.0±0.0 <sup>Ca</sup>
VP-CMC	5.0±0.0 <sup>Aa</sup>	4.72±0.0 <sup>Ac</sup>	3.97±0.11 <sup>Bd</sup>	1.72±0.07 <sup>Cb</sup>	1.0±0.0 <sup>Da</sup>
AP-CMC-BPE	5.0±0.0 <sup>Aa</sup>	4.35±0.03 <sup>Bb</sup>	2.45±0.0 <sup>Cb</sup>	1.58±0.04 <sup>Db</sup>	1.21±0.0 <sup>Da</sup>
VP-CMC-BPE	5.0±0.0 <sup>Aa</sup>	4.54±0.12 <sup>Bbc</sup>	3.11±0.15 <sup>Cc</sup>	2.0±0.06 <sup>Dc</sup>	1.41±0.11 <sup>Eb</sup>
AP-CMC-TE	5.0±0.0 <sup>Aa</sup>	4.76±0.0 <sup>Bc</sup>	4.64±0.0 <sup>Be</sup>	4.13±0.11 <sup>Cd</sup>	4.02±0.02 <sup>Cc</sup>
VP-CMC-TE	5.0±0.0 <sup>Aa</sup>	5.0±0.0 <sup>Ad</sup>	4.78±0.11 <sup>Ae</sup>	4.59±0.12 <sup>Bd</sup>	4.56±0.06 <sup>Bd</sup>

A, means with different capital letters in the same row are significantly different ( $p < 0.05$ ).

a, means with different lowercase letters in the same column are significantly different ( $p < 0.05$ ).

Table 2. General appearance and color scores of air packaged (AP) and vacuum packaged (VP) chicken breasts coated with CMC based coatings containing antimicrobial agents (black pepper seeds extract (BPE) and turmeric extract (TE)) during storage at 4°C.

Sample	Storage time (days)				
	0	4	8	12	16
AP	5.0±0.0 <sup>Aa</sup>	4.16±0.02 <sup>Ba</sup>	1.93±0.14 <sup>Ca</sup>	1.0±0.0 <sup>Da</sup>	1.0±0.0 <sup>Da</sup>
VP	5.0±0.0 <sup>Aa</sup>	4.23±0.01 <sup>Ba</sup>	2.66±0.03 <sup>Cb</sup>	1.0±0.0 <sup>Da</sup>	1.0±0.0 <sup>Da</sup>
AP-CMC	5.0±0.0 <sup>Aa</sup>	5.0±0.0 <sup>Ab</sup>	2.82±0.03 <sup>Bc</sup>	1.0±0.0 <sup>Ca</sup>	1.0±0.0 <sup>Ca</sup>
VP-CMC	5.0±0.0 <sup>Aa</sup>	5.0±0.0 <sup>Ab</sup>	2.73±0.11 <sup>Bb</sup>	1.11±0.0 <sup>Cb</sup>	1.0±0.0 <sup>Ca</sup>
AP-CMC-BPE	5.0±0.0 <sup>Aa</sup>	5.0±0.0 <sup>Ab</sup>	3.25±0.1 <sup>Bd</sup>	3.11±0.04 <sup>Bc</sup>	1.0±0.0 <sup>Ca</sup>
VP-CMC-BPE	5.0±0.0 <sup>Aa</sup>	5.0±0.0 <sup>Ab</sup>	4.14±0.05 <sup>Be</sup>	4.11±0.03 <sup>Bd</sup>	2.26±0.21 <sup>Cb</sup>
AP-CMC-TE	5.0±0.0 <sup>Aa</sup>	5.0±0.0 <sup>Ab</sup>	4.61±0.1 <sup>Bf</sup>	4.11±0.01 <sup>Cd</sup>	4.11±0.0 <sup>Cc</sup>
VP-CMC-TE	5.0±0.0 <sup>Aa</sup>	5.0±0.0 <sup>Ab</sup>	4.70±0.11 <sup>Bf</sup>	4.41±0.12 <sup>Bc</sup>	4.22±0.16 <sup>Cc</sup>

A, means with different capital letters in the same row are significantly different ( $p < 0.05$ ).

a, means with different lowercase letters in the same column are significantly different ( $p < 0.05$ ).

## 4. Conclusion

In this study, it was shown that application of turmeric extract as natural antimicrobial agent in CMC coating material was more effective than black pepper seed extract with respect of increasing the shelf life of chicken breast fillets. The turmeric extract coated fillets showed low levels of proteolysis and lipid oxidation, as deduced from the PV, TVB-N, and TBARS indices, which remained well below the limits of acceptability throughout the period studied. The application of black pepper seed extract in chicken samples was not as pleasant as compared to that of turmeric extract. Combination of vacuum packaging and edible coatings containing 2% turmeric extract increased the shelf life of chicken breast from 7 days (control) to more than 16 days, keeping total aerobic mesophilic and total aerobic psychrotrophic bacteria counts under the microbiological limits recommended for distribution and consumption. Microbiological, chemical and sensory data obtained in this study showed that the combined treatment of vacuum packaging and application of an antimicrobial edible coating exhibited a strongly synergistic interaction, extending

the shelf life of chicken breast fillets up to 8 days. However, the shelf life of TE coated and vacuum packaged chicken breast was increased to 12 days as the longest storage time. This extension is roughly equivalent to 100% shelf life extension of the product. This confirms the potential utility of the hurdle strategy for improving the shelf life of raw poultry meat.

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## Conflict of interest

The authors declare that there is no conflict of interest.



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