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CHITOSAN/CUMIN (*CUMINUM CYMINUM* L.) ESSENTIAL OIL EDIBLE BIODEGRADABLE COATING: ITS EFFECT ON MICROBIAL, PHYSICAL AND SENSORY PROPERTIES OF CHICKEN MEAT DURING REFERIGERATION

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ABSTRACT

Chicken meat is a popular food around the world due to its high nutrient content, low fat content and relatively low cost. Perishable and enrich chicken meat caused it sensitive spoilage and fat oxidation, so reduce the shelf-life of the product. The aim of this study was to investigate the effect of chitosan (Ch) and cumin essential oil (CEO) on the quality and shelf life chicken meat. Ch-CEO coatings were prepared in three treats covered chitosan, cumin essential oil / chitosan and essential oil of cumin 0.2, 0.4 and 0.6%. The microbial tests (Total count, *Enterobacteriaceae*, *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli)*, mold and yeast), the chemical tests (pH, Total volatile nitrogen (TVN), Thiobarbituric acid (TBA), Peroxide value (PV) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and sensory properties were assessed in 2, 5, 9 days. There was a significant difference in microbial load between control and treated samples with Ch-CEO (0.6%). The most antioxidant activity, TBA and PV have been shown to be CEO (0.6%). In all of concentration of CEO, pH and TVN decreased. Sensory properties in treating samples with Ch-CEO were acceptable in the second day, but in final storage period showed significant differences with the control sample. The results show that due to the antioxidant activity of CEO and the high antimicrobial activity of chitosan coating and the synergistic effect of both of them improved of sensory properties and increase shelf life chicken meat at the refrigerator temperature.

1. Introduction

Increasing the shelf life of food products, especially perishable products such as meat and dairy products have an important role in maintaining the quality and safety of food products. For this reason, researchers are looking for new methods and packaging owing to use of chemical preservative reduce the product quality and safety (Azlin-Hasim *et al*., 2018; Lomate *et al.*, 2018). Knowing that synthetic packaging materials derived from petroleum products are widely used in the food packaging due to their lower price, comfortable, extensive availability and desirable characteristics such as, brightness, plasticity and transparency, but they are not suitable in many

aspects and even dangerous. Nevertheless, the main concerns usage of these materials for food packaging includes the environmental pollution, non-degradability and environmental incompatibility, the migration of compounds from packaging to product, which can be endanger for safety of products and consumer health (Tharanathan, 2003). Therefore, finding materials and methods on new packaging, has attracted greatly for researchers. So, edible, biodegradable and friendly environmentally films for coatings are a new attitude (Alizadeh-Sani *et al*., 2020; Bagheri, *et al*., 2019). Edible coatings and films have been proposed as an appropriate packaging due to cheapness, biodegradability, environmental compatibility, nutritional value, renewable potential (Alizadeh-Sani *et al*., 2018; Noshirvani *et al*., 2018). Also, films and edible coatings are suitable carriers for additives and antimicrobial compounds, enzymes, preservatives, etc (Azizi-lalabadi *et al*., 2020; Salari *et al*., 2018; Sani *et al*., 2017). As well as biodegradable coatings and films, mainly made of natural compounds, such as proteins, lipids and polysaccharides alone or in combination with other compounds. Therefore, to use of films and coatings composite will promote achieving coating features beside that the keeping properties of the maintained products (Azlin-Hasim *et al*., 2018).

Chicken as a perishable product, is used throughout the world because of its reasonable cost and its high nutritional values (Chouliara *et al*., 2007). Fresh chicken is mainly stored at a refrigerated temperature and is freshly consumed (2-5˚C). While, microbial decay or oxidative rancidity are the main reasons the spoilage of these products. It will be worthy to improve new processing and packaging solutions to prolong shelf life of the poultry products (Babuskin *et al*., 2014). Hence, it is recommended to apply natural food preservatives such as essential oils, chitosan, nisin, etc. to be assisted in keeping poultry from spoilage and pathogenic microorganisms, because of these compounds have low processing side effects on the products (Petrou *et al*., 2012). So, it is recommended that use the polysaccharides, such as Chitosan (Ch), is considered as an excellent biopolymer, for biodegradable and edible films and coatings composite due to its non-toxic, biodegradable, biocompatible, antimicrobial properties and commonly regarded as a safe food additive (Xu *et al*., 2005). Chitosan is a cationic polysaccharide consisting of (1, 4)-linked-2-amino-deoxy-b-D-glucan, and is the deacetylated form of chitin (Petrou *et al*., 2012; Siripatrawan *et al*., 2012; Yuan *et al*., 2016). Chitosan is recognized as Generally Recognized as Safe (GRAS) by FDA and possess good antimicrobial properties against wide range of microorganisms *(Rhim et al., 2006; Yuan, et al.*, 2016). Also, it has antioxidant activity that prevents of lipid oxidation and acting as a secondary natural antioxidant for product keeping (Yuan *et al*., 2016). Chitosan films are suitable system to be used as active compounds carriers (Rhim *et al*., 2006; Yuan *et al*., 2016). Many studies reported the benefits of Ch have being applied either individually or in combination with other compounds such as essential oils in food systems. Giatrakou *et al*. (2010) extended cooked chicken shelf-life by using of Ch and thyme oil (Giatrakou *et al*., 2010). Also, Vasilatos *et al*. (2013) demonstrated the effects of Ch or rosemary oil, singly or combined, to prolong the shelf-life of turkey meat (Vasilatos *et al*., 2013); while Petrou *et al*. (2012) studied Ch dipping or oregano oil, individually or combined, on modified atmosphere packaged chicken breast meat (Petrou *et al.*, 2012).

With this attitude, EO can be considered as a good additive for production of combined chitosan films. EOs, as a natural additive, have antibacterial, antioxidant, antiviral and antifungal activities (Kedia *et al.*, 2014; Petrou *et al*., 2012; Sani *et al*., 2017). The most important characteristic of an EO is bactericidal or bacteriostatic properties against a broad range of microorganisms and/ or preventing the oxidation process (Ribeiro-Santos *et al*., 2017). Cumin also, is an annual herb that belongs to the family Apiaceae. It used extensively and afterward black pepper, is known as the second

commonly used spice in the world (Kedia *et al*., 2014; Ruby *et al*., 2012). Cumin is native to Iran, Egypt, Turkistan and East Mediterranean, China, India, Morocco, South Russia, Japan, Indonesia, Algeria and Turkey (Ruby *et al*., 2012). CEO seed exhibits antibacterial, antioxidant properties (Jirovetz *et al*., 2005; Kedia *et al*., 2014).

Based on what was said, the application of Ch with CEO, has not been reported to date, in fresh chicken meat. Thus, the purpose of this study was to evaluate the effects of Ch and CEO, applied individually or simultaneous combination use of physicochemical, microbiological and sensory properties of chicken breast meat during refrigeration.

2. Materials and methods

2.1. Chicken meat

Fresh chicken breast fillet meat was purchased from a local poultry processing company. Samples were transferred to the laboratory using insulated polystyrene boxes on ice flasks and then were divided (ca.220 g or 16cm × 8cm for each sample). Chicken meat samples were kept at refrigerated temperature for other tests.

2.2. Preparation of chitosan coating solution

Low molecular weight chitosan powder (MW; 340) with moisture content less than 10% and a deacetylation degree of 75–85% (Manufacturer's data) obtained from crab shells was purchased from Sigma Aldrich company. Chitosan coating was prepared according to Vasilatos *et al*. (2013) method with some modifications (Vasilatos *et al*., 2013). Coating-forming solution of chitosan was prepared by dissolving 1.5 g chitosan powder in 100 mL of glacial acetic acid solution (1% v/v) (as plasticizer) and was stirred 8 h at room temperature (final chitosan concentration was 1.5% w/v) (Siripatrawan *et al*., 2012).

2.3. Preparation of Cumin essential oil

To prepare CEO, about 100 g of powdered cumin seed was placed in a blender containing 500 mL distilled water for 24 h and then was transferred to our hydro-distillation facility. The distillation was performed by Clevenger apparatus for 4 h. The obtained EO dehydrated and dried using anhydrous Na_2SO_4, and then stored in dark glass bottles at 4°C for later use (Oroojalian, Kasra-Kermanshahi, Azizi, & Bassami, 2010). Different concentrations of EO (0.2, 0.4 and 0.6% (v/w) were prepared by stock concentration. Tween 80 (0.1% w/v) was added to the solution as a surfactant to assist EO dissolution in coating forming solution (Peng *et al*., 2013). The solution stirred continually for 20 min at room temperature for better homogenization.

2.4. Preparation of samples

The chicken meat samples were coated with Ch and CEO solutions, singly or in combination. Samples of meat (ca.200 g) were immersed separately and were placed inside sterile packaging pouch, containing 100 mL of Ch solution (1.5% w/v) for 1.5 min. After immersing, the excess solution was drained off on a sterilized rack (incubator) under aseptic conditions. Then, samples packaged into a clean sterile pouch. CEO in various concentration (0.2, 0.4 and 0.6% w/v) was added into the chicken meat samples (0.25 mL of EO into 100 g of chicken meat) (Petrou *et al*., 2012; Vasilatos *et al*., 2013). Finally, the same above method was used for combine Ch and CEO for samples.

2.5. Packaging of samples

Chicken breast meat samples treated with coating solutions individually (~200 g) and were transferred aseptically into the low-density polyethylene pouches. Treatments included the following groups: Blank or control (in the absence of Ch or CEO) , Ch: (samples treated with Ch 1.5% w/v), CEO: (samples treated with cumin oil 0.2, 0.4 and 0.6%), Ch-CEO: (samples treated with combined Ch 1.5% and CEO 0.2, 0.4 and 0.6%). All specimens were stored at the refrigerator temperature during the test period (9 days).

2.6. Microbiological analysis

Chicken meat samples (25 g) were blended with 225 mL of sterile peptone water (0.1%) (Merck, Darmstadt, Germany) in a stomacher bag and homogenised for 3 min. The serial dilution method was applied for microbial test. For microbial analysis, 0.1 mL from serial dilutions of homogenized chicken meats were spread on the surface of agar plates. Total viable counts (TVCs) were determined in Plate Count agar medium (PCA, Merck, Darmstadt, Germany) by incubation for 48-72 h at 30˚C (Giatrakou *et al*., 2010). *Staphylococcus aureus* count was determined in Baird-Parker agar medium (BPA, Merck, Darmstadt, Germany) by incubation for 48 h at 37˚C. To count moulds and yeasts, duplicate 0.1 mL of suitable dilutions were pour–plated on Sabouraud Dextrose agar medium (Merck, Darmstadt, Germany) and incubated at 25˚C for 3-4 days (Siripatrawan *et al*., 2012). *Enterobacteriaceae* were determined by pour-overlay method using Violet Red Bile Glucose (VRBG) agar medium by incubation for 48 h at 37˚C (Merck, Darmstadt, Germany) (Petrou *et al*., 2012; Vasilatos *et al*., 2013).

2.7. Chemical and sensory characteristics

2.7.1. pH

The pH value determined by a pH meter (Kent EIL 7020). About 25 g of chicken meat sample was homogenised with 225 mL of distilled water and the homogenised samples were used for pH estimation (Petrou *et al*., 2012).

2.7.2. DPPH assay

DPPH test is the most commonly used method for measuring antioxidant capacity. 1 mL of the CEO in different concentration was added to 0.5 mL of a standard DPPH (Sigma-Aldrich) methanolic solution. The mixture was shaken and left standing in the dark at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm (Mahdizadeh *et al*., 2020; Rebey *et al*., 2012). The Butylated hydroxytoluene (BHT) (Merck, Darmstadt, Germany) was used as standard and control sample. The capacity scavenging DPPH radical calculated by the following equation:

DPPH scavenging effect (%) = $((A_0 - A_1) / A_0) * 100$

A_0: absorbance of the control

A_1: absorbance of the sample

2.7.3. Peroxide value

Five g of meat samples and 30 mL of acid acetic and chloroform (Sigma-Aldrich) (ratio 3:2) were added to 0.5 mL Potassium iodide (KI) (Merck, Darmstadt, Germany) and was left for 1 min. Titration was performed with sodium thiosulfate ($Na_2S_2O_3$) (0.1 N) until yellow color appeared, and 0.5 mL of starch solution was added to appear purple color. Peroxide value (PV) is characterized as milliequivalents (meq) peroxide oxygen per 1 kg of lipids (Karakaya *et al*., 2011). The peroxide value calculated by the following equation:

$$\mathrm{PV}\left(\frac{\mathrm{meq}}{\mathrm{kg}}\right) = \frac{(\mathrm{S}-\mathrm{B}) * \mathrm{N} * 1000}{\mathrm{W}} \qquad (1)$$

S: The volume of titrant ($Na_2S_2O_3$ standard solution) consumed by sample (mL)

B: The volume of titrant ($Na_2S_2O_3$ standard solution) consumed by control sample (mL)

N: Normality titrant ($Na_2S_2O_3$)

W: weight sample (fat extracted, g)

2.7.4. TBARS assay

The 2-thiobarbituric acid (TBA) assay commonly used to assess lipid oxidation and expressed as mg of malondialdehyde (MDA) per kg chicken meat samples (Xiong *et al*., 2015). Ten grams of the meat sample with 50 mL of distilled water were mixed in a 100 mL tall beaker, then were stirred by a glass bar for several seconds and left for approximately 30 min. The samples were homogenized at high speed as possible for 15 sec by mixer. Then added 20 mL of 20% TBA and was placed for 10 min in ambient condition. Samples filtered through a Toyo filter paper No.42 with suction, and added distilled water until the solution level equals 100 mL. Then, the absorbance of the obtained solution was measured at 532 nm by spectrophotometry (Ultrospec 2000, Scinteck, UK) (Alizadeh-Sani *et al*., 2020). TBA content was expressed as µg MDA per g chicken meat. The ability to lipid oxidation calculated by the following equation:

MDA (µg/g) = E532 * 12.9

2.7.5. Total volatile nitrogen

To determine total volatile nitrogen (TVN), the samples (10 g) were boiled for 25 min with Magnesium oxide (MgO) (2 g), the distilled water and Ammonia (NH_3) were taken up in 0.04 M boric acid (Merck, Darmstadt, Germany) which was back titrated with 0.1 M sulfuric acid (H_2SO_4) (Merck, Darmstadt, Germany) with methyl red as indicator (Alizadeh-Sani *et al*., 2020). The control sample was without chicken meat. TVN value calculated by the following equation:

$$\mathrm{TVN}\left(\frac{\mathrm{mg}}{100\mathrm{g}}\mathrm{sample}\right) = v\,(titrant) * 14 \qquad (2)$$

2.8. Sensory analysis

For the sensory evaluation of the samples, a semi-trained 7-person panel was used (laboratory – trained and postgraduate students). Panellists were asked to assess the odour, appearance, colour and overall acceptance of uncooked chicken meat samples during the storage period. Chicken meat samples were evaluated using a 0-9 ranging score from 9 (highest score) to 1 (lowest score) (Petrou *et al*., 2012).

2.9. Statistical analysis

The statistical analysis was performed using R software. Data were subjected to analysis of nonparametric Kruskal–Wallis one-way analysis of variance. P-values less than 0.05 were considered statistically significant. All tests of this study were performed in triplicate. Results are reported as mean values ± standard deviation (S.D)

3. Results and Discussion

Seven type samples of Ch and CEO (0.2, 0.4 and 0.6% (w/v)) alone or in combination (Ch-CEO at 0.2, 0.4 and 0.6% (w/v)) were prepared and microbial and physiochemical tests were examined.

3.1. Microbiological analysis

All of microbiological results were shown in Figures 1 to 5.

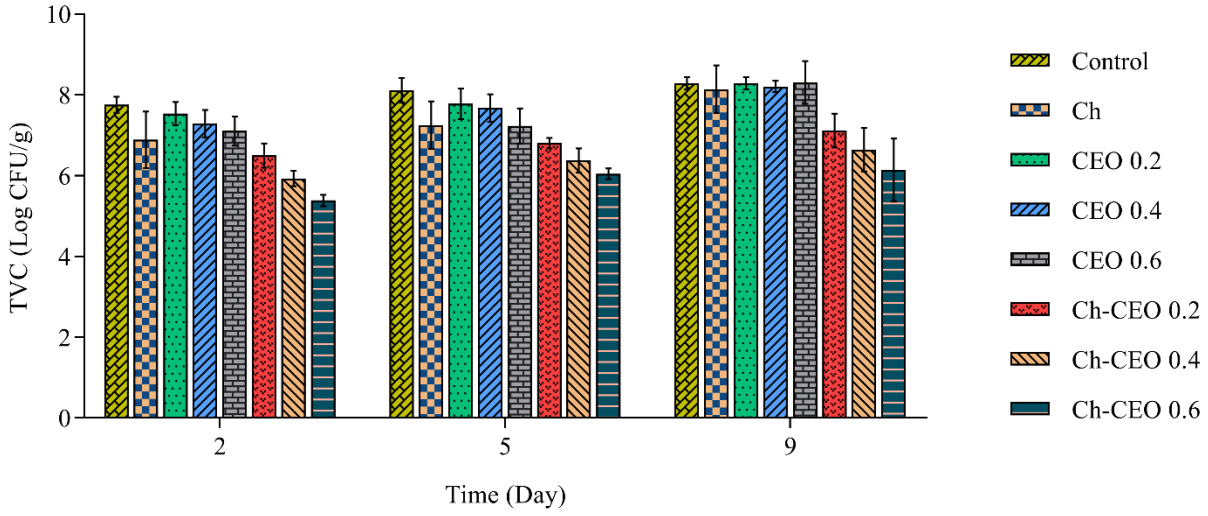

Figure 1. Effect of edible biodegradable coating containing Chitosan and CEO on the total viable counts (TVC) of chicken meat during refrigerated storage.

3.1.1. Total viable count

On the second day, the initial total count was assessed and result was shown 7.8 log cfu/g, increasing the final population during storage up (9 days) to reached ca. 8.3 log cfu/g (Chitosan samples) (Figure 1). These results indicated that

counts for treatments were about 0.5 – 1.3 log cfu/g lower than in the control samples. Data analysis was released a significant difference ($p < 0.05$) for TVC between control samples and wrapped Ch-CEO 0.2, Ch-CEO 0.4 and Ch-CEO 0.6%. TVC values of chicken meat samples exceed of 8.5 log cfu/g, which was considered as the upper acceptability limit for fresh meat on days 9th in control, Ch, CEO 0.2, 0.4 and 0.6% treatments. While samples treated with Ch-CEO 0.4 and Ch-CEO 0.6 never reached the limit value after 7days. Thus, in comparison with control samples, an increase in microbiological shelf life of 9th was achieved for Ch-CEO 0.4 and Ch-CEO 0.6 samples. This shelf life extension of these two groups could be due to the antimicrobial action CEO components (especially, cuminic alcohol) and of Ch, which increases antimicrobial activity (Allahghadri *et al*., 2010).

Recently, in a related study, a 9th microbiological shelf life increment was obtained for a fresh chicken breast meat treated with modified atmosphere (70/30 CO_2/N_2) and oregano oil (0.1%) (Chouliara *et al*., 2007). In other studies, Giatrakou *et al*. (2010) indicated that microbiological shelf life for a ready-to-eat chicken pepper kebab treated by either thyme oil (0.2% v/w) or chitosan (1.5% w/v) increased after 5 days (Giatrakou *et al*., 2010). Siripatrawan *et al*. (2012) reported a reduction of microbial counts by an average of 2.52 log cfu/g for pork sausages treated by chitosan incorporating green tea extract (20% w/v) on day 20th (Siripatrawan *et al*., 2012). Petrou *et al*. (2012) reported a shelf life extension of 5-6 days for a chicken breast meat treated with chitosan 1.5% (w/v) or oregano oil 0.25% (v/w) and modified atmosphere packaging than control samples (Petrou *et al*., 2012). Also, the combined use of chitosan and rosemary oil on the preservation of turkey meat led to a reduction of TVC by 1.0 log cfu/g, extending their shelf life at 2˚C (Vasilatos *et al*., 2013).

Similar to previous studies in present study, among all the treatments, Ch-CEO 0.6 and Ch-CEO 0.4 were the most effective on the growth inhibition of TVC (Figure 1a) in the storage period. Different antimicrobial effects were detected when using an edible coating based on chitosan combined with 0.2, 0.4 and 0.6% (v/w) of CEO against various microbial groups in fresh chicken meat stored at 4˚C. Ch prevents growth and spore germination bacteria due to the absorption of minerals and in particular calcium (Plascencia-Jatomea *et al*., 2003). The use of Ch has been proven to be a very effective way to control the microbial growth rate on chicken meat than the use of the direct addition of CEO.

3.1.2. Enterobacteriaceae count

In our study, *Enterobacteriaceae* (Coliforms), the most important part of the microbial flora of chicken meat with a psychotropic facultative anaerobic bacterial group and final counts reached to ca 8.4 log cfu/g on after 9 days (Figure 2). As previously noted, Ch-CEO 0.2, 0.4 and 0.6% treatments caused a significant reduction in coliform counts (approximately 1.5-2.2 log cycles) compared to control samples on day 9th ($p < 0.05$). Petrou *et al*. (2012) reported that a decrease of microbial counts by an average of 3-4 log cfu/g for chicken breast meat treated with chitosan/oregano oil and modified atmosphere than control samples on day 12th (Petrou *et al*., 2012). Giatrakou *et al*. (2010) showed that Enterobacteriaceae growth in ready to cook chicken product were inhibited by use of chitosan 1.5% w/v and thyme oil 0.2 w/v under aerobic packaging (Giatrakou *et al*., 2010). Chantarasataporn *et al*. (2014) showed that the total Enterobacteriaceae in minced pork control samples significant increase of 5 to 6 log cfu/g during storage while samples containing oligochitosan 0.2 and 0.4% reduced about 1 and 2 log cfu/g (Chantarasataporn *et al*., 2014).

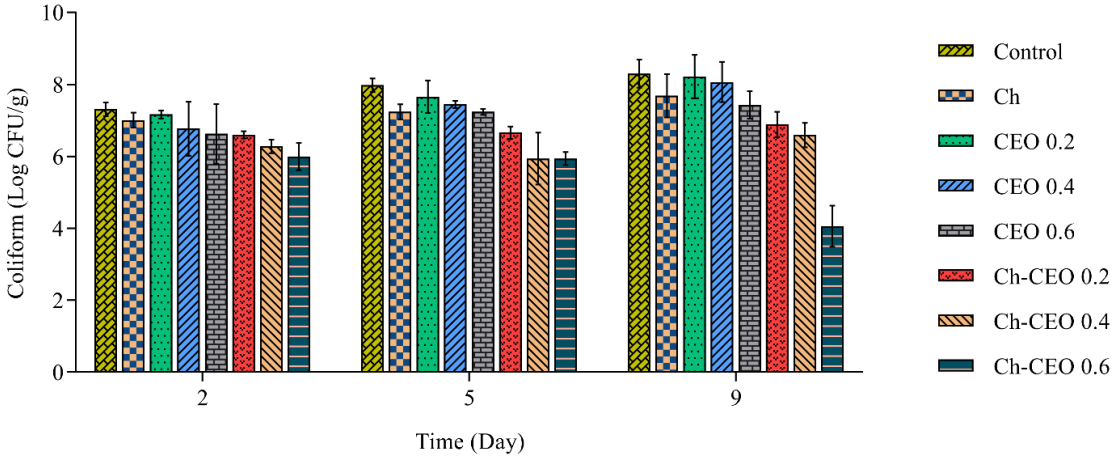

Figure 2. Effect of edible biodegradable coating containing Chitosan and CEO on the Coliforms of chicken meat during refrigerated storage.

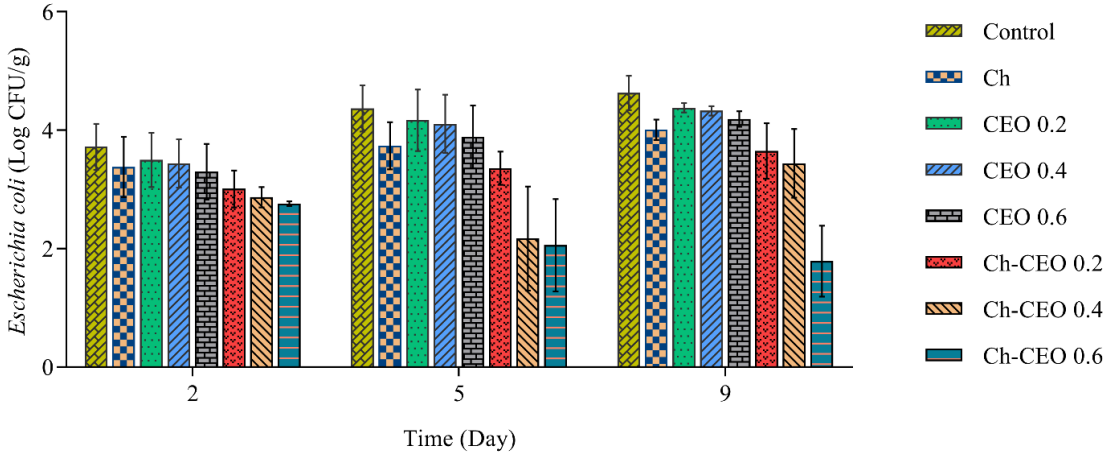

Figure 3. Effect of edible biodegradable coating containing Chitosan and CEO on the *E. coli* of chicken meat during refrigerated storage

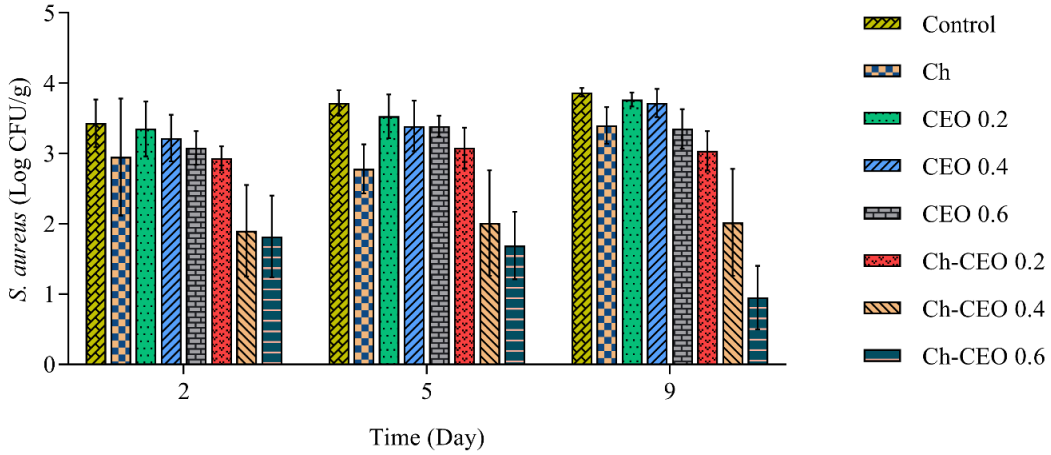

Figure 4. Effect of edible biodegradable coating containing Chitosan and CEO on the *S. aureus* of chicken meat during refrigerated storage.

3.1.3. Escherichia coli count

The initial population of *E.coli* was ca. 3.8 log cfu/g and increased to 4.7 log cfu/g at the end of the storage period (day 9th) (Figure 3).

Although, lower *E.coli* counts ($p < 0.05$) were recorded for Ch-CEO 0.6% samples stored at 4˚C. Of all the antimicrobial treatments in our study, Ch-CEO 0.6% and Ch-CEO 0.4% groups demonstrated to be the greatest effect inhibitory of growth of *E.coli* in samples, approximately resulting in a 2.1 log cycle decrease during the storage period.

These antimicrobial effects are usually attributed to the effective compounds in the CEO and the antimicrobial properties of Ch. Allahghadri *et al*. (2010) demonstrated that CEO dilutions had strong antimicrobial effects against the *E. coli* and *E. coli* was the most sensitive

3.1.4. Staphylococcus aureus count

S. aureus count in second day was 3.5 log cfu/g, increased during storage and reached final population ca. 3.9 log cfu/g for control sample (Figure 4). In contrast, counts related for Ch-CEO 0.4% and Ch-CEO 0.6% were about 0.7 – 1.2 log cfu/g lower than the control samples. *S.aureus* population was significantly ($p < 0.05$) lower in Ch-CEO 0.6% samples compared to all the other treatments. The direct addition of CEO without the use of a chitosan, in general, did not improve the microbial quality chicken meat samples. *S.aureus* count was not significantly difference ($p > 0.05$) for CEO 0.2%, CEO 0.4% and CEO 0.6% samples compared to the control samples. García-Díez *et al*. (2017) indicated that CEO inhibited the growth of *S. aureus* associated to dry-cured meat products (García-Díez *et al*., 2017). Also, Sadegi *et al*. (2012) showed that the use of cumin essential oil significantly inhibited the growth of *S. aureus* bacteria in Iranian white brined cheese (Sadeghi *et al*., 2013). In another study that investigated the effects of electro-spun chitosan-based nanofibers, it was shown that chitosan significantly inhibited the growth of *S. aureus* in meat samples (Arkoun *et al*., 2017).

bacteria to the CEO with the lowest MBC value (1 µL/mL) (Allahghadri *et al.*, 2010).

In another study, García-Díez *et al*. (2016) indicated that CEO inhibited the growth of *E. coli* related to dry-cured meat products (García-Díez *et al*., 2017). In addition, accordant with the results of this study, Shekarforoush *et al*. (2015) showed that using chitosan and oregano CEO in combination are more effective in reducing the number of spoilage and pathogenic bacteria such as *E. coli* O157:H7 in cured chicken meat (Shekarforoush *et al*., 2015).

Arkoun *et al*. (2017) also, proved that electro-spun chitosan-based nanofibers reduced the growth of spoilage and pathogenic bacteria, including *E. coli*, and resulted in an increase in the shelf life of the meat samples for one week (Arkoun *et al*., 2017)

3. 1. 5. Moulds and yeasts

Eventually, with regard to moulds and yeasts known species to be involved at the spoilage of poultry meat (Petrou *et al*., 2012). The antimicrobial treatments Ch-CEO 0.2%, Ch-CEO 0.4% and Ch-CEO 0.6% led to a significant reduction ($p < 0.05$) in yeasts and moulds count compared to the control group up to day 9th of storage (Figure 5). On the other hand, moulds and yeasts populations were significantly lower for Ch-CEO 0.6% samples compared to the wrapped chitosan samples during the storage ($p < 0.05$). Thus, while the Ch-CEO 0.6% showed effectiveness on the moulds and yeasts after 9 days, but the same result was shown in CEO 0.6% treatment without chitosan coating while did not show this parameter in microbial counts. In other studies, involving preservation of chicken breast meat treatments with chitosan or oregano oil (Petrou *et al*., 2012) led to a reduction two cycles in compared with control samples. Also, Giatrakou *et al*. (2010) reported the effects of chitosan and thymol oil beside aerobic packaging that led to count remained below 4.0 log cfu/g during the entire storage period than the control samples (Giatrakou *et al*., 2010). In another study, similarly, Siripatrawa *et al*. (2012) indicated that chitosan incorporation with green tea extract

caused shelf life extension of pork sausages 3 and 2 log cycles, respectively, compared to control samples and chitosan wrapped samples (Siripatrawan *et al*., 2012).

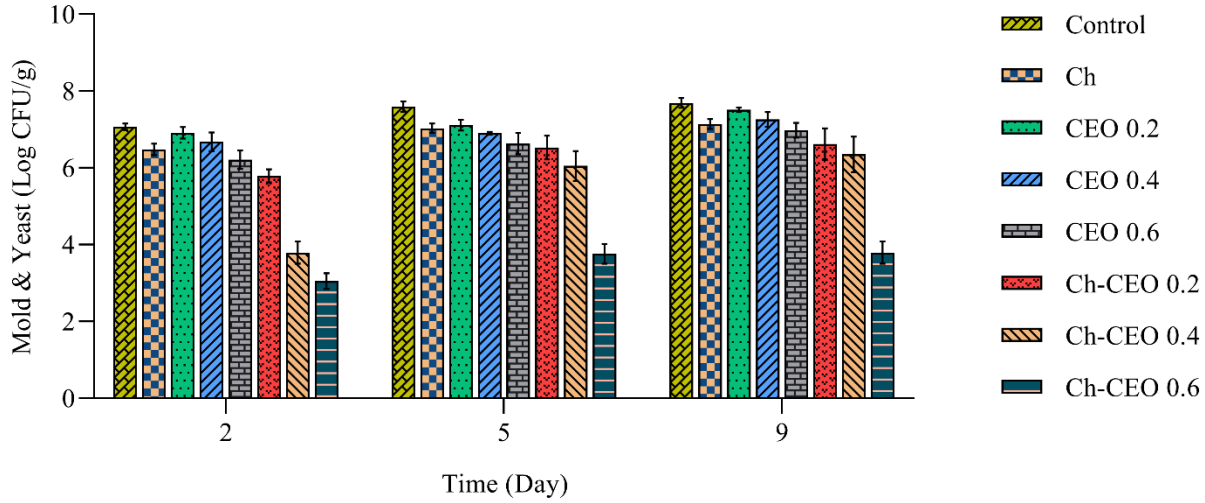

Figure 5. Effect of edible biodegradable coating containing Chitosan and CEO on the mold and yeast of chicken meat during refrigerated storage.

3.2. Physicochemical changes

3.2.1. pH value

The pH value of control and treated chicken meat samples during storage at 4˚C are shown in Table 1. The primary pH of the chicken meat samples was 6.1, whereas at the end of storage final pH was 7.5 at control samples. The pH value of treated chicken meat samples decreased during 9 days; although, between control and CEO groups, no significant difference was observed ($p > 0.05$) in this time. Treatments containing Ch-CEO 0.2, 0.4 and 0.6% resulted in lower pH value ($p < 0.05$) compared to the other groups.

Our results seem to be in agreement with those reported by Giatrakou *et al*. (2010) for reduced ready to cook chicken product containing chitosan (Giatrakou *et al*., 2010). Whereas, Petrou *et al*. (2012) reported no significant difference between chitosan, thymol oil and the combination of chitosan and thymol oil for chicken breast meat (Petrou *et al*., 2012). Also, Soultos *et al*. (2008) reported no significant difference in pH value between pork sausages samples treated with chitosan and nitrite, separately or in combination (Soultos *et al*., 2008).

Table 1. Effect of edible biodegradable coating containing Chitosan and EO on the chemical properties of chicken meat during refrigerated storage

Days	Groups	pH	PV (%)	TBARS (µg MDA/g)	TVN (mg/100g)
2	Control	6.1 ± 0.264^{a}	1.9 ± 0.115^{a}	0.006 ± 0.001^{a}	13 ± 8.08^{a}
	Ch	5.6 ± 0.251^{bcd}	1.5 ± 0.115^{b}	0.005 ± 0.0005^{a}	7.7 ± 3.89^{ab}
	CEO 0.2%	5.8 ± 0.152^{ac}	1.3 ± 0.230^{b}	0.003 ± 0.004^{ab}	9.8 ± 3.70^{ab}
	CEO 0.4%	5.7 ± 0.152^{bc}	0.9 ± 0.115^{ce}	0.002 ± 0.0006^{bc}	9.1 ± 1.85^{ab}
	CEO 0.6%	5.6 ± 0.152^{bcd}	0.4 ± 0.1^{d}	0.0004 ± 0.0004^{c}	6.3 ± 1.32^{b}
	Ch-CEO 0.2%	5.4 ± 0.057^{bde}	1.1 ± 0.1^{c}	0.004 ± 0.0005^{ad}	8.1 ± 2.65^{a}
	Ch-CEO 0.4%	5.3 ± 0.057^{de}	0.8 ± 0.05^{e}	0.004 ± 0.001^{ad}	6.9 ± 1.40^{a}
	Ch-CEO 0.6%	5.2 ± 0.057^{e}	0.4 ± 0.11^{d}	0.0026 ± 0.0005^{bcd}	5.6 ± 1.27^{b}
5	Control	6.7 ± 0.251^{a}	3.13 ± 0.230^{a}	0.010 ± 0.001^{a}	28 ± 0.00^{a}
	Ch	5.5 ± 0.076^{b}	1.8 ± 0.115^{b}	0.006 ± 0.004^{b}	6.5 ± 2.13^{bc}
	CEO 0.2%	6.6 ± 0.251^{a}	1.2 ± 0.2^{c}	0.001 ± 0.00^{cd}	14.6 ± 5.12^{d}

	CEO 0.4%	6.4 ± 0.251^{a}	1 ± 0.1^{c}	0.0007 ± 0.0001^{c}	15.8 ± 2.91^{d}
	CEO 0.6%	5.3 ± 0.115^{b}	0.6 ± 0.2^{d}	0.0004 ± 0.0001^{c}	10 ± 1.06^{b}
	Ch-CEO 0.2%	5.6 ± 0.180^{b}	1.2 ± 0.2^{c}	0.006 ± 0.001^{b}	8.1 ± 1.02^{bc}
	Ch-CEO 0.4%	5.4 ± 0.152^{b}	0.9 ± 0.05^{ce}	0.005 ± 0.001^{b}	7.3 ± 1.49^{bc}
	Ch-CEO 0.6%	5.3 ± 0.115^{b}	0.7 ± 0.1^{de}	0.004 ± 0.001^{bd}	5.4 ± 2.27^{c}
9	Control	7.5 ± 0.152^{a}	4.6 ± 0.577^{a}	0.02 ± 0.00^{a}	48.5 ± 0.80^{a}
	Ch	5.9 ± 0.229^{bd}	2.5 ± 0.503^{b}	0.01 ± 0.005^{b}	9.8 ± 1.4^{b}
	CEO 0.2%	7.2 ± 0.152^{ac}	1.5 ± 0.1^{c}	0.002 ± 0.001^{cd}	35.4 ± 14.02^{ac}
	CEO 0.4%	7.1 ± 0.115^{c}	1.2 ± 0.1^{cd}	0.001 ± 0.0008^{cd}	33.3 ± 14.09^{c}
	CEO 0.6%	7.0 ± 0.115^{c}	0.9 ± 0.057^{d}	0.0008 ± 0.001^{c}	26.6 ± 9.18^{c}
	Ch-CEO 0.2%	6.1 ± 0.346^{b}	1.6 ± 0.152^{c}	0.01 ± 0.00^{be}	12.8 ± 0.40^{b}
	Ch-CEO 0.4%	5.7 ± 0.115^{d}	1.3 ± 0.1^{cd}	0.007 ± 0.006^{de}	7 ± 1.4^{b}
	Ch-CEO 0.6%	5.6 ± 0.152^{d}	0.9 ± 0.152^{d}	0.006 ± 0.004^{ce}	4.8 ± 1.4^{b}

Any two means in the same column followed by the same letter are not significantly ($p > 0.05$) different from Duncan's multiple range tests. Ch: Chitosan, EO: Essential oil, CEO: Cumin essential oil, pH, peroxide value (PV), Malondialdehyde (MDA), Total volatile nitrogen (TVN), Thiobarbituric acid (TBARS).

3.2.2. DDPH assay

Antioxidant properties, especially radical scavenging activity, it's too important due to the deleterious role of free radicals in food and biological system (Wang *et al*., 2015). Figure 6 shows DPPH free radical scavenging activity of CEO with different concentrations compared to BHT (synthetic antioxidant). In the present study was observed statistically significant difference ($p < 0.05$) between BHT and CEO 0.2, 0.4 and 0.6%. The variety EO showed high antioxidant activities. Solvent nature had significant effect on DPPH scavenging activity of CEO. Rebey *et al*. (2012) reported highest antioxidant activities water extract cumin (Rebey *et al*., 2012). Also, Martins *et al*. (2012) stated chitosan film in combination α-tocopherol with concentration 0.1 and 0.2% the highest DPPH scavenging activity, whereas no significant difference between two α-tocopherol concentration (Martins *et al*., 2012).

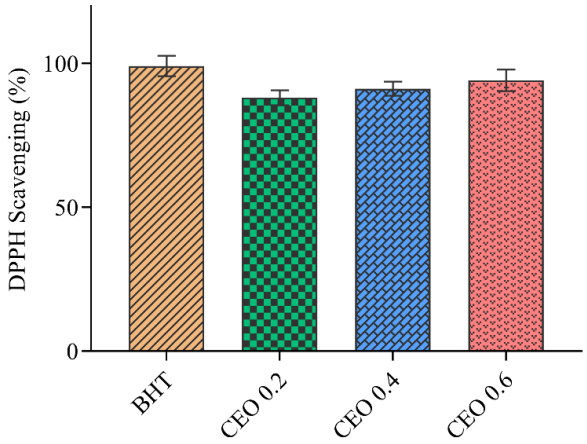

Figure 6. Antioxidant activities of Chitosan and CEO. BHT was used as standard samples. Each point represents the mean ± SD.

3.2.3. Thiobarbituric acid value

The lipid oxidation changes (TBA value) in control and treatment chicken meat samples are shown in Table1. At during storage, results were released significantly higher ($p < 0.05$) TBA in control samples than those wrapped with EO 0.2, 0.4 and 0.6%, respectively. Therefore, combination of CEO and chitosan coating increased the antioxidant properties of the coating. The TBA values of Ch-CEO 0.6% wrapped samples were lower than those wrapped with chitosan coating, whereas not showing statistically difference significantly ($p > 0.05$).

Similarly, Siripatrawan *et al*. (2012) showed that the antioxidant activities of Ch film with incorporation green tea extract increased in pork sausages (Siripatrawa *et al*., 2012). Furthermore, Ch with oregano oil and modified atmosphere packaging was shown to the lowest TBA values in 12 days of storage in chicken breast meat (Petrou *et al*., 2012). Soultos *et al*. (2008) shows low levels of lipid oxidation in sausage samples treated with chitosan concentration 0.5 and 1% in combination nitrite 150 ppm (Soultos *et al*., 2008).

3.2.4. Peroxide value

Concentration of elementary oxidation products in the lipid breakdown of the chicken meat sample measured as PV after 9 days storage at 4 ˚C are presented in Table 1. Samples containing chitosan and combination (CEO 0.2, 0.4 and 0.6%) together with those containing only CEO exhibited the lowest ($p < 0.05$) values for PV compared to the control samples. The best anti-oxidative effect ($p < 0.05$) was obtained by the combination of Ch and CEO 0.6% for the PV values that had lower at the end of storage period.

In this study, was not seen statistically difference significantly between control samples with combination of Ch and CEO 0.2 or samples containing only CEO 0.2% ($p > 0.05$).

Georgantelis *et al*. (2007) observed a decrease in the peroxide value of pork sausage samples containing Ch with rosemary EO, α- tocopherol and samples containing only Ch compared α-tocopherol and control samples (Georgantelis *et al*., 2007).

3.2.5. Total volatile nitrogen value

Changes in total volatile nitrogen value (TVN) content during the storage time are shown in Table 1. The primary TVN value was about 13 mg/100g in second day, and then it increased with time of refrigerator storage for the control samples on day 9^{th}. The control samples had the highest TVN values, while the treatment sample Ch-CEO 0.4 and 0.6% had lowered values ($p < 0.05$). In the current study was not observed statistically difference significantly between control samples with combination of Ch and CEO 0.4, 0.6% and samples containing only CEO 0.2, 0.4% with combination Ch and CEO 0.6 ($p \geq 0.05$).

Fan *et al*. (2009) reported that TVN contents increased from an initial value to 18.8 mg/100g in fish samples were given a dip treatment in 2% chitosan solution than to 30.2 mg/100g in control samples (Fan *et al*., 2009). The study conducted by Chantarasataporn *et al*. (2014) showed the amount of biogenic amines in minced pork samples of treated oligo-chitosan in the first day was about 50 mg/kg by increasing the concentration of oligo-chitosan to 0.2 and 0.4 the amount of biogenic amines reached the acceptable level on the second day (under 50 mg/kg) (Chantarasataporn *et al*., 2014).

3.2.6. Sensory analysis

Means of sensory analysis scores including odour, colour and overall acceptance of control chicken meat sample and those wrapped with Ch coating, treated (CEO 0.2, 0.4 and 0.6%) and treated (Ch-CEO 0.2%, Ch-CEO 0.4%, Ch-CEO 0.6%) during storage at 4˚C are shown in Table 2. The sensory analysis results showed significant differences ($p < 0.05$) for odour between control samples with treated samples (Ch-CEO 0.2, 0.4, and 0.6%), and colour was shown between control samples with treated samples (Ch-CEO 0.4 and 0.6%) and overall acceptance. Results showed significant differences ($p < 0.05$) for overall acceptance between control samples with treated samples that were Ch-CEO 0.4 and 0.6%.

In this study the existence of chitosan (1.5% w/v) in Ch-CEO 0.2, 0.4 and 0.6% treated samples a very desirable odour and appearance color in the chicken meat, increasing the natural freshness of the chicken meat, while addition of cumin oil (0.2, 0.4 and 0.6%) in chicken meat samples caused to off odour and slime. According to Giatrakou *et al*. (2010) study, the addition of chitosan with thyme oil to cook chicken product gave a more acceptable taste and odour as compared to the untreated samples (Giatrakou *et al*., 2010).

Table 2. Effect of edible biodegradable coating containing Chitosan and CEO on the sensory analysis of chicken meat during refrigerated storage

Sensorial indexes				
Days	**Groups**	**Colour**	**Odour**	**Acceptability**
2	Control	7.8^{a}	7.2^{b}	8.0^{a}
	Ch	7.8^{a}	8.0^{b}	8.2^{a}
	CEO 0.2%	7.8^{a}	7.8^{b}	7.8^{a}
	CEO 0.4%	7.1^{a}	7.1^{b}	7.5^{a}
	CEO 0.6%	7.0^{a}	6.8^{c}	6.5^{b}
	Ch-CEO 0.2%	8.2^{a}	8.1^{b}	8.4^{a}
	Ch-CEO 0.4%	8.2^{a}	8.2^{b}	8.5^{a}
	Ch-CEO 0.6%	8.2^{a}	8.5^{b}	9.0^{a}
5	Control	3.5^{ab}	1.4^{e}	4.1^{a}
	Ch	7.5^{d}	8.1^{f}	7.4^{b}
	CEO 0.2%	4.5^{ab}	2.4^{e}	2.4^{a}
	CEO 0.4%	3.5^{ab}	2.0^{e}	1.8^{a}
	CEO 0.6%	2.0^{ab}	1.5^{e}	1.7^{a}
	Ch-CEO 0.2%	7.8^{d}	7.7^{f}	7.5^{b}
	Ch-CEO 0.4%	8.4^{d}	8.0^{f}	8.0^{b}
	Ch-CEO 0.6%	8.4^{d}	8.2^{f}	8.5^{b}
9	Control	1.4^{g}	1.1^{c}	1.0^{ab}
	Ch	7.8^{h}	8.1^{d}	7.0^{g}
	CEO 0.2%	2.2^{g}	2.1^{c}	2.0^{ab}
	CEO 0.4%	1.8^{g}	1.0^{c}	1.5^{ab}
	CEO 0.6%	1.2^{g}	1.0^{c}	1.0^{ab}
	Ch-CEO 0.2%	8.0^{h}	8.5^{d}	8.0^{g}
	Ch-CEO 0.4%	8.4^{h}	8.4^{d}	8.2^{g}
	Ch-CEO 0.6%	8.7^{h}	8.4^{d}	8.2^{g}

Any two means in the same column followed by the same letter are not significantly ($p > 0.05$) different from Duncan's multiple range tests. Ch: Chitosan, CEO: Cumin essential oil.

4. Conclusions

The results of this study showed that the combined use of chitosan and CEO prevented the growth of spoilage and foodborne pathogenic microbial, delayed lipid oxidation and increased shelf life of chicken meat at 4˚C. The samples wrapped with Ch and CEO 0.4 and 0.6% was the most effective of all, inhibiting the growth of the microbial spoilage, decreasing lipid oxidation of 9 days. In conclusion, the use of antimicrobial coatings has been indicated to be an effective method to preserve microbial and sensory quality of meat. On the other hand, using various preservative factors in small amounts is a preferred approach, because it has physicochemical characteristics, sensory properties and economic advantages.

5. References

Alizadeh-Sani, M., Khezerlou, A., & Ehsani, A. (2018). Fabrication and characterization of the bionanocomposite film based on whey protein biopolymer loaded with TiO2 nanoparticles, cellulose nanofibers and rosemary essential oil. *Industrial crops and products, 124*, 300-315.

Alizadeh-Sani, M., Mohammadian, E., & McClements, D. J. (2020). Eco-friendly active packaging consisting of nanostructured biopolymer matrix reinforced with TiO2 and essential oil: Application for preservation of refrigerated meat. *Food chemistry*, 322, 126782.

Allahghadri, T., Rasooli, I., Owlia, P., Nadooshan, M. J., Ghazanfari, T., Taghizadeh, M., & Astaneh, S. D. A. (2010). Antimicrobial property, antioxidant capacity, and cytotoxicity of essential oil from cumin produced in Iran. *Journal of food science, 75*(2), H54-H61.

Arkoun, M., Daigle, F., Heuzey, M.C., & Ajji, A. (2017). Mechanism of action of electrospun chitosan-based nanofibers against meat spoilage and pathogenic bacteria. *Molecules, 22*(4), 585.

Azizi-lalabadi, M., Alizadeh-Sani, M., Divband, B., Ehsani, A., & McClements, D. J. (2020). Nanocomposite films consisting of functional nanoparticles (TiO2 and ZnO) embedded in 4A-Zeolite and mixed polymer matrices (gelatin and polyvinyl alcohol). *Food Research International*, 137, 109716.

Azlin-Hasim, S., Cruz-Romero, M. C., Morris, M. A., Cummins, E., & Kerry, J. P. (2018). Spray coating application for the development of nanocoated antimicrobial low-density polyethylene films to increase the shelf life of chicken breast fillets. *Food Science and Technology International, 24*(8), 688-698.

Babuskin, S., Babu, P. A. S., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M., & Sukumar, M. (2014). Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International journal of food microbiology, 171*, 32-40.

Bagheri, V., Ghanbarzadeh, B., Ayaseh, A., Ostadrahimi, A., Ehsani, A., Alizadeh-Sani, M., & Adun, P. A. (2019). The optimization of physico-mechanical properties of bionanocomposite films based on gluten/carboxymethyl cellulose/cellulose nanofiber using response surface methodology. *Polymer Testing, 78*, 105989.

Chantarasataporn, P., Tepkasikul, P., Kingcha, Y., Yoksan, R., Pichyangkura, R., Visessanguan, W., & Chirachanchai, S. (2014). Water-based oligochitosan and nanowhisker chitosan as potential food preservatives for shelf-life extension of minced pork. *Food chemistry, 159*, 463-470.

Chouliara, E., Karatapanis, A., Savvaidis, I., & Kontominas, M. (2007). Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 C. *Food Microbiology, 24*(6), 607-617.

Fan, W., Sun, J., Chen, Y., Qiu, J., Zhang, Y., & Chi, Y. (2009). Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. *Food chemistry, 115*(1), 66-70.

García-Díez, J., Alheiro, J., Falco, V., Fraqueza, M. J., & Patarata, L. (2017). Chemical characterization and antimicrobial properties of herbs and spices essential oils against pathogens and spoilage bacteria associated to dry-cured meat products. *Journal of EssEntial oil rEsEarch, 29*(2), 117-125.

Georgantelis, D., Ambrosiadis, I., Katikou, P., Blekas, G., & Georgakis, S. A. (2007). Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 C. *Meat science, 76*(1), 172-181.

Giatrakou, V., Ntzimani, A., & Savvaidis, I. (2010). Effect of chitosan and thyme oil on a ready to cook chicken product. *Food Microbiology, 27*(1), 132-136.

Jirovetz, L., Buchbauer, G., Stoyanova, A. S., Georgiev, E. V., & Damianova, S. T. (2005). Composition, quality control and antimicrobial activity of the essential oil of cumin (Cuminum cyminum L.) seeds from Bulgaria that had been stored for up to 36 years. *International journal of food science & technology, 40*(3), 305-310.

Karakaya, S., & Simsşek, S. (2011). Changes in total polar compounds, peroxide value, total phenols and antioxidant activity of various oils used in deep fat frying. *Journal of the American Oil Chemists' Society, 88*(9), 1361-1366.

Kedia, A., Prakash, B., Mishra, P. K., & Dubey, N. (2014). Antifungal and antiaflatoxigenic properties of Cuminum cyminum (L.) seed essential oil and its efficacy as a preservative in stored commodities. *International journal of food microbiology, 168*, 1-7.

Lomate, G. B., Dandi, B., & Mishra, S. (2018). Development of antimicrobial LDPE/Cu nanocomposite food packaging film for extended shelf life of peda. *Food packaging and shelf life, 16*, 211-219.

Mahdizadeh, A., Shahidi, S. A., Shariatifar, N., Shiran, M. R., & Ghorbani Hassan Saraei, A. (2020). Preparation and evaluation of chitosan-zein film containing free and encapsulated ethanol extract of kak kosh biabani Pulicaria gnaphalodes (Vent.) Boiss. *Journal of Medicinal Plants, 19*(75), 223-238.

Martins, J. T., Cerqueira, M. A., & Vicente, A. A. (2012). Influence of α-tocopherol on physicochemical properties of chitosan-based films. *Food hydrocolloids, 27*(1), 220-227.

Noshirvani, N., Hong, W., Ghanbarzadeh, B., Fasihi, H., & Montazami, R. (2018). Study of cellulose nanocrystal doped starch-polyvinyl alcohol bionanocomposite films. *International journal of biological macromolecules, 107*, 2065-2074.

Oroojalian, F., Kasra-Kermanshahi, R., Azizi, M., & Bassami, M. R. (2010). Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. *Food chemistry, 120*(3), 765-770.

Peng, Y., Li, Y., & Yin, L. (2013). Effects of edible coatings on some quality parameters of Chinese water chestnut (Eleocharis tuberosa) during storage. *International journal of food science & technology, 48*(7), 1404-1409.

Petrou, S., Tsiraki, M., Giatrakou, V., & Savvaidis, I. (2012). Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *International journal of food microbiology, 156*(3), 264-271.

Plascencia-Jatomea, M., Viniegra, G., Olayo, R., Castillo-Ortega, M. M., & Shirai, K. (2003). Effect of chitosan and temperature on spore germination of Aspergillus niger. *Macromolecular Bioscience, 3*(10), 582-586.

Rebey, I. B., Bourgou, S., Debez, I. B. S., Karoui, I. J., Sellami, I. H., Msaada, K., Limam, F., & Marzouk, B. (2012). Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin (Cuminum cyminum L.) seeds. *Food and Bioprocess Technology, 5*(7), 2827-2836.

Rebey, I. B., Jabri-Karoui, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., & Marzouk, B. (2012). Effect of drought on the biochemical composition and antioxidant activities of cumin (Cuminum cyminum L.) seeds. *Industrial crops and products, 36*(1), 238-245.

Rhim, J.-W., Hong, S.-I., Park, H.-M., & Ng, P. K. (2006). Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *Journal of agricultural and food chemistry, 54*(16), 5814-5822.

Ribeiro-Santos, R., Andrade, M., de Melo, N. R., & Sanches-Silva, A. (2017). Use of essential oils in active food packaging: Recent advances and future trends. *Trends in food science & technology, 61*, 132-140.

Ruby, K., Chauhan, R., Sharma, S., & Dwivedi, J. (2012). Polypharmacological activities of Bergenia species. *International Journal of Pharmaceutical Sciences Review and Research, 13*(1), 100-110.

Sadeghi, E., Akhondzadeh-Basti, A., Noori, N., Khanjari, A., & Partovi, R. (2013). Effect of Cuminum cyminum L. essential oil and

Lactobacillus acidophilus (a probiotic) on Staphylococcus aureus during the manufacture, ripening and storage of white brined cheese. *Journal of food processing and preservation, 37*(5), 449-455.

Salari, M., Khiabani, M. S., Mokarram, R. R., Ghanbarzadeh, B., & Kafil, H. S. (2018). Development and evaluation of chitosan based active nanocomposite films containing bacterial cellulose nanocrystals and silver nanoparticles. *Food hydrocolloids, 84*, 414-423.

Sani, M. A., Ehsani, A., & Hashemi, M. (2017). Whey protein isolate/cellulose nanofibre/TiO2 nanoparticle/rosemary essential oil nanocomposite film: Its effect on microbial and sensory quality of lamb meat and growth of common foodborne pathogenic bacteria during refrigeration. *International journal of food microbiology, 251*, 8-14.

Shekarforoush, S. S., Basiri, S., Ebrahimnejad, H., & Hosseinzadeh, S. (2015). Effect of chitosan on spoilage bacteria, Escherichia coli and Listeria monocytogenes in cured chicken meat. *International journal of biological macromolecules, 76*, 303-309.

Siripatrawan, U., & Noipha, S. (2012). Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. *Food hydrocolloids, 27*(1), 102-108.

Soultos, N., Tzikas, Z., Abrahim, A., Georgantelis, D., & Ambrosiadis, I. (2008). Chitosan effects on quality properties of Greek style fresh pork sausages. *Meat science, 80*(4), 1150-1156.

Tharanathan, R. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in food science & technology, 14*(3), 71-78.

Vasilatos, G., & Savvaidis, I. (2013). Chitosan or rosemary oil treatments, singly or combined to increase turkey meat shelf-life. *International journal of food microbiology, 166*(1), 54-58.

Wang, Q., Tian, F., Feng, Z., Fan, X., Pan, Z., & Zhou, J. (2015). Antioxidant activity and physicochemical properties of chitosan films incorporated with Lycium barbarum fruit extract for active food packaging. *International journal of food science & technology, 50*(2), 458-464.

Xiong, Z., Sun, D.-W., Pu, H., Xie, A., Han, Z., & Luo, M. (2015). Non-destructive prediction of thiobarbituric acid reactive substances (TBARS) value for freshness evaluation of chicken meat using hyperspectral imaging. *Food chemistry, 179*, 175-181.

Xu, Y., Kim, K. M., Hanna, M. A., & Nag, D. (2005). Chitosan–starch composite film: preparation and characterization. *Industrial crops and products, 21*(2), 185-192.

Yuan, G., Chen, X., & Li, D. (2016). Chitosan films and coatings containing essential oils: The antioxidant and antimicrobial activity, and application in food systems. *Food Research International, 89*, 117-128.

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