



Microbial, Chemical, and Sensorial Properties of Chicken Fillets Coated by Gelatin-Carboxymethyl Cellulose Film Containing Essential Oil of Bene (*Pistacia atlantica*)

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HIGHLIGHTS

- *Pistacia atlantica* Essential Oil (EO) showed remarkable antibacterial and antioxidant properties in chicken fillet.
- A dose-dependent manner was seen regarding antibacterial and antioxidant properties *P. atlantica* EO.
- The 0.3 and 0.6% concentrations of *P. atlantica* EO showed generally acceptable sensorial properties.

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Acronyms and abbreviations

EO=Essential Oil
CMC=Carboxymethyl Cellulose
TBARS=Thiobarbituric Acid
TVN=Total Volatile Nitrogen

ABSTRACT

Background: Fillet is a popular part of chicken carcass which its high perishability is an economic problem. The present research was designed to study the microbial, chemical, and sensorial properties of chicken fillets coated by gelatin-Carboxymethyl Cellulose (CMC) film containing Essential Oil (EO) of Bene (*Pistacia atlantica*).

Methods: The chicken fillets were coated by gelatin-CMC film containing 0.3, 0.6, and 0.8% concentrations of Bene EO. Different microbial and chemical analyses were carried out at different intervals (days 0, 3, 6, 9, and 12). Data were analyzed using SPSS, Inc, Chicago, IL software (v. 18.0).

Results: Aerobic mesophilic bacteria, psychotropic bacteria, lactic acid bacteria, and coliform counts in Bene EO treated groups were significantly ($p < 0.05$) lower than control group. Although there was not any difference between *Staphylococcus aureus* count in control and 0.3% Bene groups ($p > 0.05$), bacterial growth was significantly ($p < 0.05$) decreased in 0.6% and 0.8% Bene EO groups. There were not any meaningful difference ($p > 0.05$) between thiobarbituric acid, total volatile nitrogen, and peroxide values in control and 0.3% Bene EO treated chicken fillet groups; however, these chemical parameters were significantly ($p < 0.05$) lower in 0.6% and 0.8% Bene EO groups than control one. Both control and samples treated by 0.3% Bene EO showed the highest sensory score in all parameters while there was no significant difference between them ($p > 0.05$). Although no changes in color and texture of samples treated by 0.6% and 0.8% Bene EO were seen, but there was a slight decline in odor and taste scores.

Conclusion: Chicken fillets coated by gelatin-CMC film containing 0.6% Bene EO showed acceptable antibacterial, antioxidant, and sensorial properties. So, the outcome of the present work can be applied in the meat industries.

Introduction

Fillet is a popular part of chicken carcass which its high perishability is an economic problem for its producer-

s (Mead, 2004). In recent decades, many researchers have focused on finding the modern methods and technologies

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in order to increase shelf life of foods. On the other hand, consumer demand for healthy meal without the use of chemical preservatives has been more increased in respect to the past (Owens et al., 2010).

Antimicrobial packaging is an effective strategy in food sciences, which results in increasing the shelf life of food by reducing microbial growth. Today, natural ingredients such as gums and proteins are used to improve physical and chemical properties of foods (An et al., 2000; Cutter, 2006; Du et al., 2008). Biodegradable biopolymers are relatively inexpensive and environmentally friendly and can be used as a key ingredient for the new pragmatic packaging (Du et al., 2008). Various polysaccharides including, cellulose, pectin, starch derivatives, etc. are extensively used in film production (Chen and Lai, 2008). Carboxymethyl Cellulose (CMC) is a cellulose derivative with carboxymethyl groups ($-\text{CH}_2\text{-COOH}$) bound to some of the hydroxyl groups of the glucopyranose monomers that makes up the cellulose backbone. Cellulose is often used as sodium salt in food industry (Martelli et al., 2017). On the other hand, proteins are widely used in the formation of edible films and have a suitable resistance against the diffusion of oxygen, carbon dioxide, and fat especially in low relative humidity. Gelatin is a protein derived from collagen hydrolysate in bone and skin. In addition, gelatin is employed as a carrier for bioactive compounds. A collection of practical material can be added to increase the efficiency of films for maintaining the food materials (Cai et al., 2011; Embuscado and Huber, 2009). Different reports have revealed indirect antimicrobial activity and shelf life-enhancing of gelatin and CMC on various food products because of their role in reduction of activity water (Asma et al., 2014; Lee et al., 2004).

Essential Oils (EOs) are of natural volatile compounds which their application as food preservative is increasing in food industry due to their antimicrobial activities (Azhdarzadeh and Hojjati, 2016; Khorasany et al., 2016). Bene (*Pistacia atlantica*) is a native tree species in Iran, which its fruit has been used in traditional herbal medicine. Until now, three subspecies of *P. atlantica* have been identified in this country (especially Western areas) including, *kurdica*, *mutica* and *cabulica* (Hatamnia et al., 2014). Although some recent studies have stated that the resin of *P. atlantica* showed some antimicrobial and antioxidant characteristics, *in vitro* (Farhoosh et al., 2008; Hatamnia et al., 2014; Rezaie et al., 2015), there is still lack of data about effectiveness of these properties in food models.

The present investigation was designed to study the microbial, chemical, as well as sensorial properties of chicken fillets coated by gelatin-CMC film containing different concentrations of EO of the Bene (*P. atlantica*).

Materials and methods

Chemicals

Most of chemicals and reagents were prepared from Merck, Darmstadt, Germany. The others were included commercial gelatin obtained from Iranian commerce development organization, CMC (Santos, Japan), and commercial Bene EO (Sannandaj, Iran).

Preparing the edible coating of gelatin-CMC

The solution (4% w/v) of gelatin was prepared in deionized water and then stirred at 80 °C for 30 min. When it was completely dissolved to reach ambient temperature, 30% glycerol (90% purity) was added to this solution as a plasticizer and then stirred for 10 min. Separately, 1 g CMC was dissolved in 100 ml distilled water and 50% CMC (0.5 g) added to the solution of glycerol as a softener. Finally, a solution containing 50% CMC and 50% gelatin was prepared. Next, pH was adjusted using NaOH at the range of 5-5.5 to make a desirable interaction between gelatin and CMC. The Bene EO (0.3, 0.6, and 0.8%) was added to the mixture solution, containing 1% Tween 80. Then, it was homogenized using Ultra-Turrax T 25 (Germany) at 2400 rpm for 5 min. Each sample was placed in stationary mode to cool and leave air bubbles. After drying the film at the ambient temperature, it was placed in oven at 37 °C for 20 min (Asma et al., 2014).

Fillet coating

Chicken fillets were purchased from a local market and transferred to the laboratory in cold conditions. Samples of 50 g fillet were prepared in sterile condition and 40 fillets were considered for each treatment. To create the coating, fillets were soaked in coating solution for 30 s, taken for 2 min, and then were soaked in the solution for 30 s, again. Control (uncoated samples) was remained in distilled water until starting the tests. The fillets were placed in sterile bags and kept at 4 °C until next analysis at different intervals (days 0, 3, 6, 9, and 12).

Bacterial analysis

For bacterial analysis, each 10 g sample was put in a sterile bag containing 90 ml peptone water (0.1%), homogenized at 200 rpm/min. Decimal dilutions were prepared and plated in the appropriate media. Culturing as well as counting *Staphylococcus aureus*, aerobic mesophilic bacteria, psychotropic bacteria, lactic acid bacteria, and coliform were carried out based on Batt and Tortorello (2014).

Measurement of Thiobarbituric Acid (TBARS)

TBARS was measured based on method reported by

Botsoglou et al. (1994). For this purpose, 30 ml 4% perchloric acid was poured into a 50 ml centrifuge tube containing 10 g sample and then 1 ml 0.5% butylated hydroxytoluene homogenized in ethanol was added. The mixture was filtered by Whatman (No. 4) and 5 ml of the filtrate was mixed with 5 ml of TBARS solution (0.02 M) and then was placed in a boiling water bath for 20 min. After cooling the samples, they were read at wavelength of 532 nm using a spectrophotometer (Cecil, Swiss). The TBARS was calculated based on mg malondialdehyde per kg sample.

Total Volatile Nitrogen (TVN)

To measure TVN, 10 g sample, in addition of 2 g magnesium oxide and 500 ml distilled water were mixed in a 1 L balloon and TVN was collected in a solution containing boric acid (2%) as well as methyl red as an indicator. Titration was performed by sulfuric acid described as mg N/100 g of chicken fillet (Cai et al., 2011).

Peroxide value

For this purpose, 20 g fillet with 100 ml of chloroform-methanol was mixed at a portion of 2:1 using a blender (Waring, USA) for 1 min. After dewatering by potassium chloride, mixed aqueous phase (lower phase) was collected and used for titration by sodium thiosulfate according to Kei (1978).

Sensory analysis

The sensory analysis was conducted by a 6-member trained panel based on five-point hedonic scale to evaluate scores of color, odor, texture, taste, and overall ac-

ceptability of the fillets as described previously by Ojagh et al. (2010).

Statistical analysis

Data were analyzed using SPSS, Inc, Chicago, IL software (v. 18.0). Significance levels ($p < 0.05$) were assessed by ANOVA and mean comparison done by Duncan Multiple Range test.

Results

As illustrated in Figures 1-4, aerobic mesophylic bacteria, psychotropic bacteria, lactic acid bacteria, and coliform counts in Bene EO treated groups were significantly ($p < 0.05$) lower than control group. Although there was not any difference between *S. aureus* count in control and 0.3% Bene groups ($p > 0.05$), bacterial growth was significantly ($p < 0.05$) decreased in 0.6% and 0.8% Bene EO groups.

There were not any meaningful difference ($p > 0.05$) between TBARS, TVN, and peroxide values in control and 0.3% Bene EO treated fillet groups; However, these chemical parameters were significantly ($p < 0.05$) lower in 0.6% and 0.8% Bene EO groups than control one (Figures 5-7).

Both control and samples treated by 0.3% Bene EO showed the highest sensory score in all parameters while there was no significant difference between them ($p > 0.05$). Although no changes in color and texture of samples treated by 0.6% and 0.8% Bene EO were seen, but there was a slight decline in odor and taste scores. The 0.8% Bene EO group revealed the lowest overall acceptability (Figure 8).

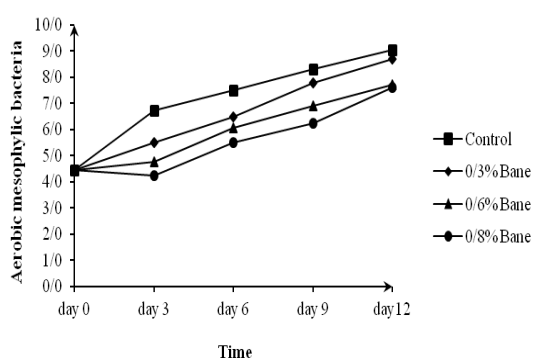


Figure 1: Aerobic mesophylic bacteria count (\log_{10} CFU/g) in control and treated chicken fillet groups at different periods of time

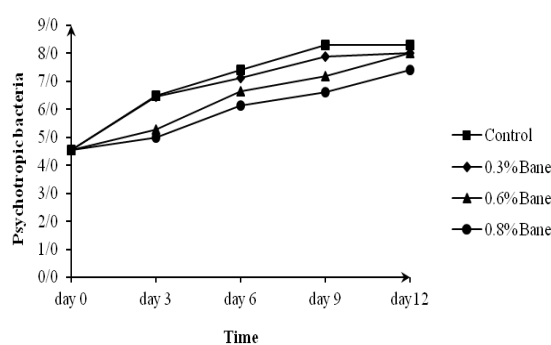


Figure 2: Psychotropic bacteria count (\log_{10} CFU/g) in control and treated chicken fillet groups at different periods of time

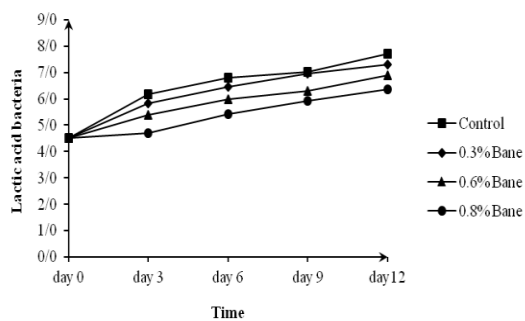


Figure 3: Lactic acid bacteria count (\log_{10} CFU/g) in control and treated chicken fillet groups at different periods of time

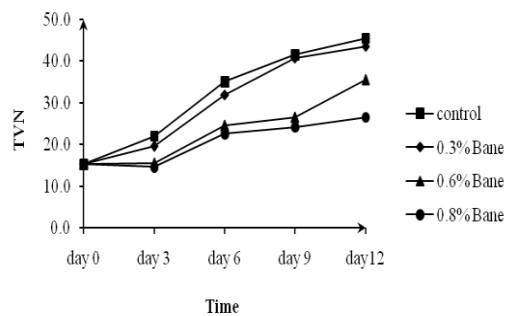


Figure 6: TVN (mg/100 g) in control and treated chicken fillet groups at different periods of time

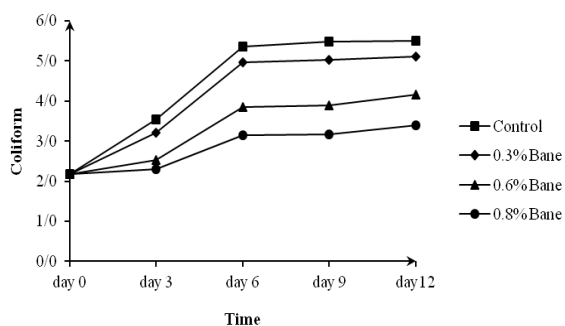


Figure 4: Coliform count (\log_{10} CFU/g) in control and treated chicken fillet groups at different periods of time

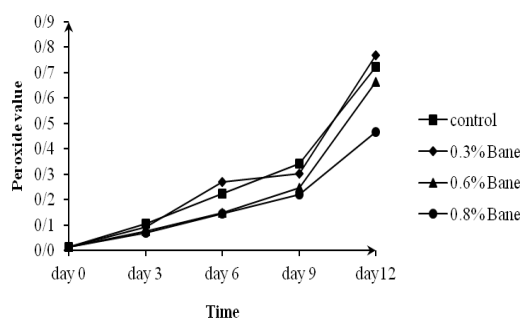


Figure 7: Peroxide value (meq/kg) in control and treated chicken fillet groups at different periods of time

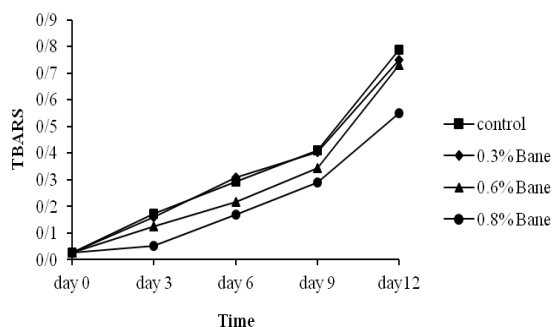


Figure 5: TBARS (mg malondialdehyde/kg) in control and treated chicken fillet groups at different periods of time

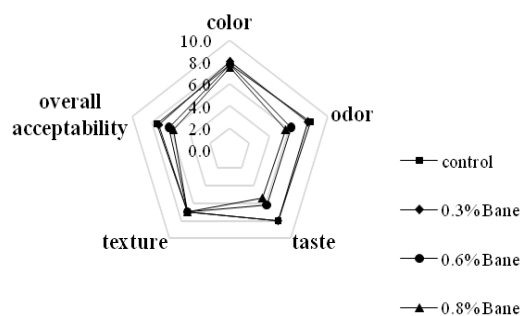


Figure 8: Sensory scores in control and treated chicken fillet groups at different periods of time

Discussion

In the present investigation, we found remarkable antibacterial and antioxidant properties of gelatin-CMC film containing different concentrations of Bene EO; however, a dose-dependent manner was seen in this regards. Our results were in accordance with those obtained by Rezaie et al. (2015) who announced that EO of *P. atlantica* subsp. *mutica* had great antibacterial impact on *E. coli* and *S. aureus* with minimum inhibitory concentration values of 12.5 and 6 $\mu\text{g/ml}$, respectively; they also attributed this antibacterial activities to some major com-

ponents determined during their study named α -pinene (20.8%), camphene (8.4%), β -myrcene (8.2%), and limonene (8%). Similarly, Hosseini et al. (2013) showed antibacterial effect of *P. atlantica* extracts on *Streptococcus mutans* biofilm. In the current research, all the treatment groups (with exception of 0.3% EO group) revealed much lower levels of TBARS, TVN, and peroxide values in comparison with control group, indicating considerable antioxidant characteristics of this EO. Our findings were in agreement with the *in vitro* studies of Farhoosh et al. (2008) and Rezaie et al. (2015) who

found appropriate antioxidant property of Bene EO in peroxide values as well as TBARS assays, respectively. Other researchers attributed antioxidant activity of Bene EO to its high phenolic ingredients including, sinapic acid, vanillic acid, and p-hydroxybenzoic acid (Hatamnia et al., 2014).

The shelf-life enhancing activities of Bene EO were detected at the end of our experiments on day 12. This finding may be attributed to this matter that the gelatin-CMC like some other films applied in food active packaging can probably maintain the chemical volatile compounds of EO for a more time (Ribeiro-Santos et al., 2017). It is assumed that if the Bene EO is used as spray, after a short time, the concentration of EO in the product is probably reduced due to high volatility resulting in reduction of EO antimicrobial/antioxidant properties (Burt, 2004). However, we found that gelatin-CMC film alone (control group) did not show any antibacterial or antioxidant activities; so, it is necessary to combine gelatin-CMC alone with an effective ingredient such as Bene EO for achievement of ideal shelf life-enhancing impact. It should be mentioned that although sensory evaluation scores of fillet contained 0.8% Bene EO were not generally acceptable, but 0.3% as well as 0.6% concentrations have acceptable sensorial characteristics and can be used as food preservative.

There was a limitation in this study that it is must be mentioned here. As we noted before, three different subspecies of *P. atlantica* have been recognized in Iran (Hatamnia et al., 2014). In this research, we used an Iranian commercial Bene EO for the experiments which its subspecies origin in unknown.

Conclusion

Chicken fillets coated by gelatin-CMC film containing 0.6% EO of Bene (*P. atlantica*) showed acceptable antibacterial, antioxidant, and sensorial properties. So, the outcome of the present work can be applied in the meat industries. To the best of our knowledge, this study is the first of its kind carried out about application of gelatin-CMC film containing Bene EO in a food model.

Conflicts of interest

All the authors declare that they have no conflicts of interest.

Acknowledgments

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