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In vitro antibacterial and antioxidant properties of *Elettaria cardamomum* Maton extract and its effects, incorporated with chitosan, on storage time of lamb meat

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ABSTRACT

In this experimental study, ethanolic extract of *Elettaria cardamomum* Maton (ECM) was prepared, the amount of phenolic compounds and antioxidant properties were measured using colorimetric methods and β-carotene bleaching assay, respectively. The effects of chitosan with a concentration of 1% and 2% (w/w) of ECM extract at refrigerated temperature (4 ± 1 °C) on the microbial quality of lamb meat was evaluated. Changes in pH level, the total count of bacteria, psychrotrophic and lactic acid bacteria, *Enterobacteriaceae*, yeast, mold counts and also the organoleptic characteristics of lamb meat in specific storage times (day zero, 1st, 3rd and 5th) were examined. The total amount of phenols of ECM ethanolic extract was 19.27 ± 0.02 mg/g Gallic acid equivalent, and antioxidant capacity was 45.7 ± 7.5%. In all treated samples, a reduction in the total count of bacteria, *Enterobacteriaceae*, psychrotrophic and lactic acid bacteria, molds and yeasts were observed ($P<0.05$). Besides, the addition of extract incorporated with chitosan to meat increased its overall acceptance rate ($P<0.05$). It may be concluded that ethanolic extract of ECM mixed with chitosan can be used as a natural antibacterial and antioxidant coating, safe to be consumed with meat products.

Key words: chitosan, *Elettaria cardamomum* Maton, storage time, lamb meat, antioxidant activity

Introduction

Meat is one of the putrefying food materials and it has a short shelf life. The rapid microbial growth of newly refrigerated meat shortens its shelf life, reduces storage time and decreases its quality, for reasons such as the non-maintenance of the cold chain during

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storage, distribution and transportation. In these circumstances, severe economic losses will be incurred relating to the meat (SHARAFATI-CHALESHTORI et al., 2014; LORETZ et al., 2011).

In addition, meat is one of the important sources of protein. Being rich in valuable proteins containing essential amino acids, minerals such as iron and zinc, as well as different types of vitamins, meat is nowadays categorized as one of the best and most complete nutritive materials in human nutrition (KARGIOTOU et al., 2011).

Currently, pollution caused by synthetic polymers is attracting public health attention regarding the use of biodegradable materials, and over the past two decades, studies on biodegradable materials made from proteins and carbohydrates have been widely expanded. Chitosan is one of the cationic polysaccharides produced by *deacetylation* of chitin obtained from the shells of crustaceans such as crabs and shrimps, produced by chemical and microbiological methods, and it is the second most frequent natural polymer in nature after cellulose (HASSANZADEH et al., 2011). This polysaccharide is characterized by features such as antibacterial, antifungal, and antioxidant properties, as well as forming an environmentally friendly, biodegradable, non-toxic, and protective film, and adhesive properties. In addition, it acts as a nutritional fiber, as well as a barrier within the exchange of CO_2 and O_2 gases and moisture utilized in the food, pharmaceutical, cosmetic, paint, and textile industries, etc. (HASSANZADEH et al., 2011; BUSATTA et al., 2008).

Nowadays, much attention has been focused on extracts and essential oils from herbs and spices, which have been used for centuries to improve the sensory characteristics and shelf life of foods (ZHANG et al., 2016). Cardamomum, with the scientific name *Elettaria cardamomum* Maton, belonging to the family of Zingiberaceae, is grown in tropical and warm regions, mostly in India. It is consumed as a spice, and an aromatic beverage, as well as a food additive. ECM is traditionally used in India for the treatment of periodontal diseases, sore throat, congestion of eyelids and digestive diseases. Its essential oil has carminative and antivirus properties (AKHAVAN et al., 2008). In previous studies, antioxidant properties (VERMA et al., 2009) and the antibacterial effect of different extracts of ECM have been shown on *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (ANEJA and SHARMA, 2010). Some studies have reported the activities of the herbal extract in the nutritional system (AKHAVAN et al., 2008; BUSATTA et al., 2008), and the decrease in economic losses resulting from deletion of this valuable nutrition, as well as the use of natural preservative methods, considering the consumers' viewpoint regarding its high quality and taste (SHARAFATI-CHALESHTORI et al., 2014; LORETZ et al., 2011; EMIROĞLU et al., 2010). The purpose of this study was to evaluate the effect of edible chitosan coatings containing ethanolic extract of *Elettaria cardamomum* Maton on increasing the storage time of meat under refrigerated conditions.

Materials and methods

Preparation of ethanolic extract of ECM and meat samples. Elettaria cardamomum Maton seeds were purchased from a reputable grocery in Shahrekord, and were identified and stored at the herbarium unit of the Medicinal Plants Research Center of Shahrekord University of Medical Sciences. After cleaning, they were dried in shade, and ground using a mill. To prepare ethanolic extract, 100 g of the dried powder of the plant was mixed with 500 cc of 80% ethanol and kept at room temperature (22 ºC) for 24 hours. The obtained extract was filtered through filter paper and placed in a rotary device (to remove solvent). The obtained alcoholic extract was dried at the temperature of 40 ºC in an incubator (SHARAFATI-CHALESHTORI et al., 2012). A male lamb leg aged 3-6 months was prepared from a slaughterhouse in Shahrekord and was used for all experiments.

Chitosan film preparation. Medium molecular weight chitosan was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Firstly, 2% acid acetic was prepared and 2% chitosan solution was dissolved with 2% (w/v) in 2% v/v acetic acid stirred at 55 °C for 150 minutes and filtered through a Whatman No. 41 filter paper. Glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added to the chitosan solution (2% v/v) as a plasticizer. Chitosan solution was then prepared along with ethanolic extract of ECM at a final concentration of 1% and 2%. To coat the samples, they were soaked in the solution for 1 minute and air-dried for 30 minutes (IBRAHIM et al., 2014). Treatment groups consisted of 1) lamb meat without chitosan and extracts coating, 2) lamb with only chitosan coating, 3) lamb coated with chitosan and extract 1% and 4) lamb coated with chitosan and extract 2%.

Analysis of Elettaria cardamomum Maton extract and chitosan

Total phenolic determination. The amount of total phenolic compounds was measured in ethanolic extract of ECM by the colorimetric method using Folin-Ciocalteu. Standard solutions with concentrations of 12.5, 25, 50, 62.5,100 and 125 ppm were prepared from Gallic acid in 60% solution. 0.1 mL of each solution was transferred to a test tube and 0.5 mL of reagent Folin-Ciocalteu solution 10% was added. After 3 to 8 minutes, 0.4 mL 7.5% sodium carbonate solution was added to it, then the tubes were kept at laboratory temperature for 30 min. The optical absorption was measured at a wavelength of 765 nm by a spectrophotometer (Unico UV-2100, USA) and a standard curve was prepared. Then 0.01 to 0.02 grams of the dried extract was dissolved in 60% methanol up to a volume of 10 mL. Total phenol content was determined using the Folin-Ciocalteu method with the difference that instead of the standard solution, 0.1 mL of the extract solution was added. The obtained absorbance rate was compared to the standard curve and thus the total phenol content of the extract in mg/g Gallic acid equivalent was estimated (SHARAFATI-CHALESHTORI et al., 2011).

Total flavonoid and flavonol determination. Total flavonoid and flavonol content were measured using the colorimetric method by aluminum chloride. At first, the standard solutions, with concentrations of 25, 50, 100, 250 and 500 ppm of Rutin were prepared in 60% methanol and 1 mL of this solution was transferred into test tubes, and then 1 mL of 2% aluminum chloride was added to it. 6 mL of 5% potassium acetate was added, the optical densities were expressed at a wavelength of 415 nm after 40 minutes for standard flavonoid and at a wavelength of 440 nm after 150 min for the standard flavonol, and a standard curve was prepared. 0.01 to 0.02 grams of the dried extract was dissolved in 60% methanol up to a volume of 10 mL and total flavonoid and flavonol content were determined using the aluminum chloride method, with the difference that instead of the standard solution, 0.1 mL of the extract solution was added. The obtained absorbance rate was compared to the standard curve and thus the total flavonoid and flavonol content of the extract in the mg/g Rutin equivalent were found (SHARAFATI-CHALESHTORI et al., 2011).

Antioxidant activity determination. To carry out this experiment, firstly, 0.5 mg β-carotene were prepared in 1 mL of chloroform and 25 µL of inolenic acid and 200 mg of Tween-40 as the stock solution, and then the chloroform was evaporated using a rotary evaporator at 50 ºC. Then, 100 mL of oxygenated water (30 min 100 mL/min) were added under vigorous shaking. 350 mL of extract (2 g/L) was added to the test tube containing 2.5 mL of aliquots of this emulsion. All these steps were carried out for butylated hydroxytoluene (BHT) as the positive control and a blank (containing only 350 µL of ethanol). After 48 h incubation at room temperature, the optical density rate of samples at 490 nm was expressed and its total antioxidant activity was measured as a percentage based on a comparison of the optical density of the samples at the time "zero" as well as with consideration of the stability of the yellow color of β-carotene (SHARAFATI-CHALESHTORI et al., 2012).

Determination of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations. The *Listeria monocytogenes* (Persian Type Culture Collection:1163), *Salmonella* Typhi (PTCC: 1609), *Streptococcus pyogenes* (PTCC: 1447) and *Shigella dysenteriae* (PTCC: 1188) were obtained from the Iranian Research Organization for Science and Technology (IROST) and cultured at 37 °C in tryptic soy broth (TSB, Merck Ink, Darmstadt, Germany). The MIC and MBC rates were measured by the microdilution method. This means that for every test, 9 wells of ELISA plates were selected. At first, 95 µL of Mueller Hinton broth (Merck Ink. Darmstadt, Germany) was added to each well and 5 µL of each bacterial suspension, equivalent to a 0.5 McFarland tube, was added to all wells. Finally, 100 µL of the consecutive dilution (serial two-fold dilutions) of extract (5.2-666 mg/mL) were added to each well.

Well No. 8, containing 195 µL Mueller Hinton broth and 5 µL of bacterial suspension without extract, was considered as the positive control, and well No. 9, containing 200 µL of Mueller Hinton broth, was considered as the negative control. The final volume in all wells was 200 µL. After the samples were mixed by Shaker (at a rate of 300 rpm per 20 s), they were placed in an incubator for 18-24 h at 37 °C. The wells were examined for the presence or lack of turbidity. The dilution plate of the well containing the lowest concentration of plant extracts that inhibited growth of bacteria (lack of turbidity) was determined as the minimum inhibitory concentration (MIC). Furthermore, the lowest concentration that showed no visible growth on Mueller Hinton agar was determined as the minimum bactericidal concentration (MBC) (SHARAFATI-CHALESHTORI et al., 2015b).

Analysis of meat samples. Microbiological analyses of meat. Samples weighing 10 g each were collected from each treatment, diluted in 90 mL of a sterile 0.9% NaCl solution, and mixed in a Stomacher (Seward Stomacher 400, Seward Medical, London, UK). The next tests were carried out with dilution tubes. Total mesophilic bacterial count (TMBC) and *Enterobacteriaceae* count were carried out in plate count agar (PCA) (Merck, Germany) by the surface culture method, and violet red-bile glucose (VRBG) agar (Merck, Germany) by the pour culture method, and they were incubated for 24 h at the temperature of 37 ºC. Counting of psychrotrophic bacteria was undertaken on the surface cultured in glutamate starch phenol red (GS P) agar (Merck, Germany) and the plates were incubated for 48 h at 35 ºC (LUCERA et al., 2014; HASSANZADEH et al., 2011). Enumeration of lactic acid bacteria (LAB) was carried out in de Man, Rogosa, Sharpe (MRS) agar (Merck, Germany) by the pour plate method, and plates were incubated for 48-72 h at 37 ºC. The yeast and mold were surface cultured in yeast extract glucose chloramphenicol (YGC) agar (Merck, Germany) and incubated for 48 h at 25 ºC and then the number of colonies was counted (EMIROĞLU et al., 2010).

pH measurement. Ten grams of meat, treated by all of the aforementioned methods were homogenized in 90 mL of deionized water for 1 minute and centrifuged for 1 minute. The pH of the supernatant solution was measured by a pH meter (CG824, Germany) (HASSANZADEH et al., 2011).

Sensory evaluation. A hedonic test was carried out by serving warm cooked samples to a panelist. The scoring scale including nine-points (1 = dislike exceedingly, 2 = dislike very much, 3 = dislike moderately, 4 = slightly dislike, 5 = neither like nor dislike, 6 = like slightly, 7 = moderately like, 8 = like very much, 9 = like extremely well) was used for sensory characters such as overall acceptability (SHARAFATI-CHALESHTORI et al., 2014).

Statistical analysis. Total chemical and microbial tests were repeated three times and obtained by Mean ± Standard deviation. The data were analyzed using SPSS software 16 through ANOVA and Duncan's tests. $P<0.05$ was considered to be significant.

Results

Fig.1 shows total phenolic, flavonoid and flavonol components of ethanolic extract of *Elettaria cardamomum* Maton. On the basis of the results, the antioxidant capacity of ethanolic extract of ECM was 45.7 ± 7.5%, which when compared to standard antioxidant butylated hydroxytoluene (BHT), with antioxidant capacity of 90.6 ± 3.30%, had lower antioxidant properties.

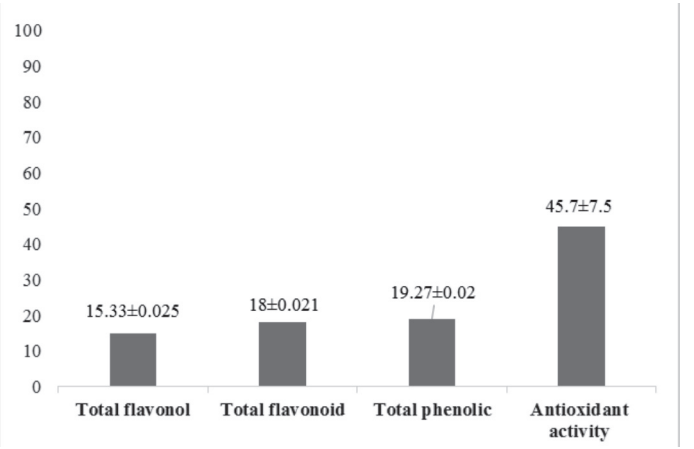

Fig.1. Flavonol, flavonoid, phenolic compounds and antioxidant activity of ethanolic *Elettaria cardamomum* Maton extract

* The total flavonol and flavonoid content of the extract were expressed in the mg/g Rutin equivalent and the total phenol content was expressed in the mg/g gallic acid equivalent. Antioxidant activity was expressed as a percentage based on the stability of the yellow color of β-carotene.

Table 1. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) activity of ethanolic *Elettaria cardamomum* Maton extract and Chitosan (prepared in 2% acetic acid) on bacteria

	Shigella dysenteriae		*Listeria monocytogenes*		*Salmonella* Typhi		*Streptococcus pyogenes*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Elettaria cardamomum Maton	41.625	83.25	166.5	333	166.5	333	83.25	166.5
Chitosan	0.5	1	0.5	1	1	2	0.25	0.5

The results related to the MIC and MBC of the aforementioned bacteria are given in Table 1. The lowest amount of MIC was 0.25 mg/mL for *Streptococcus pyogenes with chitosan,* and the highest amount was 166.5 mg/mL for *Listeria monocytogenes* and *Salmonella* Typhi with ECM. Besides, the lowest amount of MBC was 0.5 mg/mL for *Streptococcus pyogenes* and the highest amount of MBC was 333 mg/mL for *Listeria monocytogenes* and *Salmonella* Typhi. The results show that the antibacterial activity of chitosan was higher than that of the ethanolic extract of ECM *in vitro*.

Table 2. The influence of storage time on the microbiological status of lamb meat and lamb meat-film incorporated with different concentrations of ethanolic *Elettaria cardamomum* Maton extract (log cfu/g)

	Days	0	1	3	5
Total mesophilic bacterial count	C	$< 2^{A}$	$< 2^{A}$	5.84 ± 0.02^{A}	6.02 ± 0.05^{A}
	K	$< 2^{A}$	$< 2^{A}$	5.59 ± 0.01^{Y}	5.89 ± 0.01^{Y}
	KO	$< 2^{A}$	$< 2^{A}$	5.58 ± 0.01^{Y}	5.49 ± 0.02^{Z}
	KH	$< 2^{A}$	$< 2^{A}$	5.43 ± 0.07^{Z}	5.46 ± 0.01^{Z}
Psychrotrophic bacteria	C	2.19 ± 0.03^{A}	4.13 ± 0.01^{A}	4.85 ± 0.05^{A}	6.17 ± 0.01^{A}
	K	2.19 ± 0.03^{A}	$< 2^{Y}$	4.02 ± 0.02^{Y}	4.95 ± 0.05^{Y}
	KO	2.19 ± 0.03^{A}	$< 2^{Y}$	3.99 ± 0.01^{Y}	4.87 ± 0.03^{Y}
	KH	2.19 ± 0.03^{A}	$< 2^{Y}$	3.97 ± 0.05^{Y}	4.85 ± 0.05^{Y}
Mesophilic lactic acid bacteria	C	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$
	K	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$
	KO	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$
	KH	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$	$< 2^{A}$
Enterobacteriaceae	C	$< 2^{A}$	$< 2^{A}$	3.77 ± 0.05^{A}	3.90 ± 0.01^{A}
	K	$< 2^{A}$	$< 2^{A}$	$< 2^{Y}$	$< 2^{Y}$
	KO	$< 2^{A}$	$< 2^{A}$	$< 2^{Y}$	$< 2^{Y}$
	KH	$< 2^{A}$	$< 2^{A}$	$< 2^{Y}$	$< 2^{Y}$
Yeast and mold	C	$< 2^{A}$	$< 2^{A}$	3.58 ± 0.01^{A}	5.17 ± 0.01^{A}
	K	$< 2^{A}$	$< 2^{A}$	$< 2^{Y}$	$< 2^{Y}$
	KO	$< 2^{A}$	$< 2^{A}$	$< 2^{Y}$	$< 2^{Y}$
	KH	$< 2^{A}$	$< 2^{A}$	$< 2^{Y}$	$< 2^{Y}$

C; Control, K; chitosan, KO; sample coated with chitosan incorporated with 1% (w/v) ethanolic *Elettaria cardamomum* Maton extract, KH; sample coated with chitosan incorporated with 2% (w/v) ethanolic *Elettaria cardamomum* Maton extract. Letters A, Y and Z in each section in column indicate significant differences in $P<0.05$.

The results in Table 2 show that the total count of aerobic mesophilic bacteria increased in all groups within the storage time in refrigerated conditions. However, the increase in the logarithmic growth was higher in the control group than in the treated groups

(P<0.05). The logarithmic growth of psychotropic bacteria in the lamb meat increased after the third day in all groups, but the increase was lower in the treated groups than in the control group (P<0.05). Comparing the groups treated with *Elettaria cardamomum* Maton extract and chitosan, no significant difference was found in the psychrotrophic bacteria count (P>0.05) (Table 2). No logarithmic growth of lactic acid of bacteria was observed in either the control or the treatment groups (Table 2). It may be seen from the results that the control group showed an increase in the *Enterobacteriaceae* count after 3 days and it reached about log 3.9 cfu/g on the fifth day. However, in the other, treatment groups, no growth of *Enterobacteriaceae* was observed (Table 2). The results showed that there was an increase in mold and yeast counts on the third and fifth days in the control group, but in all the treated groups they were less than log 2 cfu/g on the third day. On the fifth day, logarithmic growth of molds and yeasts was seen (Table 2).

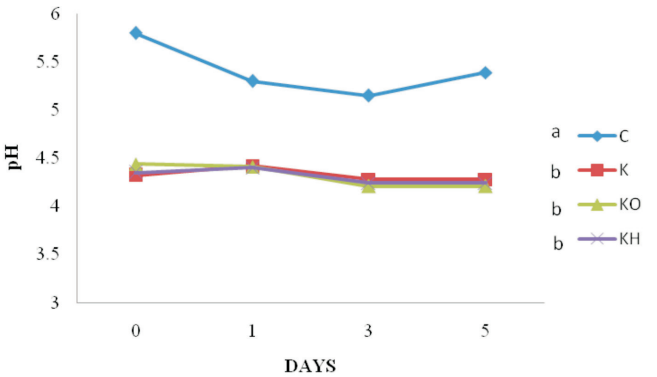

Fig. 2. pH of lamb meat and lamb meat-film stored at refrigerated temperatures (4 ± 1 °C). C; Control, K; chitosan, KO; sample coated with chitosan incorporated with 1% (w/v) ethanolic *Elettaria cardamomum* Maton extract, KH; sample coated with chitosan incorporated with 2% (w/v) ethanolic *Elettaria cardamomum* Maton extract. The letters a and b indicate significant differences where P<0.05.

In Fig. 2 shows the pH of lamb meat and lamb meat-film incorporated with ECM stored at refrigeration temperatures (4 ± 1 °C). A significant difference was observed between the control and treated groups on the third and fifth days (P<0.05). The results show that the pH level of meat first decreased during storage in the control group but then increased. There were significant differences between the negative control group and the other treatment groups (P<0.05). However, no significant difference was observed between the treatment groups.

The results show that the use of chitosan alone as a biodegradable coating reduced the total acceptance rate of meat so that it was lower than the control sample. However, the use of chitosan incorporated with ethanolic extract of the ECM increased the overall

acceptance of the samples, so the overall acceptance of chitosan incorporated with the ECM 2% was higher than the control sample (P<0.05). These results can be seen in Fig. 3.

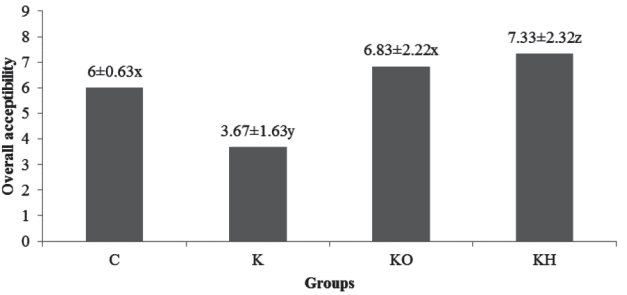

Fig.3. Overall acceptability evaluation of cooked lamb meat and lamb meat-film incorporated with different concentrations of ethanolic *Elettaria cardamomum* Maton extract.
C; Control, K; chitosan, KO; sample coated with chitosan incorporated with 1% (w/v) ethanolic *Elettaria cardamomum* Maton extract, KH; sample coated with chitosan incorporated with 2% (w/v) ethanolic *Elettaria cardamomum* Maton extract. The letters x, y and z indicate significant differences where P<0.05. Data reported (n = 10) are mean values.

Discussion

Elettaria cardamomum Maton, the aromatic herb, is traditionally used as a food additive. In this study, the antioxidant and antimicrobial properties of ethanolic extract of ECM were observed *in vitro*. In another study, the antibacterial properties of ECM fruit extracts against *S. aureus, P. mirabilis, E. coli* and *P. aeruginosa* were examined, and it was shown that the alcoholic extract had an inhibitory effect on all bacteria, but its aqueous extract had no effect (ANEJA and SHARMA, 2010).

In one other study, the antibacterial effect of various fruit extracts of ECM was examined on oral bacteria, and it was shown that the greatest effect was on *S. aureus,* and its ethanolic and acetonic extracts are a potential antibacterial source (ANEJA and JOSHI, 2009). It has been shown that ECM essential oil contains high levels of 1, 8 cineole and α-terpinyl acetate compounds (SAVAN and KÜÇÜKBAY, 2013).

The antioxidant properties of plants usually stem from their phenolic compounds, which can inhibit oxidation by supplying a hydrogen atom to free radicals (EREL et al., 2012; DEBA et al., 2008). In another study, the antibacterial and antioxidant effects of ECM essential oil were reported (GOCHEV et al., 2012).

In the present study, the antioxidant and antibacterial effects of ethanolic extract of ECM were observed *in vitro*. Previous studies have shown that monoterpene compounds containing oxygen possess antioxidant and antibacterial properties. Compounds such as linalool, eugenol, limonene and spathulenol have shown antioxidant properties that were

considered to be from a phenolic compounds (SHARAFATI-CHALESHTORI et al., 2013; EREL et al., 2012; DEBA et al., 2008). However, differences in the levels of antioxidant and antibacterial properties of extracts were suggested that could be a result of the presence of different compounds in the extracts, which are also affected by factors such as geographic location, temperature, plant growth phase, harvesting time of the plant, the land factor, as well as genetic and environmental factors related to the plant (GOZE et al., 2009).

According to the new approach by organizations responsible for food hygiene and the new attitude of food consumers, seeking to replace harmful chemical preservatives with natural substances, several studies has been carried out on the antimicrobial effects of various herbal extracts and different essential oils (SHARAFATI-CHALESHTORI et al., 2015a; SHARAFATI-CHALESHTORI et al., 2015b; SHARAFATI-CHALESHTORI et al., 2014).

Due to the possible undesired effects of extracts and essential oils in large quantities on the taste, smell and color of food, their use is limited to food preservation alone. Therefore, nowadays the technology barrier (Hurdle Technology) is used in food hygiene (SHARAFATI-CHALESHTORI et al., 2014; WU et al., 2014).

The results of this study showed the inhibitory effect of chitosan coating, and also chitosan containing ethanolic extract of ECM on populations of aerobic mesophilic bacteria, *Enterobacteriaceae,* psychrotrophic bacteria, mold and yeast during storage in refrigerated conditions (Table 2).

The antibacterial effect of chitosan coating is due to its positively charged amine groups and their reaction to anionic groups of the bacterial cell surface, which eventually causes the death of the bacteria (WU et al., 2013). Chitosan coating incorporated with extracts increases the antibacterial effects. The antibacterial effects of extracts can be attributed to their phenolic compounds. The presence of these compounds can increase permeability and remove cytoplasmic content by attacking the phospholipids of cell membrane. Furthermore, these compounds can affect the enzymes in the cellular walls of the bacteria (WU et al., 2014; WU et al., 2013). In a study, the antibacterial effects of gelatin compounds, with chitosan and oregano essential oil, indicated that the aerobic mesophilic bacteria count increased over time, but in gelatin samples with chitosan and oregano essential oil, the rate of increase was lower (WU et al., 2013). In another study, it was shown that the use of a chitosan coating on octopus caused a decrease in the growth of bacteria in terms of the total bacteria count (ATREA et al., 2009). In another study, the effect of essential oils of cloves, cinnamon and garlic on *S. aureus* bacteria with an inoculum dose of 10^5 cfu/mL in chicken meat samples at a temperature of 5 ± 1 °C in treated groups, with a negative control, reduced the number of bacteria up to the fourth day. On the sixth day, the number of bacteria increased, but the increase in the number of bacteria in the treated groups was lower than in the control group (BABU et al., 2012). In a study by RAVISHANKAR et al. (2009), the effect of coating food, using apple mixed with

antibacterial compounds, was examined for prevention of the growth of *L. monocytogenes* and *S.* Enteritidis in chicken meat, and it was proposed that it could be used to protect nutritive materials from contamination because of its antibacterial compounds.

In this study, the antibacterial effects were demonstrated of chitosan coating, along with ECM extract, and it was illustrated that the application of film containing antibacterial compounds, such as essential oils, extracts and antibiotics such as nisin, was more effective because they more gradually release these compounds into the food when compared to when these compounds are added to food alone (SÁNCHEZ-ORTEGA et al., 2014).

These results showed that pH levels of meat decreased during the storage period in the control group and then increased (Fig. 2). The initial decrease in pH level could be due to rigor mortis, but with increased storage time at a temperature of 4 ± 2 °C, the pH level of the meat increased. This may be ascribed to microorganism growth and protein breakdown (WU et al., 2014). There was a statistically significant difference between the control and treatment groups, but no statistically significant difference was observed between the groups with lamb treated with chitosan containing 1% ECM, and lamb with chitosan containing 2% ECM ($P<0.05$). However, the use of chitosan coating prepared in 2% acetic acid, creating an acidic environment, is one of the reasons for the antibacterial effects of chitosan with ECM.

From the results shown in Fig. 3 it may be seen that the use of chitosan with ethanolic extract of the ECM 2% caused a significant increase in the overall acceptance of the samples ($P<0.05$). In previous studies, the addition of essential oils and extracts triggered an increase in the acceptance of a variety of foods (BUSATTA et al., 2008). AMANY et al. (2010) reported the sensory effects of concentrations of 0.5, 1 and 1.5% of essential oils of garlic, thyme and lemon on chopped beef. It was reported to be excellent in all groups on day zero, weak on the third day of treatment by garlic, thyme and lemon, but the treatment by lemon was excellent and very good on the sixth day. In the study by KASSEM et al. (2011) addition of essential oil of thyme and jojoba cause an improvement in the sensory properties of beef burgers.

It is worth mentioning that considering the taste of chitosan and its preparation by acidic methods, essential oils and extracts can be used as harmless compounds to increase taste and aroma, but increasing the concentrations of essential oils and extracts up to an amount that does not trigger any organoleptic adverse effects is justified However, if these effects occur, it is not recommended that they be used in high concentrations.

Conclusions

Due to the antioxidant and antibacterial effects of chitosan edible coatings with *Elettaria cardamomum* Maton extract, chitosan may be recommended as a natural

coating and as an appropriate preservative material in the meat processing industry, as it ultimately enhances the safety and quality of putrefying food, reduces economic losses caused by the decomposition of valuable nutritive material, improves its taste and flavor, and increases the shelf life of meat. However, it is suggested that further toxicological, economic, and microbial studies be conducted so that it is possible to use these extracts widely food industry.

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Conflict of interest

All authors declare that they have no conflict of interests regarding the publication of this paper.

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SAŽETAK

U istraživanju je bio pripravljen etanolni iscrpak kardamoma *Elettaria cardamomum* Maton (ECM). Količina fenolnih spojeva bila je određena kolorimetrijskim metodama, a antioksidacijska svojstva postupkom

bijeljenja β-karotenom. Učinak hitozana u koncentraciji od 1 do 2 % (w/w) u iscrpku ECM pri temperaturi hladnjaka (4 ± 1 °C) bio je procijenjen s obzirom na mikrobiološku kakvoću janjećega mesa. Istražene su promjene pH vrijednosti, zatim ukupan broj bakterija, broj psihrotrofnih i bakterija kiselog vrenja, nalaz kvasaca, plijesni te organoleptička svojstva janjećeg mesa nakon određenog razdoblja pohrane (nultog, prvog, trećeg i petog dana). Ukupna količina fenola u etanolnom iscrpku ECM iznosila je 19,27 ± 0,02 mg/g ekvivalenta galne kiseline, a antioksidacijski kapacitet iznosio je 45,7 ± 7,5 %. U svim je obrađenim uzorcima bio zabilježen smanjen ukupni broj bakterija, broj bakterija porodice *Enterobacteriaceae*, psihrotrofnih bakterija, bakterija kiselog vrenja te količina kvasaca i plijesni ($P<0,05$). Dodatak iscrpka obogaćenog hitozanom janjećem mesu povećao je njegovu prosječnu kakvoću ($P<0,05$). Može se zaključiti da se etanolni iscrpak ECM pomiješan s hitozanom može rabiti kao prirodni antibakterijski i antioksidacijski sloj, siguran za uzimanje s mesnim proizvodima.

Ključne riječi: hitozan, *Elettaria cardamomum* Maton, vrijeme pohrane, janjeće meso, antioksidacijska aktivnost