ORIGINAL ARTICLE

Veterinary Research Forum. 2018; 9 (2) 153 - 161
doi: 10.30466/VRF.2018.30831
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Preservative effects of sumac hydro-alcoholic extract and chitosan coating enriched along with *Zataria multiflora* Boiss essential oil on the quality of beef during storage

Ali Mojaddar Langroodi, Hossein Tajik*, Tooraj Mehdizadeh

Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article Info

Article history:

Received: 28 July 2017
Accepted: 12 December 2017
Available online: 15 June 2018

Key words:

Beef
Chitosan
Sumac
Zataria multiflora

Abstract

Beef is susceptible to rapid spoilage due to its high amount of protein (18.00%) and moisture (72.00%). Food industries have recently found methods to extend beef shelf-life. The influence of beef dipping in hydro-alcoholic extract of sumac (SE) and chitosan (CH) coating incorporated with *Zataria multiflora* essential oil (ZEO) on microbial, chemical and sensory quality of beef was evaluated during refrigerated storage. Total viable counts (TVC), lactic acid bacteria, *Pseudomonas* spp., *Enterobacteriaceae* and yeasts-molds, total volatile nitrogen (TVN), thiobarbituric acid reactive substance values (TBARS) and peroxide value (PV) were founded to be significantly lower in all treatment groups compare to control groups during storage time. The highest level of antimicrobial effects induced by chitosan, SE 4.00% and ZEO. We found that in TVC (3.69 log CFU g^{-1} reduction compared with control group (sterile distilled water), *Enterobacteriaceae* (3.61 log CFU g^{-1} reduction) and lactic acid bacteria (2.67 log CFU g^{-1} reduction), respectively. Sumac gave a pleasant effect on sensory attributes and chitosan coating enriched with ZEO significantly improved sensory scores except for flavor factor. The results revealed the bio preservative properties of chitosan, hydro-alcoholic extract of sumac and *Z. multiflora* Boiss essential oil during refrigeration in normal packaging of beef.

اثرات نگه‌دارندگی عصاره آبی-الکلی سماق در پوشش خوراکی کیتوزان و اسانس آویشن شیرازی، بر کیفیت گوشت گاو در طی زمان نگه‌داری

چکیده

گوشت قرمز به دلیل میزان بالای پروتئین (۱۸/۰۰ درصد) و رطوبت (۷۲/۰۰ درصد) مستعد فساد سریع می‌باشد. اخیراً صنایع غذایی به دنبال یافتن روش‌هایی برای افزایش زمان ماندگاری مواد غذایی هستند. اثرات نگه‌دارندگی عصاره‌ی آبی-الکلی سماق و کیتوزان به تنهایی یا در ترکیب با اسانس آویشن شیرازی بر ویژگی‌های میکروبی، شیمیایی و حسی بر روی گوشت گاو در زمان نگه‌داری در یخچال مورد بررسی قرار گرفت. شمارش کلی باکتریایی، باکتری‌های اسید لاکتیک، *سودوموناس*، *انتروباکتریاسه* و کپک - مخمر همچون میزان نیتروژن تام، اندیس تیوباربیتوریک اسید و اندیس پراکسید به شکل معنی‌داری در همه تیمارها نسبت به تیمار کنترل (آب مقطر استریل) به میزان کمتری بود. بالاترین اثرات ضدمیکروبی به تیمار کیتوزان، چهار درصد عصاره سماق و اسانس آویشن شیرازی به ترتیب در شمارش کلی باکتریایی (کاهش ۳/۶۹ لگاریتم باکتریایی نسبت به تیمار کنترل)، *انتروباکتریاسه* (کاهش ۳/۶۱ لگاریتم باکتریایی)، باکتری‌های اسیدلاکتیک (کاهش ۲/۶۷ لگاریتم باکتریایی) مربوط می‌گردد. عصاره سماق اثر مطلوبی بر خصوصیات حسی گذاشت و پوشش کیتوزان به همراه اسانس آویشن شیرازی امتیاز حسی به غیر از فاکتور بو را افزایش داد. نتایج بدست آمده ویژگی‌های نگه‌دارندگی پوشش کیتوزان، عصاره آبی-الکلی سماق و اسانس آویشن شیرازی را در نگه‌داری گوشت گاو در بسته‌بندی معمولی نشان می‌دهد.

واژه های کلیدی: آویشن شیرازی، سماق، کیتوزان، گوشت گاو

***Correspondence:**

Hossein Tajik. DVM. PhD
Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
E-mail: h.tajik@urmia.ac.ir

Introduction

Meat is very susceptible to microbial spoilage and chemical oxidation, so it is favorable to use a natural preservative with antioxidant and antimicrobial effects. The fat content of animal meat makes meat sensitive to lipid oxidation and formation of free radical that may cause diseases in human.[1] Nasar-Abbas and Halkman showed that lipid oxidation in meat and meat products can be controlled by antioxidants.[1] To achieve the best shelf-life of meat, especially red meat, it is necessary to reduce the levels of microbial contamination. The use of synthetic chemicals as food preservatives such as sodium benzoate, benzoic acid, sodium nitrite, sodium sorbate, potassium sorbate and sulphur dioxide have been restricted because of their adverse effects on human health.[2] The raising concerns about the use of chemicals preservative, have been change the attitude of food manufacture to replace them with natural preservatives. Therefore, natural additives such as sumac (SE) extract and *Zataria multiflora* essential oil (ZEO), not only give flavor to foods but they also have the advantage of being a natural preservative to reduce the pathogenic bacteria and to increase the shelf life of processed foods.[3,4]

Among the natural antioxidant and antibacterial agents, the antibacterial and antioxidant activities of the herbal extracts such as thyme, rosemary, garlic, sumac, ginger, pepper and mustard have been investigated previously.[5] *Sumac* (*Rhus coriaria L.*) has been shown to have antimicrobial effects on foodborne bacteria. Antibacterial activity of sumac is mainly attributed to the tannins and other compounds.[6] The medicinal *Zataria multiflora* Boiss. belongs to the *Lamiaceae* family which extensively grows in tropical regions of Iran, Afghanistan and Pakistan.[7] Antioxidant and antibacterial activities of sumac is correlated to the high level of carvacrol and thymol in sumac.[8,9] Chitosan as a potentially attractive natural food preservative, has antimicrobial effects against a widespread range of different pathogenic and spoilage organisms in meat and processed meat products.[10] Commercial chitosan is mostly a crab by product and has been used as a food preservative.[10] Most recently the edible chitosan coating has been used to protect the foods from microbial and chemical spoiling agents.[11] Darmadji and Izumimoto have investigated the effects of chitosan on shelf life of fresh minced beef patties.[11] The aims of the current study were to investigate the preservative effects of chitosan coating enriched with *Z. multiflora* Boiss essential oil and hydro-alcohol extract of sumac on beef during refrigeration in commercial packaging.

Materials and Methods

Preparation of sumac hydro-alcoholic extract. Fresh sumac fruits (*Rhus coriaria L.*) were purchased from local shops. The sumac fruits were washed, dried and powdered. An amount of 700 mL alcohol and 300 ml distilled water were added to 250 g of sumac powder. The mixture was shaken for 24 hr and incubated for 1 hr at 40 ˚C in a water bath. After cooling and filtration using a paper filter, the solvent was removed in a rotary evaporator (Laborata 4003; Heidolph, Schwabach, Germany) and the extract was stored at 4 ˚C until use.[1]

Extraction of *Z. multiflora* Boiss essential oil. The *Zataria multiflora* Boiss that has been collected from the central part of Iran was purchased from a local market and confirmed by Institute of Medicinal Plants, Urmia, Iran, and the plant materials with the voucher number 41754. As described previously,[12] hydrodistilation of dried parts of plant was performed in a Clevenger-type apparatus for 3 hr. Sodium sulfate anhydrous was used for dehydration of the oil, then oil was filtered using 0.22 µm filters and stored inside colored glass tubes at 4 ˚C.

Meat samples. Boneless fresh beef samples (Quadriceps femoris) were purchased from a local slaughterhouse and refrigerated at 4 ˚C for 1 hr until analysis.

Preparation of coating solutions and treatment groups. To prepare chitosan solution, 2 g of chitosan (medium molecular weight, Sigma-Aldrich Chemical Co., Steinheim, Germany), was added to 100 mL of distilled water, then 1 mL of glacial acetic acid and was slightly shaken for 8 hr. Then, glycerol (Merck & Co. Inc., Kenilworth, USA) as a plasticizer, was added at 0.75 mL g^{-1} concentration and stirred for 10 min. For treatments containing ZEO, 1g Tween 80 in 1000 mL (Sigma-Aldrich Chemical Co.) was added, to dissolve the essential oil.[13,14] Beef samples were divided into six groups including the control (sterile distilled water), CH (chitosan), SE 2.00%-CH (chitosan), SE 4.00%-CH , SE 2.00%-CH-Z 1.00% (*Z. multiflora* essential oil 1.00%), SE 4.00%-CH-Z 1.00%. Three min after treating the samples were drained, aerobically packaged and stored at refrigerator. Chemical, microbiological and sensorial assays were carried out to assess the total quality of beef samples (amount of each samples was related to kind of examination) up to 20 days at 5-day intervals. [9]

Microbiological analysis. Ten grams of each sample was suspended in 90 mL sterile 0.1 mL peptone water (Merck, Darmstadt, Germany) and homogenized using stomacher (Lab Blender 400; Seward Medical Ltd., London, UK) for 3 min at room temperature. Then, 0.10 mL of serial dilutions (0.10% peptone water) of beef homogenates were transferred to agar plates. *Pseudomonas* spp. were counted on *Pseudomonas* agar supplemented with cephaloridine fucidin cetrimide (CFC; Merck) at 25 ˚C for 48 hr. *lactobacilli* were enumerated on De Man, Rogosa and Sharpe agar (MRS; Merck) after incubation at 30 ˚C for 48 hr.[15] *Enterobacteriaceae* were counted by the pour-overlay method using violet red bile glucose (VRBG) agar at 37 ˚C for 24 hr. Yeasts-molds were

counted on Rose Bengal Chloramphenicol (RBC, Merck) selective agar after incubation at 25 ˚C for 3 to 5 days in the dark. Finally, total viable counts (TVC) were determined using plate count agar (Merck), after 2 days incubation at 30 ˚C.[10]

Sensory analysis. The sensory analyses of meat samples were carried out by 10 experienced PhD students. After preparing the treatments, the samples were separately presented to each panelist. Fresh beef was used as control. Panelists were asked to evaluate texture, color, flavor and odor and provide their overall acceptance score on a nine-point Hedonic scale, with nine being so good and 1 being so poor. [20] At day 0, meat samples were cooked at 160 ˚C for 15 min and then served.

Determination of thiobarbituric acid value (TBA). Level of lipid oxidation was investigated using thiobarbituric acid reactive substances (TBARS) method as described by Pikul *et al*.[16] Ten grams of each meat samples were homogenized using 1 mL butylated hydroxytoluene (BHT) and 35 mL trichloroacetic acid (5.00%) in a blender. The mixture was filtered using No. 1 filter (Whatman Ltd., Maidston, UK). Five mL TBA solution (0.02 M) was added to 5 mL of filtrate solution and incubated in a water bath at 100 ˚C for 60 min to expand the malondialdehyde–TBA complex. After cooling, the absorbance of the samples was determined at 532 nm wave length.[16]

Determination of peroxide value. Another method for detection of lipid oxidation was peroxide value determination method as described by International Dairy Federation (IDF) standard method 74a.[17] To determine the peroxide value, the meat sample between 0.01 to 0.30 gram depending on the level of peroxidation was mixed with 9.80 mL chloroform-methanol (30.00% - 70.00%) by a shaker (BV1000; Benchmark Scientific, Michigan, Netherlands). After that 50 µL ammonium thiocyanate solution was added. Then, 50 µL iron solution (0.4 g barium chloride + 0.5 g iron (II) sulfate + 2 mL HCL + 100 mL distilled water) was added and mixed. After 5 min incubation at room temperature, the absorbance of the sample was determined at 500 nm (LKB Novaspec II; Pharmacia, Sweden).

Determination of total volatile nitrogen (TVN). The TVN content of the meat samples was investigated using a macro Kjeldahl apparatus. Ten grams of each beef samples was steam distilled in 300 mL of water containing 3 g magnesium oxide (Merck).[18]

Determination of pH value. The pH values were determined using a digital pH meter (pH-Meter E520, Metrohm Herisau, Switzerland). Five grams of each beef sample was homogenized in 25 mL of distilled water was homogenized for 1 min.[19]

Statistical analysis. All experiments were carried out in triplicates. Data were analyzed using the SPSS statistical package (version 21.0; IBM Corp, Armonk, USA). Differences between treatments were tested for significance by one-way ANOVA followed by with Tukey's post-test. A statistical difference at $p < 0.05$ were considered significant.

Results

Microbiological analysis. Changes in TVC, *Pseudomonas* spp., lactic acid bacteria (LAB), *Enterobacteriaceae* and yeast-mold populations of meat samples stored at 4 ˚C were determined up to 20 days. The antimicrobial activity of sumac was decreased with increasing the concentrations of sumac extract. The initial TVC of beef samples was 4.63 log CFU g^{-1}. A significant decrease in TVC was found among treatment groups ($p < 0.05$; Table 1). The initial LAB, *Enterobacteriaceae* and *Pseudomonas* spp. counts in all samples were in the range of 3.63–3.78, 3.57–3.80 and 2.69–2.94 log CFU g^{-1}, respectively. Also the initial findings for the number of mold and yeast were 2.43–3.09 and 3.03–3.29 log CFU g^{-1}, respectively.

The effects of SE and ZEO and chitosan on LAB count is presented in Table 1. The initial count for LAB was 3.78 log CFU g^{-1} in the control sample and experienced increasing trend to 7.40 log CFU g^{-1} on final day of storage. At the end of storage, LAB counts were reached to 6.15 and 4.73 log CFU g^{-1} in CH and CH-SE 4.00%-Z treated samples, respectively. LAB counts were 5.94 log CFU g^{-1} on day 20 for CH-SE 2.00% samples.

The values for viable *Enterobacteriaceae* count in beef samples are shown in Table 1. The initial count for *Enterobacteriaceae* was between 3.57 to 3.8 log CFU g^{-1} in all treatment. In all treatment groups *Enterobacteriaceae* counts were reduced approximately 1.00–3.00 log CFU g^{-1} cycles ($p < 0.05$) compared to the control samples on day 20, indicating that both extract and EO significantly inhibited *Enterobacteriaceae* after 12 days. The *Enterobacteriaceae* counts in SE treatment group were 2.15 - 3.61 log CFU g^{-1} less than control treatment.

The initial counts for *Pseudomonas spp*., was 2.69–2.94 log CFU g^{-1} in all groups. At the end of storage time, *Pseudomonas* counts reached 5.96, 4.60 and 4.18 log CFU g^{-1} in control, CH-SE 4.00% and CH-SE 4.00%-Z groups, respectively. After 12 days, the *Pseudomonas* count in CH-SE 4.00%-Z treated sample was 2.29 log CFU g^{-1} that was lower than control sample. Table 1 shows the effect of chitosan and SE and ZEO on yeasts-molds count in beef. Samples treated in distilled water contained 2.11 log CFU g^{-1} more yeasts-molds counts than CH-SE 4.00%-Z group.

Changes of pH value. Changes in samples pH values during storage under refrigeration condition are shown in Figure 1. The initial pH of control group on day 0 was 5.76 indicating the acceptable conditions offer of beef samples. The pH values of all samples were increased during the storage period. The samples treated with chitosan, 4.00% sumac and ZEO showed significantly lower pH values compared with other groups ($p < 0.05$).

Table 1. The changes in total viable counts, lactic acid bacteria, *Enterobacteriacea*, *Pseudomonas* and yeasts-molds counts of beef meat during storage at 4 ˚C at different time points.

Parameter	Treatment*	Storage time (day)					
		0	4	8	12	16	20
Total viable counts	**C**	4.63 ± 0.07^{Aab}	7.10 ± 0.11^{Ba}	8.26 ± 0.06^{Ca}	8.39 ± 0.07^{Ca}	8.53 ± 0.06^{Ca}	8.95 ± 0.14^{Da}
	CH	4.59 ± 0.09^{Aa}	5.61 ± 0.15^{Bb}	5.90 ± 0.05^{Bb}	6.12 ± 0.01^{Bb}	6.29 ± 0.06^{Bb}	7.01 ± 0.13^{Cb}
	CH-SE 2.00%	4.50 ± 0.08^{Abc}	4.86 ± 0.04^{Ac}	5.26 ± 0.05^{Bc}	5.60 ± 0.03^{Bc}	5.86 ± 0.04^{Bc}	6.38 ± 0.06^{Cc}
	CH-SE 4.00%	4.48 ± 0.03^{Abc}	4.67 ± 0.04^{Ad}	4.92 ± 0.04^{Ad}	5.32 ± 0.04^{Bd}	5.62 ± 0.06^{Cd}	5.91 ± 0.06^{Cd}
	CH-SE 2.00%-Z	4.45 ± 0.06^{Ac}	4.59 ± 0.02^{Aa}	4.76 ± 0.03^{Aa}	4.93 ± 0.03^{Ae}	5.21 ± 0.05^{Be}	5.40 ± 0.06^{Be}
	CH-SE 4.00%-Z	4.50 ± 0.03^{Abc}	4.60 ± 0.02^{Ae}	4.71 ± 0.01^{Ae}	4.85 ± 0.05^{Ae}	5.30 ± 0.06^{Af}	5.26 ± 0.03^{Bf}
Lactic acid bacteria	**C**	3.78 ± 0.01^{Aa}	4.75 ± 0.03^{Ba}	5.40 ± 0.07^{Ca}	5.84 ± 0.02^{Ca}	6.28 ± 0.02^{Ca}	7.40 ± 0.04^{Da}
	CH	3.69 ± 0.01^{Ab}	3.94 ± 0.03^{Ab}	4.40 ± 0.02^{Bb}	4.66 ± 0.02^{Bb}	5.33 ± 0.03^{Cb}	6.15 ± 0.02^{Db}
	CH-SE 2.00%	3.73 ± 0.03^{Aa}	3.81 ± 0.01^{Ac}	4.15 ± 0.03^{Ac}	4.54 ± 0.03^{Bc}	4.85 ± 0.03^{Bc}	5.94 ± 0.05^{Cc}
	CH-SE 4.00%	3.66 ± 0.03^{Abc}	3.76 ± 0.01^{Ad}	3.93 ± 0.02^{Ad}	4.28 ± 0.03^{Bd}	4.60 ± 0.02^{Bd}	5.13 ± 0.02^{Cd}
	CH-SE 2.00%-Z	3.63 ± 0.03^{Ac}	3.72 ± 0.01^{Ac}	3.81 ± 0.00 ^{Ac}	3.92 ± 0.10^{Ac}	4.25 ± 0.03^{Bc}	4.87 ± 0.05^{Bc}
	CH-SE 4.00%-Z	3.67 ± 0.01^{Acb}	3.72 ± 0.00^{Ac}	3.79 ± 0.01^{Ac}	3.89 ± 0.01^{Ac}	4.12 ± 0.00^{Af}	4.73 ± 0.02^{Bf}
Enterobacteriaceae	**C**	3.74 ± 0.11^{Aa}	6.84 ± 0.12^{Ba}	7.21 ± 0.09^{Ca}	7.34 ± 0.03^{Ca}	7.44 ± 0.04^{Ca}	7.64 ± 0.08^{Ca}
	CH	3.08 ± 0.08^{Aa}	5.73 ± 0.11^{Bb}	6.14 ± 0.04^{Cb}	6.33 ± 0.03^{Cb}	6.46 ± 0.05^{Cb}	6.73 ± 0.05^{Cb}
	CH-SE 2.00%	3.57 ± 0.06^{Ab}	4.66 ± 0.05^{Bc}	4.88 ± 0.04^{Bc}	5.19 ± 0.03^{Cc}	5.32 ± 0.01^{Cc}	5.48 ± 0.03^{Cc}
	CH-SE 4.00%	3.59 ± 0.07^{Ab}	3.92 ± 0.04^{Ad}	4.16 ± 0.04^{Ad}	4.36 ± 0.03^{Bd}	4.48 ± 0.08^{Bd}	4.72 ± 0.05^{Bd}
	CH-SE 2.00%-Z	3.57 ± 0.05^{Ab}	3.67 ± 0.00^{Ac}	3.71 ± 0.01^{Ac}	3.81 ± 0.01^{Ac}	3.93 ± 0.02^{Ac}	4.18 ± 0.07^{Bc}
	CH-SE 4.00%-Z	3.58 ± 0.06^{Ab}	3.65 ± 0.00^{Ac}	3.68 ± 0.00^{Ac}	3.73 ± 0.01^{Af}	3.83 ± 0.01^{Af}	4.00 ± 0.08^{Af}
Pseudomonas	**C**	2.94 ± 0.03^{Aa}	3.24 ± 0.02^{Aa}	4.88 ± 0.03^{Ba}	5.43 ± 0.04^{Ca}	5.74 ± 0.03^{Ca}	5.96 ± 0.00^{Ca}
	CH	2.81 ± 0.02^{Abc}	3.14 ± 0.02^{Bb}	3.95 ± 0.03^{Cb}	4.33 ± 0.02^{Cb}	4.57 ± 0.04^{Db}	5.16 ± 0.04^{Db}
	CH-SE 2.00%	2.69 ± 0.02^{Ad}	2.96 ± 0.01^{Ad}	3.52 ± 0.02^{Bc}	3.86 ± 0.03^{Bc}	4.30 ± 0.02^{Cc}	4.79 ± 0.01^{Cc}
	CH-SE 4.00%	2.82 ± 0.02^{Ab}	2.92 ± 0.01^{Ad}	3.34 ± 0.02^{Ad}	3.54 ± 0.03^{Bd}	4.02 ± 0.06^{Bd}	4.06 ± 0.02^{Bd}
	CH-SE 2.00%-Z	2.76 ± 0.04^{Ac}	2.89 ± 0.01^{Ad}	3.14 ± 0.03^{Ac}	3.37 ± 0.01^{Bc}	3.67 ± 0.04^{Bc}	4.51 ± 0.01^{Cc}
	CH-SE 4.00%-Z	2.76 ± 0.02^{Abc}	2.85 ± 0.03^{Ac}	3.00 ± 0.00^{Af}	3.14 ± 0.02^{Af}	3.46 ± 0.03^{Bf}	4.18 ± 00.01^{Bf}
Yeasts–molds	**C**	3.03 ± 0.06^{Ac}	4.77 ± 0.05^{Ba}	5.30 ± 0.03^{Ca}	7.57 ± 0.04^{Da}	8.32 ± 0.01^{Ea}	8.57 ± 0.04^{Ea}
	CH	3.14 ± 0.03^{Ab}	4.57 ± 0.04^{Bb}	4.95 ± 0.03^{Bb}	6.94 ± 0.03^{Cb}	7.92 ± 0.00^{Db}	8.13 ± 0.01^{Db}
	CH-SE 2.00%	3.17 ± 0.01^{Ab}	4.35 ± 0.03^{Bc}	4.65 ± 0.03^{Bc}	6.66 ± 0.04^{Cc}	7.28 ± 0.01^{Dc}	7.63 ± 0.04^{Dc}
	CH-SE 4.00%	3.13 ± 0.01^{Ab}	3.91 ± 0.02^{Ad}	4.29 ± 0.02^{Bd}	6.24 ± 0.01^{Cd}	6.96 ± 0.02^{Cd}	7.19 ± 0.01^{Dd}
	CH-SE 2.00%-Z	3.29 ± 0.01^{Aa}	3.64 ± 0.03^{Ac}	3.92 ± 0.01^{Ac}	5.64 ± 0.05^{Bc}	6.02 ± 0.07^{Bc}	6.63 ± 0.03^{Cc}
	CH-SE 4.00%-Z	3.16 ± 0.02^{Ab}	3.77 ± 0.03^{Ac}	3.94 ± 0.02^{Ac}	5.46 ± 0.01^{Bf}	5.88 ± 0.00^{Bf}	6.46 ± 0.03^{Cf}

*Treatments: Control (C), chitosan (CH), sumac extract 2% with chitosan (CH-SE 2.00%), sumac extract 4.00% with chitosan (CH-SE 4.00%), sumac extract 2.00% and *Z. multiflora* essential oil with chitosan (CH-SE 2.00%-Z) and sumac 4.00% and *Z. multiflora* essential oil with chitosan (CH-SE 4.00%-Z).

Different uppercase letters in the same row and lowercase letters in the same column indicate significant differences ($p < 0.05$).

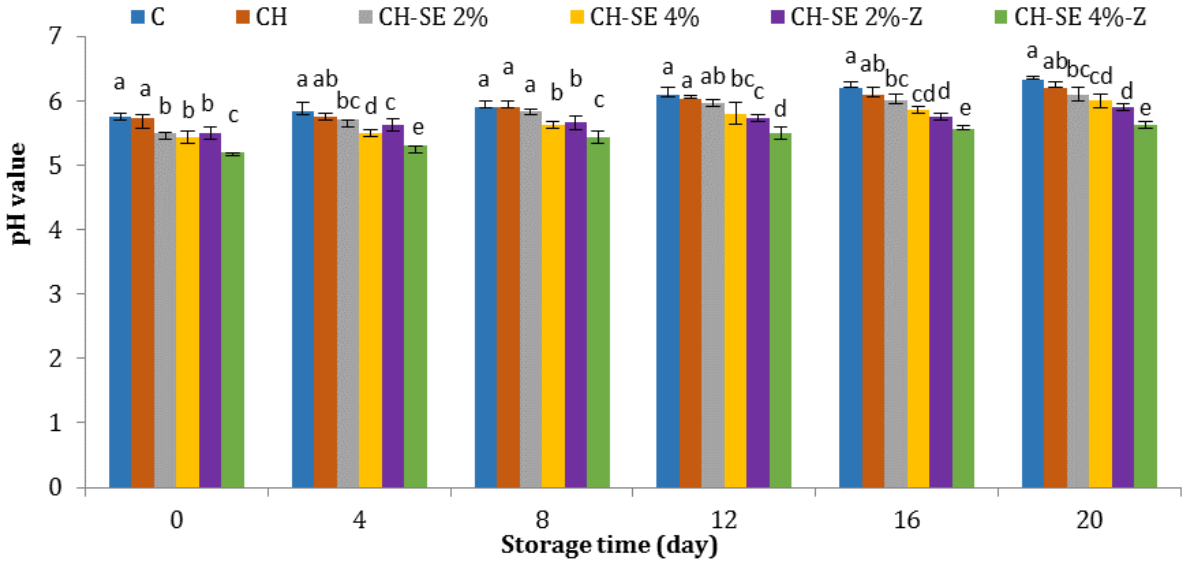

Fig. 1. The changes in pH beef meat in different treatment groups during storage at different time points. Treatments: Control (C), chitosan (CH), sumac extract 2.00% with chitosan (CH-SE 2.00%), sumac 4.00% with chitosan (CH-SE 4.00%), sumac 2.00% and *Z. multiflora* essential oil with chitosan (CH-SE 2.00%-Z) and sumac 4.00% and *Z. multiflora* essential oil with chitosan (CH-SE 4.00%-Z).

Different letters indicate a statistically significant difference at each time point ($p < 0.05$).

Changes in TBARS. The results of TBARS analyses of beef samples during storage time are showed in Table 2. The TBA determination, shows the level of secondary damage of lipids. During 20 days of storage, the TBA values of treated samples were significantly lower than those in the control ($p < 0.05$). The lowest lipid oxidation was determined for the samples treated with chitosan, sumac extract 4.00% and ZEO. The lipid oxidation was occurred quickly in the control sample with the greatest changes between 16 and 20 days of the storage.

Sensory analysis. The addition of SE and ZEO changed the color of meat samples and improved the acceptability scores. Apart from the color, which had the lowest scores, CH-SE 4.00%-Z-treated samples had the highest acceptability scores ($p < 0.05$) in sensory characteristics including flavor, odor and texture with the mean values of 8.30, 8.00 and 8.90 during 20 days of storage time, respectively, but for the color factor, the treated samples had the lowest scores. The control group showed to be the most perishable group during the storage (Table 3).

Table 2. The changes in TBARS (mg MDA per kg^{-1}) of beef meat in different treatment groups during storage at 4 ˚C at different time points.

Treatment*	Storage time (day)					
	0	4	8	12	16	20
C	0.28 ± 0.02^{a}	0.62 ± 0.04^{b}	1.13 ± 0.08^{a}	1.56 ± 0.09^{a}	2.22 ± 0.17^{a}	2.65 ± 0.36^{a}
CH	0.27 ± 0.01^{a}	0.52 ± 0.06^{bc}	0.64 ± 0.03^{c}	1.14 ± 0.09^{b}	1.39 ± 0.02^{b}	1.58 ± 0.34^{bc}
CH-SE 2.00%	0.28 ± 0.01^{a}	0.79 ± 0.06^{a}	0.87 ± 0.09^{b}	1.08 ± 0.05^{bc}	1.28 ± 0.06^{bc}	1.48 ± 0.02^{bc}
CH-SE 4.00%	0.27 ± 0.01^{a}	0.56 ± 0.09^{bc}	0.72 ± 0.10^{c}	1.12 ± 0.08^{b}	1.32 ± 0.07^{b}	1.82 ± 0.19^{b}
CH-SE 2.00%-Z	0.24 ± 0.01^{b}	0.52 ± 0.05^{bc}	0.71 ± 0.03^{c}	0.94 ± 0.11^{cd}	1.09 ± 0.07^{cd}	1.30 ± 0.18^{c}
CH-SE 4.00%-Z	0.23 ± 0.02^{b}	0.48 ± 0.06^{c}	0.66 ± 0.03^{c}	0.88 ± 0.08^{d}	1.13 ± 0.06^{d}	1.13 ± 0.03^{d}

*Treatments: Control (C), chitosan (CH), chitosan with sumac extract 2.00% (CH-SE 2.00%), chitosan with sumac extract 4.00% (CH-SE 4.00%), chitosan with sumac extract 2.00% and ZEO (CH-SE 2.00%-Z) and chitosan with sumac extract 4.00% and ZEO (CH-SE 4.00%-Z). Different letters in each column indicate a statistically significant difference ($p < 0.05$).

Table 3. The changes in sensory attributes of beef meat in different treatment groups during storage at 4 ˚C at different time points.

Sensory attributes	Treatment*	Storage time (day)					
		0	4	8	12	16	20
Taste	**C**	8.50 ± 0.52^{ab}	-	-	-	-	-
	CH	8.60 ± 0.69^{a}	-	-	-	-	-
	CH-SE 2.00%	8.60 ± 0.32^{a}	-	-	-	-	-
	CH-SE 4.00%	7.90 ± 0.99^{bc}	-	-	-	-	-
	CH-SE 2.00%-Z	7.80 ± 0.63^{c}	-	-	-	-	-
	CH-SE 4.00%-Z	8.30 ± 0.48^{abc}	-	-	-	-	-
Color	**C**	6.70 ± 0.67^{a}	6.20 ± 1.03^{ab}	5.70 ± 0.82^{ab}	4.10 ± 0.73^{b}	1.3 ± 0.67^{c}	0.00 ± 0.00^{d}
	CH	6.30 ± 0.67^{ab}	6.30 ± 1.49^{a}	6.00 ± 1.15^{a}	5.20 ± 1.03^{a}	2.00 ± 1.24^{c}	0.00 ± 0.00^{d}
	CH-SE 2.00%	5.90 ± 0.73^{bc}	5.80 ± 1.13^{ab}	5.50 ± 0.97^{abc}	5.40 ± 1.07^{a}	3.60 ± 1.26^{b}	2.20 ± 0.91^{c}
	CH-SE 4.00%	5.70 ± 0.63^{bc}	5.80 ± 1.13^{ab}	5.60 ± 0.96^{abc}	5.30 ± 1.05^{a}	5.00 ± 1.33^{a}	3.20 ± 1.03^{b}
	CH-SE 2.00%-Z	5.40 ± 0.94^{cd}	5.20 ± 1.39^{ab}	5.00 ± 0.94^{bc}	4.90 ± 0.99^{ab}	4.50 ± 1.08^{ab}	4.50 ± 1.08^{a}
	CH-SE 4.00%-Z	5.00 ± 0.66^{d}	5.00 ± 1.24^{b}	4.70 ± 0.94^{c}	4.50 ± 0.84^{ab}	4.40 ± 1.07^{ab}	4.00 ± 0.94^{a}
Odor	**C**	8.70 ± 0.48^{a}	7.50 ± 0.52^{c}	6.00 ± 0.47^{c}	4.30 ± 0.48^{d}	1.50 ± 0.70^{d}	0.20 ± 0.00^{f}
	CH	8.70 ± 0.48^{a}	8.20 ± 0.63^{ab}	8.00 ± 0.81^{ab}	6.20 ± 0.78^{c}	5.10 ± 0.99^{c}	2.60 ± 0.51^{e}
	CH-SE 2.00%	8.60 ± 0.51^{a}	8.20 ± 0.42^{ab}	7.90 ± 0.73^{ab}	6.50 ± 0.52^{bc}	60.00 ± 0.00^{b}	4.50 ± 0.70^{d}
	CH-SE 4.00%	8.50 ± 0.52^{a}	8.40 ± 0.69^{a}	8.20 ± 0.63^{a}	6.90 ± 0.31^{ab}	6.70 ± 0.94^{a}	5.10 ± 0.31^{c}
	CH-SE 2.00%-Z	7.70 ± 0.67^{b}	7.70 ± 0.67^{bc}	7.50 ± 0.52^{b}	7.10 ± 0.99^{ab}	6.00 ± 0.00^{b}	5.80 ± 0.63^{b}
	CH-SE 4.00%-Z	8.00 ± 0.00^{b}	7.90 ± 0.56^{abc}	7.60 ± 0.51^{ab}	7.00 ± 0.67^{a}	6.60 ± 0.51^{ab}	6.30 ± 0.48^{a}
Texture	**C**	9.00 ± 0.00^{a}	8.30 ± 0.94^{a}	6.50 ± 0.52^{c}	4.00 ± 0.94^{d}	1.50 ± 0.70^{d}	0.00 ± 0.00^{e}
	CH	8.70 ± 0.48^{ab}	8.50 ± 0.70^{a}	8.10 ± 0.99^{ab}	5.60 ± 1.34^{c}	1.20 ± 0.42^{c}	3.10 ± 0.73^{d}
	CH-SE 2.00%	8.40 ± 0.51^{bc}	8.40 ± 0.69^{a}	8.10 ± 0.87^{ab}	7.90 ± 0.73^{ab}	4.80 ± 0.91^{c}	4.00 ± 1.05^{c}
	CH-SE 4.00%	8.40 ± 0.69^{bc}	8.40 ± 0.96^{a}	8.20 ± 0.78^{ab}	8.10 ± 0.87^{a}	5.70 ± 1.33^{ab}	5.30 ± 0.94^{b}
	CH-SE 2.00%-Z	8.00 ± 0.66^{d}	8.00 ± 0.81^{a}	7.50 ± 0.97^{b}	7.00 ± 1.15^{b}	7.60 ± 1.34^{b}	6.30 ± 0.67^{a}
	CH-SE 4.00%-Z	8.90 ± 0.31^{a}	8.70 ± 0.48^{a}	8.60 ± 0.51^{a}	8.10 ± 1.10^{a}	6.80 ± 1.13^{a}	7.00 ± 1.33^{a}
Overall	**C**	7.10 ± 0.73^{d}	5.10 ± 0.87^{a}	3.70 ± 0.67^{c}	1.50 ± 0.52^{d}	1.30 ± 0.48^{e}	1.10 ± 0.31^{e}
	CH	8.00 ± 0.81b^{c}	8.00 ± 0.81^{b}	5.20 ± 0.63^{b}	4.80 ± 0.78^{c}	4.60 ± 0.84^{d}	2.60 ± 0.84^{d}
	CH-SE 2.00%	8.60 ± 0.51^{ab}	8.60 ± 0.69^{b}	6.70 ± 0.82^{a}	6.10 ± 0.73^{b}	6.10 ± 0.73^{c}	3.90 ± 0.73^{c}
	CH-SE 4.00%	8.70 ± 0.48^{a}	8.60 ± 0.51^{b}	6.90 ± 0.99^{a}	6.50 ± 1.08^{ab}	6.20 ± 0.78^{bc}	5.10 ± 0.73^{b}
	CH-SE 2.00%-Z	8.10 ± 0.73^{abc}	8.00 ± 0.81^{b}	7.00 ± 1.15^{a}	7.00 ± 0.81^{a}	6.80 ± 0.63^{ab}	5.90 ± 0.99^{a}
	CH-SE 4.00%-Z	7.90 ± 0.73^{c}	7.90 ± 0.87^{b}	7.30 ± 0.94^{a}	7.20 ± 0.78^{a}	7.00 ± 0.81^{a}	6.30 ± 0.67^{a}

* Treatments: Control (C), chitosan (CH), chitosan with sumac extract 2.00% (CH-SE 2.00%), chitosan with sumac extract 4.00% (CH-SE 4.00%), chitosan with sumac extract 2.00% and ZEO (CH-SE 2.00%-Z) and chitosan with sumac extract 4.00% and ZEO (CH-SE 4.00%-Z). Different letters in each column indicate a statistically significant difference ($p < 0.05$).

Peroxide value. The PV of the control sample was significantly higher than the other treatment groups during storage (p <0.05; Fig. 2). The mean PV of all samples increased during the first 12 days of storage and then decreased afterward. In control samples, the PV increased from 0.11 to 2.67 meq peroxides kg^{-1} lipid after 12 days and decreased thereafter to 0.39 at days 20 of storage.

Total volatile nitrogen. The TVN 100 g^{-1} values of 7.93 mg in control samples at the first day indicate the acceptable quality for the freshness of the beef samples (Fig. 3). Changes in the TVN values were time dependent in all treatment groups. TVN contents increased gradually and reached to final values of 29.40 and 14.93 mg for control and CH-SE 4.00%-Z samples, respectively. This reduction may be related to low initial TPC count (4.63 log CFU g^{-1}). In the control group (TVN was 17.26 mg 100 g^{-1} of meat) during the first 8 days.

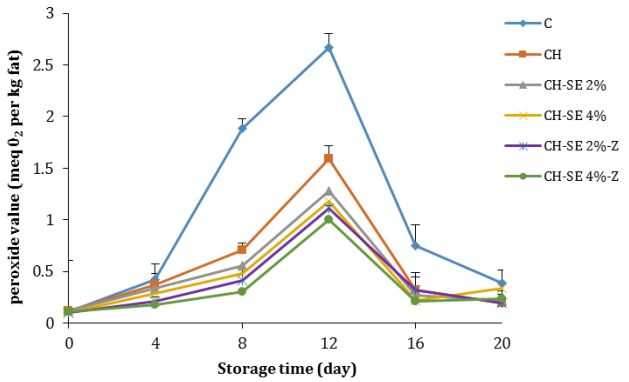

Fig. 2. The changes in peroxide value (meq O_2 per kg fat) of beef meat in different treatment groups during storage at 4 ℃ at different time points.
Treatments: Control (C), chitosan (CH), sumac extract 2.00% with chitosan (CH-SE 2.00%), sumac 4.00% with chitosan (CH-SE 4.00%), sumac 2.00% and *Z. multiflora* essential oil with chitosan (CH-SE 2.00%-Z) and sumac 4.00% and *Z. multiflora* essential oil with chitosan (CH-SE 4.00%-Z).

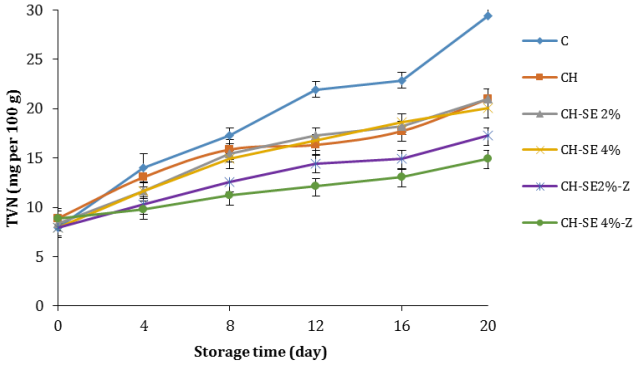

Fig. 3. The TVN content of beef meat in different treatment groups during storage at 4 ℃ at different time points. Treatments: Control (C), chitosan (CH), sumac extract 2.00% with chitosan (CH-SE 2.00%), sumac 4.00% with chitosan (CH-SE 4.00%), sumac 2.00% and *Z. multiflora* essential oil with chitosan (CH-SE 2.00%-Z) and sumac 4.00% and *Z. multiflora* essential oil with chitosan (CH-SE 4.00%-Z).

Discussion

The gas chromatography–mass spectrometry (GC–MS) analysis of ZEO showed that the major constituent of ZEO is carvacrol (46.82%).[21] Antimicrobial activity of ZEO and SE was attributed to the phenolic compounds such as thymol and carvacrol. Chitosan is believed to act on the spoilage microorganisms and pathogens, by changing the permeability of the cytoplasmic membrane, leading to the leakage of intracellular electrolytes, and finally destroying the cell.[10] The initial count of TVC was agreement with the results for beef (4.89 log CFU g^{-1}),[22] however, it was in consistent with Emiroğlu *et al*. study for beef meat (6.90 log CFU g^{-1}).[23] Based on the International Commission on Microbiological Specifications for Foods (ICMFS), the highest microbial level for acceptability of meat is 7.00 log CFU g^{-1}.[24] In the all treatment groups the population of TVC, LAB, *Enterobacteriaceae*, *Pseudomonas spp*. and yeast-mold were significantly decreased compared with the control group at the end of storage time. It has been shown that the use of EOs and extracts in combination with each other may have an additive, synergistic or antagonistic effects.[25] In the present study, the CH-SE 4.00%-Z treatment group was the most effective treatment on TVC. A previous study has shown that TVC reached 7.00 log CFU g^{-1} on days 4–5 for control samples while in samples containing CH, TVC reached the same level as the control samples on day 20.[26] Some species of LAB such as *Lactobacillus* and *Carnobacterium* have detrimental effect in meat.[27,28] In a previous study, LAB count was 5.95 log CFU g^{-1} on 9 day for 0.10% grape seed extract and 0.1% ZEO.[29] In present study among all, CH-SE 4.00%-Z and CH-SE 2.00%-Z treatments were found to be the most effective in controlling LAB. Initial count for *Entero-bacteriaceae* in the previous studies were 3.50 log CFU g^{-1}, 2.30 log CFU g^{-1}, and 2.00 log CFU g^{-1}.[30-32] In consistent with our findings, previously antimicrobial effects have been reported for thyme essential oil in beef and beef burger.[33,34] *Pseudomonas spp.* are known to compete for nutrients by forming siderophores, that may reduce the level of several bacteria.[35] The effect of *Z. multiflora* Boiss essential oil and grape seed extract on the shelf life of raw buffalo for *Pseudomonas* spp. count has been shown to be 1.62 log CFU g^{-1}.[30] In another study, antibacterial synergistic effect of pomegranate juice and chitosan with *Z. multiflora* Boiss essential oil on chicken meat has been found during refrigerated storage.[10] In a previous study, the antifungal effect for ZEO against several molds and yeasts has been shown.[30]

In the present study, a significant increase in pH value was observed from day 3 to day 21 (p < 0.05).[36] Consistent with the results of this study, Banon *et al.* reported that the combinations of ZEO and grape seed extract had a synergistic effect causing increase of pH value in beef patties.[37]

Georgantelis *et al*. showed that combination of chitosan and rosemary had stronger antioxidative effects on fresh pork sausage.[38] Antioxidant activities of ZEO could be related to its high level of phenolic agents such as tymol and carvacrol.[39] The beef treated samples presented overall lower levels of changes in TBA values. The present study showed a higher antioxidant effect of sumac than ZEO in beef. Effect of SE on the shelf life of sucuk (Turkish dry-fermented sausage) was previously studied and it was shown that SE decreased TBARS value (0.22 ± 0.02 and 0.47 ± 0.03 at the end of storage time in sumac treated and control samples, respectively,[40] while in this study TBARS values were 0.13 ± 0.00 and 0.06 ± 0.00 at the 20th day in control and CH-SE 4.00%-Z treatment groups, respectively).

The increase of PV during storage time may be caused by the faster rate of formation of new peroxides than the decay of peroxides products into secondary oxidation products. Similar results were shown by Ojagh *et al*. (0.21 and 0.24 mg at the 16th day of storage in coating and control groups, respectively)[13] and Zakipour Rahimabadi and Divband (0.85 ± 0.04 and 0.52 ± 0.01 at 15th day in control and treated samples, respectively) indicating that *Z. multiflora* Boiss essential oil and coating are capable to defer the factor of lipid oxidation.[41] In the present study SE, chitosan and ZEO improved antioxidant activity which may be caused by the protecting effect of coating against oxidation of phenolic compounds.[26]

The protein breakdown leads to formation of TVN.[42] The results for TVN showed that the best treatment for beef was gained in CH-SE 4.00%-Z group which was in agreement with the previous report.[41]

The effects of the addition of chitosan in food samples have been investigated before.[43] Jo *et al.* showed that chitosan has a positive effect on the color of pork sausages, while in the control group the odor, color texture and overall acceptability of sausages were given 'unacceptable' scores after eight days.[44] Darmadji and Izumimoto showed that the use of chitosan improved the sensory quality in the assessment of meat.[11]

In conclusion, it can be concluded that hydro-alcoholic extract of sumac has the ability to delay microbial and chemical changes and produce desirable sensory attributes including taste, color, odor and texture in beef meat. The results also revealed that ZEO and different concentration of SE compare with other treatment and control samples were most effective and were able to inhibit the bacterial growth, and apart from the color, they improved the chemical characteristics and the sensory quality of meat except color factor. The present study demonstrates the efficacy of chitosan and ZEO and SE as a potent antibacterial and antioxidant agents that can be used for the preservation and shelf life extension of meat. The effect of SE on the other meat products needs to be assessed. Meanwhile, using other kind of coatings or packaging for long-time storage of this new product is proposed.

Acknowledgments

This work was financially supported by the Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Nasar-Abbas S, Halkman AK. Antimicrobial effect of water extract of sumac (Rhus coriaria L.) on the growth of some food borne bacteria including pathogens. Int J Food Microbiol 2004; 97(1): 63-69.
2. Davidson p. Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci 2001; 26(3): 174-181.
3. Mehdizadeh T, Narimani R, Mojaddar Langroodi A, et al. Antimicrobial effects of *Zataria multiflora* essential oil and *Lactobacillus acidophilus* on *Escherichia coli* O157 stability in the Iranian probiotic white-brined cheese. J Food Saf 2018; doi.org/10.1111/jfs.12476.
4. Oussalah M, Caillet S, Saucier L. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli O157: H7, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes*. Food Control 2007; 18(5): 414-420.
5. Aliakbarlu J, Sadaghiani, SK, Mohammadi S. Comparative evaluation of antioxidant and anti food-borne bacterial activities of essential oils from some spices commonly consumed in Iran. Food Sci Biotechnol 2013; 22(6): 1487-1493.
6. Chorianopoulos N, Kalpoutzakis E, Aligiannis, et al. Essential oils of satureja, origanum, and thymus species: Chemical composition and antibacterial activities against foodborne pathogens. J Agric Food Chem 2004; 52(26): 8261-8267.
7. Hosseinzadeh H, Ramezani M, Salmani G. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats. J Ethnopharmacol 2000; 73(3): 379-385.
8. Akhondzadeh Basti A, Aminzare M, Razavi Rohani SM, et al. The combined effect of lysozyme and *Zataria multiflora* essential oil on *Vibrio Parahaemolyticus*. J Med Plants Res 2014; 2(50): 27-34.
9. Ahn J, Grün IU, Mustapha A. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. J Food Prot 2004; 67(1): 148-155.
10. Bazargani-Gilani B, Aliakbarlu J, Tajik H. Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. Innov Food Sci Emerg Technol 2015; 29: 280-287.

11. Darmadji P, Izumimoto M. Effect of chitosan in meat preservation. Meat Sci 1994; 38(2): 243-254.
12. Moradi M, Tajik H, Rohani SMR, et al. Characterization of antioxidant chitosan film incorporated with *Zataria multiflora* Boiss essential oil and grape seed extract. J Food Sci Technol 2012; 46(2); 477-484.
13. Ojagh SM, Rezaei M, Razavi SH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chem 2010; 120(1): 193-198.
14. Petrou S, Tsiraki M, Giatrakou V, et al. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. Int J Food Microbiol 2012; 156(3): 264-271.
15. Russo F, Ercolini D, Mauriello G, et al. Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. Food Microbiol 2006; 23(8): 797-802.
16. Pikul J, Leszczynski DE, Kummerow FA. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. J Agric Food Chem 1989; 37(5): 1309-1313.
17. IDF. International Dairy Federation, IDF-Square Vergote 41, Brussels, Belgium: IDF 1991; Sec: 74A.
18. Bell RG, Garout AM. The effective product life of vacuum-packaged beef imported into Saudi Arabia by sea, as assessed by chemical, microbiological and organoleptic criteria. Meat Sci 1994; 36(3): 381-396.
19. Brannan, RG. Effect of grape seed extract on physicochemical properties of ground, salted, chicken thigh meat during refrigerated storage at different relative humidity levels. J Food Sci 2008; 73(1): 36-40.
20. Ehsani A, Jasour MS, Hashemi M, et al. *Zataria multiflora* Boiss essential oiland sodium acetate: How they affect shelf life of vacuum-packaged trout burgers. Int. J. Food Sci. Technol 2014; 49(2): 1055-1062.
21. Mojaddar Langroodi A, Tajik H, Mehdizadeh T. Physico-chemical, antibacterial and antioxidant characteristics of *Zataria multiflora* Boiss essential oil and comparison of its antioxidative and antibacterial activity with hydroalcohol extract of *Rhus coriaria* L. (sumac). J Food Qual Hazards Control 2017 ; 4(3): 66-71.
22. Peng Y, Zhang J, Wang W, et al. Potential prediction of the microbial spoilage of beef using spatially resolved hyperspectral scattering profiles. J Food Eng 2011; 102(2): 163-169.
23. Emiroğlu ZK, Yemiş GP, Coşkun BK, et al. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Sci 2010; 86(2): 283-288.
24. Brocklehurst T, Zaman-Wong C, Lund BM. A note on the microbiology of retail packs of prepared salad vegetables. J Appl Microbiol 1987; 63(5): 409-415.
25. Fu Y, Zu Y, Chen L, et al. Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother Res 2007; 21(10): 989-994.
26. Aliakbarlu J, Mohammadi S, Khalili S. A study on antioxidant potency and antibacterial activity of water extracts of some spices widely consumed in Iranian diet. J Food Biochem 2014; 38(2): 159-166.
27. Hammes WP, Tichaczek PS. The potential of lactic acid bacteria for the production of safe and wholesome food. J Lebensm Unters Forsch 1994; 198(3): 193-201.
28. Moraes PM, Vicosa GN, Yamazi AK. Foodborne pathogens and microbiological characteristics of raw milk soft cheese produced and on retail sale in Brazil. Foodborne Pathog Dis 2009; 6(2): 245-249.
29. Tajik H, Aminzare M, Mounesi Raad T, et al. Effect of *Zataria multiflora* Boiss essential oil and grape seed extract on the shelf life of raw buffalo patty and fate of inoculated *Listeria monocytogenes*. J Food Process Preserv 2015; 39(6): 3005-3013.
30. Bingol EB, Ergun O. Effects of modified atmosphere packaging (MAP) on the microbiological quality and shelf life of ostrich meat. Meat Sci 2011; 88(4): 774-785.
31. Seydim AC, Acton JC, Hall MA, et al. Effects of packaging atmospheres on shelf-life quality of ground ostrich meat. Meat Sci 2006; 73(3): 503-510.
32. Fernández-López J, Sayas-Barberá E, Munoz, T, et al. Effect of packaging conditions on shelf-life of ostrich steaks. Meat Sci 2008; 70(1-2): 143-152.
33. Kassem G, Atta-Alla O, Ali F. Improving the quality of beef burger by adding thyme essential oil and jojoba oil. Arch Zootec 2011; 60(231): 787-795.
34. Solomakos N, Govaris A, Koidis P, et al. The antimicrobial effect of thyme essential oil, nisin, and their combination against Listeria monocytogenes in minced beef during refrigerated storage. Food Microbiol 2008; 25(1): 120-127.
35. Wei H, Wolf G, Hammes WP. Indigenous micro-organisms from iceberg lettuce with adherence and antagonistic potential for use as protective culture. Innov Food Sci Emerg Technol 2006; 7(4): 294-301.
36. Brenesselová M, Koréneková B, Mačanga J, et al. Effects of vacuum packaging conditions on the quality, biochemical changes and the durability of ostrich meat. Meat Sci 2015; 101: 42-47.
37. Banon S, Díaz P, Rodríguez M, et al. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. Meat Sci 2007; 77(4): 626-633.
38. Georgantelis D, Ambrosiadis I, Katikou P. Effect of rosemary extract, chitosan and α-tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 ˚C. Meat Sci 2007; 76(1): 172-181.
39. Zakipour Rahimabadi E, Rigi M, Rahnama M. Combined effects of *Zataria multiflora* Boiss essential oil and nisin on the shelf-life of refrigerated rainbow trout (*Onchorynchus mykiss*) fillets. Iran J Fish Sci 2013; 12(1): 115-126.

40. Bozkurt, H. Investigation of the effect of sumac extract and BHT addition on the quality of sucuk (Turkish dry-fermented sausage). J Sci Food Agric 2006; 86(5): 849-856.
41. Zakipour Rahimabadi E, Divband M. The Effects of coating and *Zataria multiflora* Boiss essential oil on chemical attributes of silver carp fillet stored at 4 ˚C. Int Food Res J 2012; 19(2): 685-690.
42. Ajewole K, Adeyeye A. Characterisation of Nigerian citrus seed oils. Food Chem 1993; 47(1): 77-78.
43. No HK, Meyers SP, Prinyawiwatkul W, et al. Applications of chitosan for improvement of quality and shelf life of foods: A review. J Food Sci 2007; 72(5): 87-100.
44. Jo C, Lee JW, Lee KH, et al. Quality properties of pork sausage prepared with water-soluble chitosan oligomer. Meat Sci 2001; 59(4): 369-375.