LWT - Food Science and Technology 64 (2015) 898–904

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effect of carboxymethyl cellulose-based coatings incorporated with *Zataria multiflora* Boiss. essential oil and grape seed extract on the shelf life of rainbow trout fillets

Mojtaba Raeisi [a, b, *], Hossein Tajik [c], Javad Aliakbarlu [c], Seyed Hamed Mirhosseini [d], Seyed Mohammad Hashem Hosseini [e]

[a] *Cereal Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran*
[b] *Department of Public Health, School of Health, Golestan University of Medical Sciences, Gorgan, Iran*
[c] *Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran*
[d] *Department of Food Technology, Faculty of Food Science and Technology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[e] *Department of Food Science and Technology, College of Agriculture, Shiraz University, 71441-65186 Shiraz, Iran*

ARTICLE INFO

Article history:
Received 16 February 2015
Received in revised form
28 April 2015
Accepted 9 June 2015
Available online 18 June 2015

Keywords:
Carboxymethyl cellulose
Zataria multiflora Boiss. essential oil
Grape seed extract
Rainbow trout fillet

ABSTRACT

To prolong the shelf life of seafood products, lipid oxidation and growth of microorganisms should be retarded. The objective of the current study was evaluating the potential application of carboxymethyl cellulose (CMC) coatings incorporated with *Zataria multiflora* Boiss. essential oil (ZMEO) and grape seed extract (GSE) on chemical (thiobarbituric acid reactive substances (TBARS) and total volatile basic nitrogen (TVB-N)), microbial (total viable count, lactic acid bacteria and *Pseudomonas* spp.) and organoleptic attributes of rainbow trout fillets during refrigerated storage for twenty days. GC–MS analysis showed that ZMEO is rich in monoterpene phenols such as thymol and carvacrol. The following results were obtained after 20 days of storage: The minimum level of TVB-N was measured in the fillets coated with CMC + 2% v/v ZMEO + 0.5% v/v GSE. The minimum number of total viable bacteria, lactic acid bacteria and *Pseudomonas* spp. were determined in the fillets coated with CMC + 2% v/v ZMEO + 1% v/v GSE. The fillets coated with CMC + 1% v/v ZMEO + 1% v/v GSE showed the best organoleptic properties. Our results revealed that CMC-based coatings incorporated with ZMEO and GSE could improve chemical, microbial and sensorial characteristics of rainbow trout fillets during cold storage.

1. Introduction

Fresh fish is one of the most perishable seafood products. It has been reported that the spoilage of fish muscle is a combination of different spoilage mechanisms including lipid oxidation, microbial and endogenous enzymes activities as well as enzymatic browning. These events lead to a decrease in the shelf life of fish meat and other seafood products (Arashisar, Hisara, Kayab, & Yanik, 2004; Mace et al., 2013). In recent years, new techniques have been tried by many researchers to prolong the shelf life of food products. Among different applied methods, application of bio-based films and coatings was the most promising technique (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007; Jouki, Tabatabaei-Yazdi, Mortazavi, Koocheki, & Khazaei, 2014; Ojagh, Rezaei, Razavi, & Hosseini, 2010).

Edible films and coatings of particular characteristics can be produced from different sources including polysaccharides, proteins and lipids (Sayanjali, Ghanbarzadeh, & Ghiassifar, 2011). Polysaccharides have been frequently used to develop films and coatings because of appropriate film forming properties. CMC (E466) is a cellulose derivative of wide applications in food technology such as thickening, stabilizing and mouthfeel improving. It is composed of linear chains of β (1–4) glucosidic units with methyl and carboxyl substituents (Togrol & Arsalan, 2004). The obtained film from aqueous solutions of CMC has moderate strength; however, has high water vapor permeability because of the inherent hydrophilic nature. In contrast to some biopolymers such as chitosan, CMC does not have any intrinsic antimicrobial properties. A way to improve the moisture barrier properties of CMC together

* Corresponding author. Department of Public Health, School of Health, Golestan University of Medical Sciences, Gorgan, Iran.
E-mail address: drmraeisi@goums.ac.ir (M. Raeisi).

http://dx.doi.org/10.1016/j.lwt.2015.06.010
0023-6438/

with developing antimicrobial characteristics would be the incorporation of hydrophobic compounds such as essential oils. According to Dashipour et al. (2015), *Zataria multiflora* essential oil addition into CMC-based films could improve physical, mechanical and antibacterial properties.

In recent years, there has been an increased interest in the use of natural antimicrobial agents instead of chemical ones. CMC and the other biopolymers can be used as a suitable carrier for natural antimicrobial and antioxidant compounds. Essential oils and their components show promising activities against many food-borne pathogens and spoilage microorganisms. *Z. multiflora* Boiss. (Shirazi thyme) is a member of Labiatae family which grows in some parts of Iran, Pakistan and Afghanistan. *Z. multiflora* Boiss. essential oil (ZMEO) shows strong antimicrobial and antioxidant activities because of having large quantities of phenolic oxygenated monoterpenes. Thymol and carvacrol are the main constituents of this essential oil (Moradi et al., 2012). Grape seed is a byproduct of winery and grape juice industry (Ignea et al., 2013). Grape seed extract (GSE) has different amounts of lipid, protein, carbohydrates and 5–8% polyphenols. Main polyphenol compounds present in GSE include monomeric flavon-3-ols such as catechin, epicatechin and procyanidin dimmers and trimers (Chedea, Braicu, & Socaciu, 2010; Nakamura, Tsuji, & Tonogai, 2003; Peng et al., 2001). In practical applications of biopolymer-based antimicrobial films, higher concentrations of essential oils are needed to exert similar functional effects as those obtained during in vitro assays, which is due to the entrapment of the essential oil components within film matrix. This may result in unfavorable sensory characteristics. Formulating different types of natural preservatives mixtures is thus a new solution to increase the efficacy of essential oils and decrease the unfavorable organoleptic properties by taking the advantage of their synergistic and additive effects. Essential oils containing carvacrol, cinnamaldehyde, cinnamic acid, eugenol and thymol have a synergistic effect (positive interaction) in combination with other polyphenols. The constituents of essential oils may act synergistically by affecting multiple targets and by physicochemical interactions (Bassolé & Juliani, 2012). The efficacy of antimicrobial coatings of different origins has been demonstrated in previous studies (Jouki et al., 2014; Ojagh et al., 2010). Liu, Han, Zhang, Li, and Li (2012) utilized CMC films contained rosemary extract to inhibit microbial and oxidative degradations of fresh beef during cold storage.

To the best of our knowledge the simultaneous incorporation of ZMEO and GSE into CMC-based film and evaluating its potential application in real food systems (such as fish fillets) has not been studied previously. Therefore, the objective of the current study was exploring the antibacterial and antioxidant properties of CMC coatings incorporated with ZMEO and GSE on improving different quality characteristics of rainbow trout fillets during cold storage.

2. Material and methods

2.1. Chemicals and materials

CMC (average MW of 41 kDa) was purchased from Caragum Parsian Co. (Tehran, Iran). Commercial GSE powder was obtained from Mega Natural Inc. (Madera, CA, USA). Stock solution (10% w/v) of GSE powder was prepared by dissolving GSE in distilled water. Glycerol (analytical grade), Standard Plate Count Agar (PCA) and de Man Rogosa Sharpe Agar (MRSA) culture media were purchased from Merck Co. (Darmstadt, Germany). Pseudomonas Isolation Agar (PIA) culture medium was purchased from Oxoid Co. (Cambridge, UK). All other used reagents were of analytical grade.

2.2. Fish sample preparation

Fresh aqua cultured rainbow trout (*Oncorhynchus mykiss*) with average weight of 400–500 g were purchased from a cold water aqua culture farm in Urmia, Iran. The samples were transferred in ice boxes to the laboratory. In the first step, all fishes were eviscerated and filleted and then remained at 4 °C before coating and subsequent analysis.

2.3. Essential oil extraction and its GC/MS analysis

To prepare ZMEO, the plant was purchased from a local grocery and authenticated at the Faculty of Agriculture, Urmia University (Urmia, Iran). Hydrodistillation method using a Clevenger type apparatus was utilized to extract essential oil during a 3 h distillation. The obtained essential oil was dehydrated using sodium sulfate and after filtration, it was stored at 4 °C and darkness. Analysis of ZMEO was performed using an Agilent 6890N instrument equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness). The carrier gas was helium with a flow rate of 1 ml/min. The column temperature was initially set at 50 °C, and then gradually increased to 120 °C at a 2 °C/min rate, held for 3 min at this temperature, and finally increased to 300 °C. The detection procedure was operated at 70 eV. The compounds were identified by comparing their retention indices with those of authentic samples and mass spectral data available in the library (Wiley 2001 data software).

2.4. Preparation of CMC coating solutions

To prepare coating solution, 1 g carboxymethyl cellulose was dispersed in 100 ml distilled water. Glycerol, as a plasticizer, was added into CMC coating solution at 0.5% v/v. The dispersion was heated at 85 °C for 5 min with subsequent cooling at room temperature (Sayanjali et al., 2011). ZMEO (1% and 2% v/v) and GSE (0.5% and 1% v/v) were incorporated into CMC coating solutions. Fillet coating was performed using immersion method. The coated samples were then allowed to lose excess biopolymer solution before coating development. In this study, nine samples including the blank sample (without any coating) and those coated with CMC contained different amounts of ZMEO, GSE and their possible mixtures were prepared. The samples were stored at 4 °C for 20 days and analyses were carried out during 5 day intervals.

2.5. Chemical analysis

2.5.1. Proximate composition

The AOAC method was used to determine the moisture and the crude ash contents at 103 and 550 °C, respectively (AOAC, 2002). Total crude protein was assessed using Kjeldahl method (AOAC, 2005). The lipid content was measured according to the method described by Bligh and Dyer (1959).

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

Determination of TVB-N values was performed based on the micro-diffusion method described by Pikul, Lesztzynski, and Kummerow (1989). This method was carried out by distillation using a Kjeldahl type apparatus after MgO addition into the homogenized samples. A flask containing aqueous solution of boric acid of 3% concentration as well as indicator (produced by dissolving 0.1 g methyl red and 0.1 g methylene blue in 100 ml absolute ethanol) was utilized to collect the distillate. To determine TVB-N values, the boric acid solution was titrated using 0.05 M sulfuric acid solution. Results have been expressed in milligram of nitrogen per 100 g of sample.

2.5.3. Determination of thiobarbituric acid reactive substances (TBARS)

TBARS were determined using the method described by Jeon, Kamil, and Shahidi (2002). This colorimetry technique is based on the reaction between thiobarbituric acid and the secondary products of the lipid oxidation such as aldehydes and ketones. The experiment was performed at 530 nm and TBA values were expressed as mg of malonaldehyde equivalents/kg of meat.

2.6. Microbiological analysis

Microbiological analyses were conducted using surface spread technique (Arashisar et al., 2004; Sallam, 2007). A portion (10 g) of fillets was drawn aseptically and transferred into 90 ml sterile peptone water solution (0.1% w/v). All samples were homogenized using a stomacher. To enumerate bacteria, 0.1 ml of serially diluted homogenates was spread on the specific agar plates. To determine total viable count (TVC), spread plate technique was performed on Standard PCA. The plates were incubated at 30 °C for 48 h. To enumerate the *Pseudomonas* spp. plates containing PIA were incubated at 10 °C for 7 days. Lactic acid bacteria (LAB) were enumerated on MRSA incubated at 25 °C for 5 days under anaerobic conditions. The experiments were performed in triplicate and the counts were expressed as $\log_{10}$ cfu/g.

2.7. Sensory evaluation

The organoleptic properties of steamed (10–20 min at 98 °C) samples were evaluated by a panel of seven laboratory trained judges. The judges were not aware of the experimental approach and the samples were blind. The evaluation of odor, texture, taste and overall acceptability were carried out based on a nine point hedonic scale (1, dislike extremely to 9, like extremely). Samples obtained average sensorial scores of more than 4 were considered as acceptable ones (Jouki et al., 2014).

2.8. Statistical analysis

In this study, all experiments were done in triplicate and analyzed by ANOVA using SPSS software (version 19). A completely randomized design (CRD) was considered to create all samples. Type and concentration of ZMEO and GSE were considered as independent variables. Comparison of means was carried out using Tukey's multiple range tests at a confidence level of 0.05.

3. Results and discussion

3.1. Identification of essential oil components

The essential oil content of the aerial parts of *Z. multiflora* Boiss. was around 1.2% v/w. The main chemical constituents of ZMEO are given in Table 1. In this study, thymol (27.4%) and carvacrol (41.2%) were the most representative components of ZMEO. Similar results have been reported in previous studies (Moradi, Tajik, Razavi Rohani, & Oromiehie, 2011; Shariffar, Mosha, Mansouri, Khodashenas, & Khoshnoodi, 2007). Although, Azizkhani, Misaghi, AkhondzadehBasti, Gandomi, and Hosseini (2013) and Moosavy et al. (2008) reported that the phenolic monoterpene carvacrol is the major component. Tepe et al. (2004) suggested that the observed differences between the essential oils compositions extracted from similar plants could be attributed to the difference in climates, seasons, geography and geology in which the plants have grown. Phenolic compounds have strong antimicrobial activity, among which, carvacrol has been shown to have the highest antimicrobial efficacy. The hydrophobic nature and the presence of a free hydroxyl group are essential factors for the carvacrol activity on the cell membranes (Ali, Saleem, & Ahmad, 2000).

Table 1
Chemical composition of *Zataria multiflora* Boiss. essential oil (ZMEO).

Number	Compounds	Retention time (RT)	%
1	α-Thujene	17.325	0.30
2	α-Pinene	17.675	0.84
3	Camphene	18.187	0.63
4	β-pinene	19.242	0.58
5	α-Phellandrene	20.222	0.52
6	δ-3-Carene	23.560	0.54
7	α-Terpinene	44.793	0.59
8	*p*-Cymene	44.644	0.23
9	Limonene	21.240	0.40
10	Gamma terpinene	26.891	4.11
11	Terpinolene	23.138	0.90
12	Linalool	23.432	3.40
13	Terpinene-4-ol	20.681	0.45
14	Thymol methyl ether	27.908	1.55
15	Thymol	30.183	27.40
16	Carvacrol	30.831	41.2
17	Thymol acetate	31.939	0.50
18	Eugenol	32.067	0.34
19	β-Caryophyllene	33.604	0.49
20	Aromadendrene	34.509	0.20
21	α-Humulene	34.366	0.11
22	Spathulenol	37.447	1.20
23	Caryophyllene oxide	37.583	0.90
24	O-isopropyltoluene	21.179	4.54
25	1-Cycloheptene	38.216	2.18
26	3-Methylresacetophenone	33.846	2.21
27	Myrcene	19.687	0.32
	Total		96.63

3.2. Proximate composition

The average moisture, protein, lipid and ash contents (%) of the rainbow trout fillets were 66.4 ± 1.12, 22.82 ± 0.44, 3.1 ± 0.2 and 3.89 ± 0.4, respectively. The obtained results are in good agreement with those reported by Ojagh et al. (2010) and Jouki et al. (2014). Celik et al. (2007) and Gokoglu, Yerlikaya, and Cengiz (2004) reported the average values (%) of 19.60 ± 0.06 and 19.80 ± 0.04 for protein content as well as 4.43 ± 0.16 and 1.35 ± 0.01 for lipid content, respectively. These differences which can be related to the factors such as nutrition, sexual variations, fish size and living area are able to change the sensory attributes and the microbial growth conditions of fish meat (González-Fandos, Villarino-Rodríguez, García-Linares, García-Arias, & García-Fernández, 2005).

3.3. Effect of CMC-based coating incorporated with ZMEO and GSE on TVB-N of rainbow trout fillets

TVB-N value is used as an indicator for the fish spoilage, since different compounds including ammonia, primary, secondary and tertiary amines can be totally measured (Fan, Chi, & Zhang, 2008). The activity of fish spoilage bacteria and also the endogenous enzymes present in fish tissue accelerate the deterioration process of the muscle tissue, consequently lead to an increase in TVB-N values (Jouki et al., 2014). TVB-N values of the samples are given in Table 2. The results indicated that the initial TVB-N values ranged from 8.67 ± 0.58 mg N/100 g in the sample coated with the CMC + 2% v/v ZMEO + 1% v/v GSE to 12.67 ± 0.58 mg N/100 g in control sample. Jouki et al. (2014) and Ojagh et al. (2010) reported similar results; however, the values obtained by Arashisar et al. (2004) were relatively lower. According to Gimenez, Roncales, and Beltran (2002) TVB-N value of 25 mg N/100 g is the highest acceptable level. The

Table 2
The amounts of total volatile basic nitrogen (TVB-N, milligram of nitrogen per 100 g of sample) in the rainbow trout fillets coated with CMC during cold storage.

Coating formulation	Storage time (day)				
	0	5	10	15	20
GSE 0.5%	11.34 ± 0.58^{A}	15.33 ± 0.58^{E}	23.33 ± 0.58^{D}	32.33 ± 0.58^{D}	40.3 ± 0.58^{C}
GSE 1%	11.33 ± 0.58^{A}	14.33 ± 0.58^{DE}	21.67 ± 0.58^{C}	29.67 ± 0.58^{D}	37.57 ± 0.58^{C}
ZMEO 1%	10.33 ± 0.58^{A}	13.67 ± 0.58^{CD}	18.33 ± 0.58^{BC}	28.67 ± 0.58^{C}	35.33 ± 0.58^{B}
ZMEO 2%	9.33 ± 0.58^{A}	12.67 ± 0.58^{ABC}	15.33 ± 1.15^{B}	23 ± 1.00^{BC}	31.6 ± 1.15^{A}
ZMEO 1% + GSE 0.5%	10.33 ± 0.58^{A}	13.33 ± 1.15^{BC}	17.33 ± 1.53^{BC}	22.33 ± 1.15^{BC}	33.33 ± 0.58^{B}
ZMEO 1% + GSE 1%	9.67 ± 0.58^{A}	12.67 ± 0.58^{BC}	18 ± 1.00^{BC}	23 ± 1.00^{BC}	34.33 ± 0.58^{B}
ZMEO 2% + GSE 0.5%	8.67 ± 0.58^{A}	12 ± 1.00^{AB}	14 ± 2.00^{A}	20.67 ± 3.79^{AB}	26.3 ± 0.58^{A}
ZMEO 2% + GSE 1%	8.33 ± 1.15^{A}	12.33 ± 1.15^{A}	15.33 ± 0.73^{A}	21.33 ± 1.00^{A}	31 ± 1.00^{A}
Control	12.67 ± 0.58^{A}	17.33 ± 0.58^{E}	25.33 ± 0.58^{D}	36.33 ± 0.58^{D}	52 ± 1.00^{C}

A–D: different letters in the same columns indicate significant differences ($P < 0.05$).
ZMEO: *Zataria multiflora* essential oil; GSE: grape seed extract; TVBN: total volatile basic nitrogen.

samples coated with CMC + 2% v/v ZMEO + 1% v/v GSE and CMC + 2% v/v ZMEO + 0.5% v/v GSE showed the lowest TVB-N values. TVB-N values of uncoated sample significantly ($P < 0.05$) increased during storage time and reached to 52 mg N/100 g after 20 days. TVB-N values of the most coated samples (CMC + 2% v/v ZMEO + 1% v/v GSE, CMC + 2% v/v ZMEO + 0.5% v/v GSE, CMC + 1% v/v ZMEO + 1% v/v GSE, CMC + 1% v/v ZMEO + 0.5% v/v GSE and CMC + 2% v/v ZMEO) did not reach to the maximum acceptable level until fifteenth day of storage. This phenomenon could be explained by the fact that incorporating ZMEO and GSE into CMC coating resulted in a decrease in microbial growth and/or in the ability of bacteria for oxidative de-amination of non-protein compounds (Fan et al., 2008; Jouki et al., 2014). The synergistic and additive effects of simultaneous incorporating GSE and ZMEO into CMC-based solution were obvious in the current work.

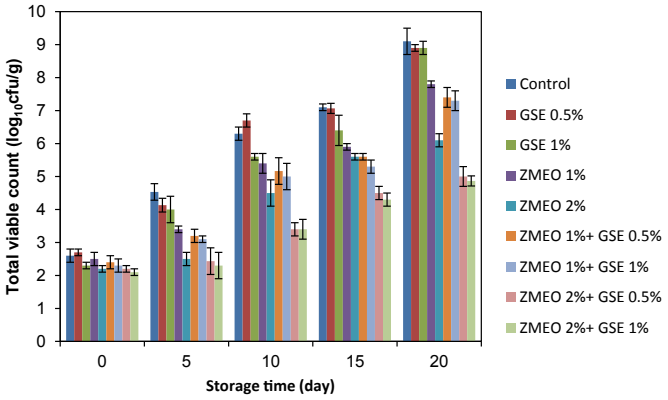

Fig. 1. Changes in total viable count (TVC) of rainbow trout fillets during refrigerated storage (ZMEO: *Zataria multiflora* essential oil; GSE: Grape seed extract).

3.4. Effect of CMC-based coating incorporated with ZMEO and GSE on TBARS of rainbow trout fillets

TBA can be used as an indicator to determine the secondary products of lipid oxidation (Jeon et al., 2002). The results are shown in Table 3. The initial TBARS values ranged from 0.16 ± 0.007 to 0.21 ± 0.018 mg malonaldehyde equivalents/kg in the fillets coated with CMC + 2% v/v ZMEO + 1% v/v GSE and in control samples, respectively. The results revealed that TBARS values of all samples increased with storage time; however, the increase was sample dependent. For example, the increment in TBARS values of the samples coated with CMC + 2% v/v ZMEO + 1% v/v GSE was significantly ($P < 0.05$) lower than that of other samples. In addition, TBARS values of uncoated samples were significantly higher than those of other samples during storage time. The detection threshold of objectionable odor in fish flesh and the maximum tolerated level of TBARS value which cannot adversely affect the quality of the fish (frozen, chilled or stored with ice) are around TBARS values of 1–2 and 5 mg malonaldehyde equivalents/kg, respectively (Sallam, 2007). The results showed that TBARS values of all samples were lower than the accepted level until the fifteenth day. Our results are in agreement with previous findings. Ojagh et al. (2010) and Jouki et al. (2014) reported that TBARS values of rainbow trout meat did not reach to 2 mg malonaldehyde equivalents/kg during 16 and 18 days of storage, respectively. The lower TBARS values of coated samples compared to those of control ones might be due to the strong antioxidant activity of individual ZMEO and GSE components (phenolic and polyphenolic compounds), their synergistic and additive effects and also the oxygen barrier properties of CMC coating.

Table 3
TBA values (malonaldehyde equivalents/kg of meat) in the fillets of rainbow trout coated with CMC during cold storage.

Coating formulation	Storage time (day)				
	0	5	10	15	20
GSE 0.5%	0.21 ± 0.018	0.37 ± 0.032*	0.92 ± 0.010*	1.07 ± 0.039*	1.24 ± 0.035*
GSE 1%	0.19 ± 0.007	0.30 ± 0.210*	0.68 ± 0.033*	1.04 ± 0.025*	1.15 ± 0.025*
ZMEO 1%	0.19 ± 0.014	0.24 ± 0.010*	0.54 ± 0.017*	0.59 ± 0.018*	0.95 ± 0.010*
ZMEO 2%	0.16 ± 0.008*	0.23 ± 0.007*	0.33 ± 0.014*	0.42 ± 0.010*	0.70 ± 0.004*
ZMEO 1% + GSE 0.5%	0.18 ± 0.004	0.24 ± 0.004*	0.49 ± 0.007*	0.52 ± 0.004*	0.84 ± 0.025*
ZMEO 1% + GSE 1%	0.18 ± 0.007	0.25 ± 0.021*	0.53 ± 0.004*	0.56 ± 0.020*	0.91 ± 0.035*
ZMEO 2% + GSE 0.5%	0.17 ± 0.004*	0.23 ± 0.007*	0.34 ± 0.004*	0.42 ± 0.014*	0.60 ± 0.008*
ZMEO 2% + GSE 1%	0.16 ± 0.007*	0.21 ± 0.007*	0.29 ± 0.007*	0.44 ± 0.021*	0.55 ± 0.008*
Control	0.19 ± 0.014	0.44 ± 0.033	1.20 ± 0.014	1.59 ± 0.028	2.05 ± 0.047

*This symbol in the same columns indicates significant differences with control sample ($P < 0.05$).
ZMEO: *Zataria multiflora* essential oil; GSE: grape seed extract; TBA: thiobarbituric acid.

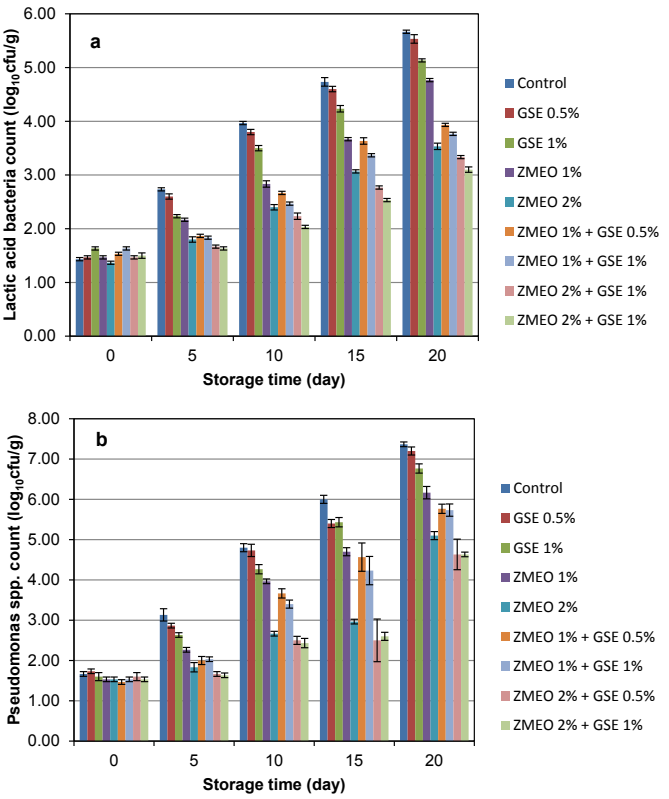

Fig. 2. Changes in (a) lactic acid bacteria (LAB) and (b) *Pseudomonas* spp. counts of rainbow trout fillets during refrigerated storage (ZMEO: *Zataria multiflora* essential oil; GSE: grape seed extract).

3.5. Effect of CMC-based coating incorporated with ZMEO and GSE on microbiological quality of rainbow trout fillets

Changes in TVC, LAB count and *Pseudomonas* spp. count are shown in Figs. 1 and 2a, b, respectively. The initial TVC of control sample was 2.6 ± 0.2 $\log_{10}$ cfu/g while the sample coated with CMC + 2% v/v ZMEO + 1% v/v GSE had lower initial TVC (2.2 ± 0.1 $\log_{10}$ cfu/g). Another researchers (Arashisar et al., 2004; Frangos, Pyrgotou, Giatrakou, Ntzimani, & Savvaidis, 2010; Ojagh et al., 2010) reported that the initial TVC of control samples varied between 3 and 4 $\log_{10}$ cfu/g. In this study, the low amount of the initial TVC of fish fillets confirmed that the samples were of high hygienic quality. According to Sallam (2007), the maximum accepted value of TVC in raw fish is 7 $\log_{10}$ cfu/g. As shown in Fig. 1, except for control sample, TVCs of the other samples were below 7 $\log_{10}$ cfu/g until the fifteenth day. TVC of control sample was much higher than 7 $\log_{10}$ cfu/g after 10 days of storage, while TVCs of three samples coated with CMC + 2% v/v ZMEO + 1% v/v GSE, CMC + 2% v/v ZMEO + 0.5% v/v GSE and CMC + 2% v/v ZMEO, did not exceed 7 $\log_{10}$ cfu/g. The significant ($P < 0.05$) differences among TVCs of control samples and coated ones and among TVCs of coated samples of different formulations could be attributed to the antibacterial effect of natural preservative as well as preservative type and concentration. Jouki et al. (2014) and Ojagh et al. (2010) reported similar trends in TVCs of coated rainbow trout fillets.

Facultative anaerobic LAB are the main cause of meat spoilage. Particular species of LAB (such as *Lactobacillus* spp., *Carnobacterium* spp. and *Leuconostoc* spp.) have been reported as the most effective bacteria in meat spoilage (Giatrakou, Ntzimani, Zwietering, & Savvaidis, 2010). In our study, LAB counts varied from 1.4 to 5.7 $\log_{10}$ cfu/g during 20 days of storage. The lowest amounts of LAB count were enumerated in the samples coated with CMC + 2% v/v ZMEO + 1% v/v GSE and amounted to 1.5–3.1 $\log_{10}$ cfu/g at first and last days of storage, respectively. Statistically significant ($P < 0.05$) differences were found between control sample and the others during cold storage. Jouki et al. (2014) reported that the application of quince seed mucilage edible films incorporated with oregano essential oil for extending the shelf life of rainbow trout fillets could reduce only 1 $\log_{10}$ cfu/g of LAB count, which may be attributed to the differences in natural flora of the prepared samples. It has been reported that LAB are the most resistant Gram positive bacteria towards the antimicrobial action of essential oils (Burt, 2004). In this study, the samples included high concentrations of ZMEO and GSE revealed appropriate resistance toward LAB growth which is may be due to the synergistic effects of ZMEO and GSE. However, antibacterial effects of ZMEO and GSE against LAB were relatively stronger than those against *Pseudomonas* spp. (discussed later). Frangos et al. (2010) suggested that the greater resistance of LAB is due to their better ability to face with osmotic stress conditions and also more efficient response to K^+ efflux caused by many essential oils.

Gram negative psychrotrophic bacteria are the major group of microorganisms responsible for aerobically spoilage of fresh fish at low temperatures and *Pseudomonas* spp. are the main bacteria in this group (Wei, Wolf, & Hammes, 2006). The initial *Pseudomonas* spp. counts of samples ranged from 1.5 to 1.7 $\log_{10}$ cfu/g in CMC + 2% v/v ZMEO + 1% v/v GSE coated and control samples, respectively. As shown in Fig. 2b, gradual increase was occurred in *Pseudomonas* spp. count of samples. However, the increase rate in control samples was significantly ($P < 0.05$) higher than that of coated ones as reached to 7.4 $\log_{10}$ cfu/g at day 10. Coating formulations containing ZMEO and GSE revealed appropriate antibacterial effects against *Pseudomonas* spp. The minimum amount of *Pseudomonas* spp. count (4.6 $\log_{10}$ cfu/g) was obtained in samples coated with CMC + 2% v/v ZMEO + 1% v/v GSE and CMC + 2% v/v ZMEO + 0.5% v/v GSE.

It has been reported that monoterpene phenols (such as thymol and carvacrol) and polyphenols (such as hydroquinone, caffeic acid, gallic acid and resveratrol) present in ZMEO and GSE, respectively, show effective antimicrobial activity against both Gram positive and Gram negative bacteria (Moradi et al., 2012). Simultaneous incorporating ZMEO and GSE into CMC-based coatings resulted in more resistance of fillets toward microbial and oxidative degradations due to the synergistic and additive effects. One of the mechanisms behind the antimicrobial action of essential oils such as ZMEO includes the direct contact between essential oil constituents and phospholipid bilayer of bacterial membrane results in the ion permeability and consequently the leakage of vital intracellular components (Burt, 2004). The antimicrobial efficacy of polyphenols may be related to their effects on the hydrolytic enzymes, interactions with cell envelope transport proteins and nonspecific interactions with carbohydrates (Cowan, 1999).

3.6. Effect of CMC-based coating incorporated with ZMEO and GSE on sensory properties of steamed rainbow trout fillets

Essential oils should be used at high concentrations to achieve desired antibacterial and antioxidant effects. One of the main concerns regarding the application of plant extracts and essential oils (especially those release strong odor and flavor such as oregano and *Z. multiflora* Boiss. essential oils) is the negative effects on the organoleptic properties of applied food (Jouki et al., 2014). The results of taste, texture, odor and overall acceptability evaluations of fillets are shown in Table 4. Sensorial scores of different

Table 4
Changes in sensory attributes scores of fish samples during refrigerated storage.

Coating additive	Storage time (day)					
		0	5	10	15	20
GSE 0.5%	Taste	8.5 ± 0.3^{C}	6.7 ± 0.3^{C}	3.7 ± 0.5^{A}	1.3 ± 0.4^{A}	–
	Texture	8.5 ± 0.2^{A}	5.8 ± 0.3^{A}	4.3 ± 0.8^{A}	2.9 ± 0.6^{A}	1.1 ± 0.2^{A}
	Odor	8.8 ± 0.2^{B}	6.8 ± 0.3^{AB}	5.3 ± 0.7^{BC}	3.3 ± 0.5^{BC}	–
	Overall	8.4 ± 0.8^{AB}	7.4 ± 0.7^{AB}	4.8 ± 0.4^{A}	2.3 ± 0.4^{AB}	2.1 ± 0.7^{AB}
GSE 1%	Taste	8.5 ± 0.1^{C}	6.8 ± 0.3^{C}	3.6 ± 0.2^{A}	1.8 ± 0.1^{A}	–
	Texture	8.5 ± 0.8^{A}	6.3 ± 0.1^{AB}	5.7 ± 0.1^{AB}	3.4 ± 0.6^{AB}	1.4 ± 0.7^{AB}
	Odor	9.0 ± 0.1^{B}	7.3 ± 0.5^{B}	5.8 ± 0.2^{BC}	4.3 ± 0.3^{C}	–
	Overall	8.7 ± 0.4^{AB}	7.4 ± 0.3^{AB}	5.3 ± 0.2^{AB}	3.4 ± 0.4^{BC}	2.1 ± 0.3^{AB}
ZMEO 1%	Taste	7.8 ± 0.2^{BC}	6.5 ± 0.7^{C}	4.5 ± 0.8^{B}	2.7 ± 0.2^{A}	–
	Texture	8.8 ± 0.2^{A}	7.4 ± 0.3^{B}	6.3 ± 0.3^{B}	5.6 ± 0.9^{CD}	2.8 ± 0.7^{BC}
	Odor	8.5 ± 0.8^{B}	7.7 ± 0.7^{B}	6.3 ± 0.3^{C}	4.3 ± 0.7^{C}	1.7 ± 0.5^{A}
	Overall	9.0 ± 0.7^{B}	8.4 ± 0.7^{B}	7.2 ± 0.5^{C}	5.2 ± 0.2^{D}	3.5 ± 0.9^{BC}
ZMEO 2%	Taste	7.4 ± 0.2^{BC}	5.7 ± 0.6^{BC}	3.7 ± 0.1^{A}	1.6 ± 0.7^{B}	–
	Texture	8.4 ± 0.1^{A}	7.6 ± 0.5^{B}	6.8 ± 0.1^{B}	6.3 ± 0.3^{D}	2.9 ± 0.4^{C}
	Odor	7.9 ± 0.5^{AB}	6.3 ± 0.9^{AB}	4.3 ± 0.1^{AB}	1.7 ± 0.4^{A}	–
	Overall	7.4 ± 0.4^{A}	6.7 ± 0.3^{A}	5.3 ± 0.7^{AB}	3.3 ± 0.9^{BC}	1.0 ± 0.1^{A}
ZMEO 1% + GSE 0.5%	Taste	7.6 ± 0.6^{BC}	6.7 ± 0.1^{C}	3.8 ± 0.5^{A}	3.7 ± 0.4^{D}	2.7 ± 0.5^{BC}
	Texture	8.6 ± 0.5^{A}	7.6 ± 0.4^{B}	6.6 ± 0.6^{B}	4.9 ± 0.7^{CD}	2.7 ± 0.6^{AB}
	Odor	8.6 ± 0.5^{AB}	7.8 ± 0.1^{B}	6.7 ± 0.7^{C}	4.3 ± 0.7^{C}	3.7 ± 0.7^{B}
	Overall	9.0 ± 0.6^{B}	8.4 ± 0.1^{B}	7.3 ± 0.9^{C}	5.1 ± 0.3^{D}	4.1 ± 0.3^{BC}
ZMEO 1% + GSE 1%	Taste	7.5 ± 0.3^{BC}	5.8 ± 0.2^{BC}	4.7 ± 0.3^{B}	4.6 ± 0.5^{B}	3.8 ± 0.6^{C}
	Texture	8.8 ± 0.1^{A}	7.8 ± 0.3^{B}	5.7 ± 0.2^{AB}	5.2 ± 0.5^{CD}	3.8 ± 0.9^{BC}
	Odor	8.7 ± 0.8^{AB}	7.8 ± 0.4^{B}	6.3 ± 0.3^{C}	4.3 ± 0.2^{C}	2.8 ± 0.6^{AB}
	Overall	9.0 ± 0.9^{B}	8.4 ± 0.8^{B}	6.2 ± 0.3^{BC}	4.6 ± 0.5^{CD}	4.4 ± 0.5^{C}
ZMEO 2% + GSE 0.5%	Taste	7.3 ± 0.2^{C}	4.3 ± 0.3^{AB}	3.3 ± 0.7^{A}	2.7 ± 0.3^{C}	1.3 ± 0.2^{A}
	Texture	8.8 ± 0.5^{A}	6.6 ± 0.3^{AB}	6.7 ± 0.4^{B}	5.9 ± 0.3^{CD}	1.3 ± 0.2^{BC}
	Odor	7.9 ± 0.5^{AB}	6.3 ± 0.7^{AB}	4.6 ± 0.7^{AB}	3.1 ± 0.9^{BC}	–
	Overall	7.6 ± 0.2^{AB}	7.1 ± 0.9^{AB}	5.9 ± 0.4^{AB}	4.8 ± 0.7^{CD}	2.2 ± 0.2^{A}
ZMEO 2% + GSE 1%	Taste	7.2 ± 0.2^{C}	3.6 ± 0.7^{A}	4.5 ± 0.2^{A}	4.4 ± 0.6^{B}	1.4 ± 0.6^{A}
	Texture	8.5 ± 0.2^{A}	7.6 ± 0.8^{AB}	6.8 ± 0.2^{B}	5.9 ± 0.1^{CD}	1.4 ± 0.5^{C}
	Odor	7.7 ± 0.3^{A}	5.7 ± 0.4^{A}	3.7 ± 0.5^{A}	2.1 ± 0.6^{AB}	–
	Overall	7.4 ± 0.6^{A}	6.2 ± 0.7^{A}	6.0 ± 0.6^{BC}	3.2 ± 0.7^{BC}	1.3 ± 0.7^{A}
Control	Taste	8.7 ± 0.5^{C}	5.3 ± 0.5^{BC}	3.6 ± 0.5^{A}	–	–
	Texture	8.7 ± 0.2^{A}	6.6 ± 0.2^{AB}	4.8 ± 0.3^{A}	2.7 ± 0.3^{A}	1.3 ± 0.4^{AB}
	Odor	9.0 ± 0.2^{B}	7.8 ± 0.3^{B}	5.1 ± 0.2^{BC}	–	–
	Overall	8.4 ± 0.4^{AB}	7.8 ± 0.4^{AB}	4.3 ± 0.5^{A}	1.2 ± 0.4^{A}	1.0 ± 0.6^{A}

A–D: different letters in the same column for each sensorial characteristic indicate significant differences ($P < 0.05$).
ZMEO: *Zataria multiflora* essential oil; GSE: grape seed extract.
–: The evaluation was not performed because of inappropriate organoleptic properties.

organoleptic properties of fillets showed a similar pattern of decreasing during storage time. Introducing higher concentrations of ZMEO and GSE led to decrease in taste and odor scores of coated samples compared to those of control sample during initial days of storage. Both samples contained 1% v/v ZMEO + 0.5% v/v GSE and 1% v/v ZMEO + 1% v/v GSE obtained the highest scores during sensory evaluation. It should be noted that taste and odor evaluations of some samples at 15 and 20 days of storage was impossible because of inappropriate organoleptic characteristics. During 20 days of storage, texture properties as well as overall acceptability of the samples enriched with CMC + 1% v/v ZMEO + 0.5% v/v (1% v/v) GSE were more superior. The samples were considered to be acceptable for consumption until the sensory score was above 4 (Fan et al., 2008; Jouki et al., 2014; Mexis, Chouliara, & Kontominas, 2009). The overall acceptability score of the control sample reached to unacceptable values (below 4) before the 10th day, while the overall acceptability scores of the samples coated with CMC + 1% v/v ZMEO + 0.5% v/v GSE and CMC + 1% v/v ZMEO + 1% v/v GSE remained above 4 even after the end of storage time. Ojagh et al. (2010) and Jouki et al. (2014) reported that the application of biopolymer-based antimicrobial coatings led to significant increase in overall acceptability of fish fillets. They also reported that the shelf life of control sample is 9 days during cold storage. Mexis et al. (2009) reported that essential oil addition could improve the overall quality of fish fillets and eventually prolong the shelf life of the product.

4. Conclusion

As a summary, the application of CMC-based edible coatings incorporated with ZMEO and GSE resulted in extending the shelf life of rainbow trout fillets. The results of the current study showed that microbiological and chemical characteristics of coated fillets were better than those of control samples during refrigerated storage. The higher concentrations of ZMEO and GSE resulted in stronger antimicrobial and antioxidant activities. However, the increase in the concentrations of natural preservatives led to decrease in the sensorial scores of the samples. Taking into account the appropriate sensorial attributes and also required antibacterial and antioxidant effects, CMC + 1% v/v ZMEO + 0.5% v/v GSE and CMC + 1% v/v ZMEO + 1% v/v GSE formulations can be used for extending the shelf life of fish fillets during refrigerated storage.

Acknowledgments

Faculty of Veterinary Medicine, Urmia University financially supported this work. The authors would like to appreciate Dr. Moradi for his technical assistance.

References

Ali, M. S., Saleem, M., & Ahmad, V. U. (2000). Chemistry of *Zataria multiflora*. *Phytochemistry, 55*, 933–936.

AOAC. (2002). *Official method of analysis* (14th ed.). Washington, DC: Association of Official Analytical Chemists.
AOAC. (2005). *Official method of analysis* (17th ed.). Washington, DC: Association of Official Analytical Chemists.
Arashisar, S., Hisara, O., Kayab, M., & Yanik, T. (2004). Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. *International Journal of Food Microbiology, 97*, 209–214.
Azizkhani, M., Misaghi, A., Akhondzadeh Basti, A., Gandomi, H., & Hosseini, H. (2013). Effects of *Zataria multiflora* Boiss. essential oil on growth and gene expression of enterotoxins A, C and E in *Staphylococcus aureus* ATCC 29213. *International Journal of Food Microbiology, 163*, 159–165.
Bassolé, I. H. N., & Juliani, H. R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules, 17*, 3989–4006.
Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.
Burt, S. (2004). Essential oil: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology, 94*, 223–253.
Celik, M., Gökçe, M. A., Başusta, N., Küçükgülmez, A., Tasbozan, O., & Tabakoğlu, S. S. (2007). Nutritional quality of rainbow trout (*Oncorhynchus mykiss*) caught from the Atatürk Dam Lake in Turkey. *Journal of Muscle Foods, 19*, 50–61.
Chedea, V. S., Braicu, C., & Socaciu, C. (2010). Antioxidant/prooxidant activity of a polyphenolic grape seed extract. *Food Chemistry, 121*, 132–139.
Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*, 564–582.
Dashipour, A., Razavilar, V., Hosseini, H., Shojaee-Aliabadi, S., Bruce German, J., Ghanati, K., et al. (2015). Antioxidant and antimicrobial carboxymethyl cellulose films containing *Zataria multiflora* essential oil. *International Journal of Biological Macromolecules, 72*, 606–613.
Fan, W., Chi, Y., & Zhang, S. (2008). The use of a tea polyphenol dip to extend the shelf life of silver carp (*Hypophthalmichthys molitrix*) during storage in ice. *Food Chemistry, 108*, 148–153.
Frangos, L., Pyrgotou, N., Giatrakou, V., Ntzimani, A., & Savvaidis, I. N. (2010). Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets. *Food Microbiology, 27*, 115–121.
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, 75*, 256–264.
Giatrakou, V., Ntzimani, A., Zwietering, M., & Savvaidis, I. N. (2010). Combine chitosan-thyme treatments with modified atmosphere packaging on a Greek ready to cook (RTC) poultry product. *Journal of Food Protection, 73*, 663–669.
Gimenez, B., Roncales, P., & Beltran, J. A. (2002). Modified atmosphere packaging of filleted rainbow trout. *Journal of Science and Food Agricultural, 84*, 1154–1159.
Gokoglu, N., Yerlikaya, P., & Cengiz, E. (2004). Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). *Food Chemistry, 84*, 19–22.
González-Fandos, E., Villarino-Rodríguez, A., García-Linares, M. C., García-Arias, M. T., & García-Fernández, M. C. (2005). Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. *Food Control, 16*, 77–85.
Ignea, C., Dorobantu, C. M., Mintoff, C. P., Branza-Nichita, N., Ladomery, M. R., Kefalas, P., et al. (2013). Modulation of the antioxidant/pro-oxidant balance, cytotoxicity and antiviral actions of grape seed extracts. *Food Chemistry, 141*, 3967–3976.
Jeon, Y. J., Kamil, J. Y., & Shahidi, F. (2002). Chitosan as an edible invisible film for quality preservation of herring and atlantic cod. *Journal of Agricultural and Food Chemistry, 50*, 5167–5178.
Jouki, M., Tabatabaei-Yazdi, F., Mortazavi, S. A., Koocheki, A., & Khazaei, N. (2014). Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets. *International Journal of Food Microbiology, 174*, 88–97.
Liu, Q., Han, J. C., Zhang, Y. G., Li, S. M., & Li, J. (2012). Antimicrobial and antioxidant activities of carboxymethyl cellulose edible films incorporated with rosemary extracts on fresh beef during refrigerated storage. *Advance Material Research, 554*, 1187–1194.
Mace, S., Joffraud, J. J., Cardinal, M., Malcheva, M., Cornet, J., Lallane, V., et al. (2013). Evaluation of the spoilage potential of bacteria isolated from spoiled raw salmon (*Salmo salar*) fillets stored under modified atmosphere packaging. *International Journal of Food Microbiology, 160*, 227–238.
Mexis, S. F., Chouliara, E., & Kontominas, M. G. (2009). Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4 °C. *Food Microbiology, 26*, 598–605.
Moosavy, M. H., Akhondzadeh Bastia, A., Misaghi, A., ZahraeiSalehi, T., Abbasifar, R., EbrahimzadehMousavi, H. A., et al. (2008). Effect of *Zataria multiflora* Boiss. essential oil and nisin on *Salmonella typhimurium* and *Staphylococcus aureus* in a food model system and on the bacterial cell membranes. *Food Research International, 41*, 1050–1057.
Moradi, M., Tajik, H., Razavi Rohani, S. M., & Oromiehie, A. R. (2011). Effectiveness of *Zataria multiflora* Boiss. essential oil and grape seed extract impregnated chitosan film on ready-to-eat mortadella-type sausages during refrigerated storage. *Journal of the Science of Food and Agriculture, 91*, 2850–2857.
Moradi, M., Tajik, H., RazaviRohani, S. M., Oromiehie, A. R., Malekinejad, H., Aliakbarlu, J., et al. (2012). Characterization of antioxidant chitosan film incorporated with *Zataria multiflora* Boiss. essential oil and grape seed extract. *LWT – Food Science and Emerging Technology, 46*, 477–484.
Nakamura, Y., Tsuji, S., & Tonogai, Y. (2003). Analysis of proanthocyanidins in grape seed extracts, health foods and grape seed oils. *Journal of Health Science, 49*, 45–54.
Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chemistry, 122*, 161–166.
Peng, Z., Hayasaka, Y., Iland, P. G., Sefton, M., Høj, P., & Waters, E. J. (2001). Quantitative analysis of polymeric procyanidins (tannins) from grape (*Vitis vinifera*) seeds by reverse phase high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 49*, 26–31.
Pikul, J., Lesztzynski, D. E., & Kummerow, F. A. (1989). Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *Journal of Agricultural and Food Chemistry, 37*, 1309–1313.
Sallam, K. I. (2007). Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon. *Food Control, 18*, 566–575.
Sayanjali, S., Ghanbarzadeh, B., & Ghiassifar, S. H. (2011). Evaluation of antimicrobial and physical properties of edible film based on carboxymethyl cellulose containing potassium sorbate on some mycotoxigenic *Aspergillus* species in fresh pistachios. *LWT – Food Science and Technology, 44*, 1133–1138.
Shariffar, F., Mosha, M. H., Mansouri, S. H., Khodashenas, M., & Khoshnoodi, M. (2007). In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multiflora* Boiss. *Food Control, 18*, 800–805.
Tepe, B., Donmez, E., Unlu, M., Candan, F., Daferera, D., & Vardar-Unlu, G. (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (montbretetaucher ex benth.) and *Salvia multicaulis* (vahl). *Food Chemistry, 84*, 519–525.
Togrol, J., & Arsalan, N. (2004). Carboxymethyl cellulose from sugar beet pulp cellulose as a hydrophilic polymer in coating of mandarin. *Journal of Food Engineering, 62*, 271–279.
Wei, H., Wolf, G., & Hammes, W. P. (2006). Indigenous microorganisms from iceberg lettuce with adherence and antagonist potential to use as protective culture. *Innovative Food Science and Emerging Technologies, 7*, 294–301.