Volume 8, Issue 5, 2018, 3505 - 3513 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 03.09.2018 / Revised: 10.10.2018 / Accepted: 12.10.2018 / Published on-line: 15.10.2018

Antimicrobial and antioxidant effect of nanoliposomes containing *Zataria multiflora* boiss essential oil on the rainbow trout fillets during refrigeration

Ghazaleh Bahramian [1], Leila Golestan [1], Kianoush Khosravi-Darani [2,*]

[1]Department of Food Science and Technology, Ayatollah Amoli branch, Islamic Azad University, Amol, Iran

[2]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, P. O. Box 19395-4741, Tehran, Iran

*corresponding author e-mail address: ***k.khosravi@sbmu.ac.ir, kiankh@yahoo.com***

ABSTRACT

Application of natural preservatives in the food industry has been taken into account more than in previous decay. *Zataria multiflora* Boiss essential oil (ZtEO), which has been paid attention by researchers, has a widerange of antimicrobial and antioxidant activity. But the essential oils required to be encapsulated due to the problematic usage of free from due low dispersion in the aqueous phase, sensitivity to oxidation and decreasing of sensorial properties of incorporated food. Fish and other seafoods, despite the high nutritional value, are considered as quick-corrupted food due to having chemical compounds e.g. unsaturated fatty acids, a high percentage of proteins and high pH; In this research, the impact of nanoliposomal encapsulated ZtEO was investigated on the shelf life of rainbow trout fillet. So, ZtEO, was encapsulated in liposome using the thermal method and the most suitable formulation was achieved which includes 2.5 %w/w, phosphatidylcholine, process temperature 35˚C, at time 42 min and 0.81 ratio of essential oil to phosphatidyl choline. Then fish fillets were encountered with nanoliposomes containing ZtEO in concentration 0.5, 1, 2, 4, and 8 %w/w. The best concentrations included 1, 2 and 4%, were subjected under chemical test and microbial evaluation test (total and psychrophilic counts). Finally, sensory evaluation tests were conducted in triplicate in days 0, 3, 6, 9, 12 at 4°C. The results of chemical, microbial and sensory evaluations show that in rainbow trout fillet incorporated with nanoliposomal ZtEO, the quality and the shelf life of fillet in cold storage was increased. Also results of showed that incorporation of fillet with 4% nanoliposomal ZtEo leads to more suitable condition in term of antioxidant and microbial properties as well as sensory evaluation.

Keywords: *liposome, nanocarrieres, essential oil, fish products, antimicrobial and antioxidant activities.*

1. INTRODUCTION

Food is undoubtedly one of the first and main needs of human beings and provision of healthy food is in direct relationship with the health of society. Meat products are among the fast-spoiling products and fishes are also included in this group. In spite of their high nutritional value, fishes will be spoiled fast due to their chemical compounds such as fatty acids and high protein percentage in high pH values [1].

Among the various types of fishes, rainbow trout with annual production of 126,515 tons with per capita consumption of 1.6 kg, availability (in many parts of the world including Iran) is of crucial importance. As this fish with a scientific name of *Oncorhyncus mykiss* is among cold water fishes, rainbow trout is considered among oily fishes from salmon family[2]. This fish possess bacteria resistant to cold storage and due to having poly unsaturated fatty acids (PUFA), it is highly spoilable. So any investigation to prolong its shelf life while preserving its quality is worthwhile. It has also of unsaturated omega 3 mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [3]. On the other hand, due to having a substantial amount of PUFA, fishes are highly vulnerable to oxidation-induced spoilage [4]. Lack of proper preservation techniques for seafoods and fishes will result in rapid changes in chemical, biochemical and microbiological properties of the product which will lead to the complicated phenomenon of fish spoilage [5].

Application of natural preservatives is one of the most applicable methods to preserve the foods; in this regard, herbal essential oils (also called etheric or volatile oils) can be mentioned. Plants' essential oils are a good source of natural antioxidants and can be a good choice to replace conventional synthetic ones [6]. *Zataria multiflora* is one of these plants which has been studied by numerous researchers due to its antimicrobial and anti-oxidative activities. *Zataria multiflora* is an aromatic plant from the family of Lamiaceae.

Strong odor of the essential oils is one of the main important problems for their widespread use in food products, as well as their instability under light irradiation, heat and oxygen exposure. In addition, they are poorly soluble in water and can be weakly bound to the target. In this regard, their coating and surface modification was applied to increase their efficiency and improve their release rate [7].

Recently, liposome technology has drawn a considerable attention. Application of liposomes in disease diagnosis, drug release, cosmetics, genetic engineering, food industry and anti-cancer drugs has been widely proven [8]. In the food industry, this system has been used to improve the taste and odor [9]), food enrichment by different micronutrients [10] and protection of antioxidants [11].

In recent years, liposomes have been extensively studied as carrier systems in medical [12-16] and food [17-24] purposes, but only a few works dealing with meat have been published.

To our knowledge, this the first report of the application of liposome encapsulated Essential oil to increase the shelf life of rainbow filler in cold storage. For this purpose, a green method of heating method was selected to produce liposomal ZEo to decrease the possibility of any solvent residue in liposome structures.

2. EXPERIMENTAL SECTION

Materials. The phosphatidylcholine (Across, Belgium); and tween 80, Thiobarbituric acid, glycerol, sodium carbonate, chloroform, ethanol were purchased from Merk Co, (Germany). ZtEO was purchased from (Barij, Iran); plate count agar was also purchased from Merk Co (Germany).

***Zataria multiflora* essence preparation.** Essence of *Zataria multiflora* was prepared by Barij Essence Company (Kashan, Iran) by water vapor distillation technique. During the study, the essence was kept in dark capped container away from light in the refrigerator.

Identification of chemical composition of the essence. Chemical compounds of *Zataria multiflora* was investigated by gas chromatography (GC)-mass spectrophotometry (MS). The features and thermal regulation of the device were as follows: A gas chromatograph (Agilent 7890A) attached to a mass spectrometer (Agilent 5975C) equipped with a capillary column of HP-5 with the length of 30 m and an internal diameter of 0.25 mm and film thickness of 0.25 micron was used. The oven temperature increase from 40 to 140°C by the rate of 5°C/min and maintained in that temperature for 1 h. Then it was increased to 280°C with the rate of 30°C/min and kept at that temperature for 18 min. The carrier gas was used (flow rate of 0.5 ml/min) and the ionization energy was 70 eV while the injection volume was 2 μl. The levels of compounds were determined by injecting a specific amount of their standards.

Fish specimen preparation. Rainbow trout with the weight of 500-700 g were purchased from the local market and transferred to the refrigerator in an ice container. After cutting the head of the fish and discharging its viscera, the bones were removed and after washing, 200 or 300 g fillets were prepared from each fish under septic condition. It must be noted that all the fillets were provided from the fish cross-section.

Preparation of nano-liposomal *Zataria multiflora* Boiss essential oil. Liposomal components such as phosphatidyl colin and *Zataria multiflora* essential oil (with different weight percentages) were hydrated by addition of deionized water and glycerol (3 %v/v)[25].

In the next stage, they were mixed at 35°C for 42 min at 1000 rpm. For stability of the liposomal solution, it was kept at room temperature for 1 h and then transferred to refrigerator at 4 ℃ [25-28].

Partixle measurement of liposomal *Zataria multiflora* Boiss essential oil. Mean particle size was measured by dynamic light scattering technique which is used for investigation of particles size in a liquid environment [27].

Fish fillet preparation. Each 100 g fish fillet was placed in sterilized bags and was exposed to a nanoliposome solution containing different percentages of essential oil. The samples were kept at 4°C for 12 days for further chemical, microbial and organoleptic tests.

Sensory evaluations. Sensory tests were carried out in three stages: first, finding the best concentration of nano-liposome (among 0.5, 1, 2, 4 and 8% w/w concentration) and finding tolerable levels of this essence for the consumer. The second stage involved comparing the total acceptance data of this test with that of samples exposed to a free form of essence. In the third stage, the exposed samples with acceptable levels of essence underwent sensory tests in a 12-day period to find the effect of microbial and chemical changes on sensory features of the samples.

Chemical tests

pH measurement. A 10 g sample of the fish was uniformed in 90 ml of distilled water by a glass bar. Then its pH was measured by a digital pH meter at room temperature.

Approximated compounds. Three replicates were performed for each sample. Humidity measurement was carried out by drying the fish in the oven at 105℃ until reaching to constant weight [29]. Fat measurement was done by two solutions of chloroform and methanol [30] and protein content was assessed based on nitrogen content by Kjedahl method (conversion coefficient of 6.25) [25].

Fat extraction for FFA and TBA tests.

First, the fishes were transformed to paste condition by an electric mill. Then 15 g of paste sample was weighted and added to decanter along with 60 cc methanol and completely homogenized. Then 30 ml of chloroform was added and the decanter was shaken well. After 5 min, 30 ml of chloroform was again added and the decanter was rested for 24 h until the fat was extracted. After 24 h, 36 ml distilled water was added for phase separation. The extracted oil was used for free fatty acid (FFA) and tiobarbi- turic acid (TBA) measurements[31].

FFA measurement. For fatty acid measurements, firstly, 75 ml ethanol (96%) was poured into an Erlenmeyer flask, 2-3 drops of Phenolphthalein was then added. The mixture was titrated by 0.1 N NaOH A pink color appeared. The flask was placed on the hot plate at atemperature of 60-70 ℃ to prepared ward neutral ethanol. 10 g oil was weight in Erlenmeyer Flask and then 75 ml warm neutral ethanol was added to it 2-3 drops of Phenolphthalein was then added and the mixture was titrated by 0.1N NaOH. The end of titration was the emergence of a pale pink color. Acidity was measured by oleic acid based on Eq. 1 [32].

$$\text{Acidity}=[V \times N \times (28/2)]/\text{w} \qquad \textbf{Eq. 1}$$

where V is the volume of NaOH in the second stage, N is NaOH normality; W is the amount of sample.

Measurement of thiobarbituric acid. For this purpose, first 200 mg of the extracted fish oil was transferred to a 25-ml flask and the volume was reached by addition of 1-butanol. 5 ml of this

solution was transferred to a capped tube and thiobarbituric acid was added to it. The mentioned tubes were then placed in a bath at 95 ℃ for 2 h and then cooled down to the room temperature. Their optical absorbance was read by spectrophotometer at the wavelength of 530 nm. Thiobarbituric was calculated by Eq. 2 [33].

$$TBA=\frac{(As-Ab)}{200}\times 50 \qquad \textbf{Eq. 2}$$

TVN measurement. For measurement of total volatile nitrogen, 10 g of sample, 2 g magnesium oxide, 2 drops of anti-foam and 300 ml distilled water was added to Kjeldahl balloon. The distilled vapor entered to 25-ml Erlenmeyer flask containing boric acid (2%) and 2 drops of methyl red and then titrated by sulfuric acid (0.1 N). TVB-N was calculated by [34].

Microbiological tests.

Aerobic mesophyllic bacteria counting. To count mesophyll bacteria, pour plate and Plate Count Agar (PCA) were used. The samples were placed upside-down in incubator at 37 ℃ f b or 48 h. then the colony counting was performed [35].

Counting *Psychrophillic* bacteria. Psychrophiles bacteria were cultured on PCA. Plates were placed in an incubator at 7℃ for 10 days and the numbers of colonies were then counted [35].

Statistical analysis. In this study, the effect of *Zataria multiflora* essence-containing nano-liposome on microbial and chemical properties of rainbow trout fillet was investigated at refrigerator temperature by application of a completely randomized test at different concentrations of 0, 1, 2, 4% w/w/ for 12 days by repeated measurements at 4 ℃ at probability level of ($p\leq0.05$). Each treatment was measured in three replicas and the results were investigated by one-way ANOVA method at a probability level of ($p\leq0.05$). The mean comparison was also carried out by Duncan's multiple range test ($p\leq0.05$). All the analyses were carried out by SPSS V.22 software.

3. RESULTS SECTION

GC/Ms analysis results for *Zataria multiflora* Boiss essential oil. The main composition of *Zataria multiflora* essential oil includes carvacrol and thymol. Determination of chemical composition of *Zataria multiflora* showed that phenolic compounds comprise the majority of its composition [36] which include thymol (53.2%), carvacrol (12.4%), alpha-peptin (1.2%) beta mircin (0.6%), pisimen (9.3%), gamma tripinen (4%). In the study of Zakkipour and Divband (2012)[37], GC MS analysis also reported that Iranian Zataria contains thymol and carvacrol amounts as 59.50 and 5.6%, respectively. Variation in the amounts of these compounds could be due to harvesting season, plant's age, soil, water and geography and method of drying and extraction [38,39].

Size analysis of the liposomes containing *Zataria multiflora* essential oil. The size of the produced liposome is one of the main features of particle formation because encapsulation efficiency closely tied to the size of capsules. Dynamic light scattering technique has been known as a photon-dependent spectroscopic technique. According to the applied method, the mean size of the particles was determined as 254.4±0.210 nm. There is no specific definition for describing the dimension of nano-liposomes. As the literature referred to the term 'nano-liposome' when the dimension of the particles was below 300 nm, therefore, the results of size analysis of essential oil-containing liposomes showed that the particles' size is 254.4 ±0.210 nm. Therefore, these particles could be named *Zataria multiflora* essential oil-containing nano-liposomes. Colas et al managed to produce nisin containing nano-liposome particles with the dimension of 190±5 to 284±10.6 nm [27].

Determining tolerable concentration of liposomal *Zataria multiflora* essential oil in fish fillet. Table 1. shows the sensory evaluation of rainbow fish containing a different concentration of liposomal ZtEO, then the other research about antioxidant activity should be conducted on the result of this table. Variance analysis of data obtained from sensory evaluation showed that the best concentration of *Zataria multiflora* essential oil capsulated in nano-liposomes on the taste of fish was about 1 and 2%. These treatments had the highest taste score and the 8% treatment had the lowest taste score.

Results of the organoleptic evaluation of best *Zataria multiflora* essence-containing nano-liposomes concentration on odor score showed that 1 and 2% treatments had no significant difference with each other. 1 and 2% treatments had the highest odor score and the 8% treatment had the lowest odor score. Results of the organoleptic evaluation of best *Zataria multiflora* essence-containing nano-liposomes concentration on overall acceptance of fish score showed that 1, 2 and 4% treatments had no significant difference with each other. These three mentioned treatments showed the highest and the 8% treatment had the lowest overall acceptance scores.

Table 1. Finding the best concentration of *Zataria multiflora* Boiss encapsulated in nanoliposome in term of sensory properties of fish ($p\leq0.05$)[§]

test / oil (%w/w)	taste	odor	color	Overall acceptance
0.5	2.9±0.31^{c}	2.7 ± 0.67^{c}	2.6 ± 0.69^{c}	**2.9 ± 0.32** ^{b}
1	4.8±0.14^{a}	4.7 ± 0.67^{a}	4.8 ±0.42^{a}	**4.9 ±0.32** ^{a}
2	4.8±0.14^{a}	4.8 ± 0.42^{a}	4.7 ±0.67^{a}	**5.00 ±0.00**^{a}
4	3.9±0.32^{b}	3.8 ±0.42^{b}	4.8 ±0.42^{a}	**5.00 ±0.00**^{a}
8	1.7±0.48^{d}	1.8 ± 0.42^{d}	3.7 ± 0.67^{b}	**1.80 ±0.42** ^{c}

[§]The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p\leq0.05$). The numbers (mean ± standard deviation) with the small letters in each row have not statistically significant differences ($p\leq0.05$).

pH. Most of the types of fish have small amounts of carbohydrates in their muscular tissue; in a way that after the death of the organism, the amount of produced lactic acid as the result of glycolysis will be decreased. Consequently, pH of fish will increase beyond 6 after rigor mortis. The reason for low initial pH could be due to the improvement of lactic acid after glycolysis reaction. This is one of the specific and important features of fish [40]. In most of the species, hemoglobin acts as a per-oxidant in pH range of 6-7 and therefore, oxidation intensity will be decreased in pH values above 7. pH range of 6.8 to 7 has been

recognized as an acceptable limit. Although pH index alone is not suitable for evaluation of fish quality [40,41] , but it can be effective beside other indices. Initial pH of the fish after their rigor mortis varies from 5.4 to 7.2, depending on their species [42].

pH variation of *Oncorhynchus mykiss* fillet in this study (Table 2) showed that it was 6.23, 6.26, 6.26 and 6.26 in the day 0 for control, 1%, 2% and 4% treatments, respectively. At the end of the period, these values reached to 6.20, 6.23, 6.20 and 6.20 for the same order; which had no statistically significant difference. Only 1% treatment had a significant difference in days 0 and 6 which showed the minimum and maximum pH values, respectively. All the treatments were in acceptable range till day 12. The results of this study are in agreement with the results of Zakkipour and Divband and also Angiz and Uzhqan who investigated the effect of *Zataria multiflora* essential oil on chemical and microbiological properties of fresh *Oncorhynchus mykiss* during refrigerator storage chain (18 days at 4℃)[37,43].

Proximate Analysis. Approximated composition in 100 g fresh *Oncorhynchus mykiss* included 77.6 ± 0.057 humidity, 19.10 ±0.100 protein and 8.26 ±0.030 fat.

Free fatty acids. Fish fat is a major source of unsaturated fatty acids with several double bonds of omega 3, mainly DHA and EPA [3]. Amount of free fatty acids is an index for fat spoilage. Formation of FFA will not reduce the nutrition value of food products. However, its evaluation is important in thw investigation of fish spoilage [44]

Glycerides, glycolipids and phospholipids could be hydrolyzed by lipase enzyme and be converted to free fatty acids. Fats will be transformed into aldehydes and ketones in continue of oxidation process whose production will result in undesirable taste in fish [45].Allowed limit of FFA has been defined as 5% [46].FFA production has a direct negative impact on the taste of fish. It was also mentioned that FFA production can intensify fat oxidation in fish [47].

Results of this study about the impact of *Zataria multiflora* essence-containing nano-liposomes on the amount of free fatty acids in *Oncorhynchus mykiss* fillet at refrigerator temperature for 12 days (Table 2) showed that passing of time will result in increasing trend of FFA amount. This increase was more profound in the control sample. Treatments exhibited significant changes from day 3. FFA content of all treatments remained in allowed level. But the lowest value was observed in 4% treatment. At the end of the period, control and 4% treatment samples had maximum and minimum FFA content, respectively. Formation of FFA during a limited period of storage is due to fats catalysis by internal enzymes, mainly lipase and phosphatase [44].

Erkan et al (2010) investigated the effect of *Zataria multiflora* and *Laurus nobilis* essential oils on the durability of bluefish stored in ice for 13 days. They realized that the level of FFA increased by time which was more profound in control sample [48]. These findings are in accordance with our results.

Shabanpoor et al (2012) expressed that the level of fat hydrolysis and formation of FFA were lower in thyme extract treatment in comparison with the control group. Higher concentration of extract resulted in more inhibition of FFA formation [49]. However, a regular trend couldn't be established for this index; but generally, FFA content increased during storage which was in agreement with the presented results.

Determination of Thiobarbituric acid . Lipid oxidation is one of the main factors of undesirable taste of products [50].Fat oxidation can be evaluated by their malondialdehyde content (MDA) [51]. For evaluation of lipid oxidation in fishes, thiobarbituric acid has been widely employed which has been recognized as the indicator of lipid secondary oxidation and is due to the presence of thiobarbituric-reactive materials due to the second stage of auto-oxidation. During this process, peroxides will be converted to aldehydes and ketones [52]. Moreover, >2 mg Malondialdehyde indicates a drop in quality [44].

Results of this study about the effect of *Zataria multiflora* essential oil -containing nano-liposomes on thiobarbituric acid content of *Oncorhynchus mykiss* fillet (Table 2) showed that during 12 days, this index increased in a way that control and 4% treatment samples had maximum and minimum levels of thiobarbituric acid, respectively. Overall, TBA content was less than the allowed limit during the storage period. Increase f TBA could be due to lipid oxidation and production of volatile metabolites at the presence of oxygen [53]. Results of this study showed that *Zataria multiflora* essential oil -containing nano-liposomes have anti-oxidant properties.

Comparison of TBA index of samples indicated that after passing of 12 days, control sample had the highest TBA level and experienced more oxidation and more aldehydes were created in it. Increasing trend of this index is due to enhancement of peroxides in muscles and also the production of aldehyde from secondary products of hydroperoxides [54].TBA increased in other samples, but the increasing trend of *Zataria multiflora* essential-containing treatment was lower and the higher the concentration of essential oil, the lower the TBA increase. Reduction of TBA could be attributed to the reduction of hydroperoxides and reaction between Malondialdehyde and proteins, amino acids or glycogens [45,55]. Shabanpoor et al (2012) studied the effect of *Zataria multiflora* extract on the durability of *Oncorhynchus mykiss* in the refrigerator. They showed that TBA content increased during the storage period. But the level of this index was significantly lower in *Zataria multiflora*-treated samples ($p \leq 0.05$) [49]. The results of the mentioned study are in agreement with our results.

Angis and Oguzhan (2013) investigated the effect of *Zataria multiflora* in packaging on chemical and microbiological properties of fresh *Oncorhynchus mykiss* during refrigerator storage [43]. They showed that TBA content increased gradually which agree with our results.

Determination of Total volatile basic nitrogen. Total volatile basic nitrogen (TVB-N) is mainly trimethylamine, dimethyl amine, ammonia and other volatile nirogenic compounds which are related to seafood spoilage, which is produced by spoilage bacteria, autolytic enzymes, amino acids deamination and nucleotides, respectively. They are one of the indices of meat proteins destruction and degradation [56]. However, some researchers have mentioned that this index can't be regarded as the only index for fish evaluation[57]. TVB-N content is dependent on bacterial content and therefore bacterial destruction. Fish degradation is a progressive proteolytic process which is mainly conducted by microorganisms and at lower levels by autolytic

enzymes. According to the reports, mgTVB-N/25 is the highest acceptable level for farmed fishes including *Oncorhynchus mykiss* [45]. TVB-N content of fish fillet not only differs between different species but it could be also different between different ages, gender, in different seasons and environments [58].

Results of this study (Table 2) indicated that initial TVB-N level at day 0 was 14.36 mg/100g for control sample; while it increased over time in all concentrations of *Zataria multiflora* essence. However, an increase of *Zataria multiflora* essential oil concentration resulted in a slower increase of TVB-N. This index exceeded the allowed limit in all samples at day 12 except for the sample with 4% *Zataria multiflora* essential oil. After 12 days, control sample and the one treated with 4% *Zataria multiflora* essential oil had the maximum and minimum TVB-N contents, respectively. This could be due to the inhibitory impact of *Zataria multiflora* essential oil on bacterial growth as one of the main underlying reasons for TVB-N formation. During all days, except day 12, 2% and 4% treatments had no significant difference with each other.

During all days, the control and 4% treatment samples had the highest and lowest TVB-N, respectively. Therefore, bypassing of them, TVB-N content increased. The increase of TVB-N is related to spoilage bacteria and Endogenous enzymes [59].

Results of this study are in accordance with that of Shabanpoor et al (2012) on the effect of *Zataria multiflora* on the durability of salty and packed *Oncorhynchus mykiss* fillet in vacuum and refrigerator. In all the studied samples, TVB-N contents of *Zataria multiflora*-treated samples were significantly less than control ($p \leq 0.05$) and TBV-N formation had an increasing trend in all the samples[49]. Control sample and treated samples were usable for 12-15 and 18-20 days, respectively. TVB-N factors exist in meats enzymes and microorganisms [60].

Table 2. Comparing the chemical analyses in *Zataria multiflora* Boiss sample during refrigeration temperature storage for 12 days ($p \leq 0.05$)*

Storages (days)	0	3	6	9	12
Ph					
Control	6.23 ± 0.057^{aA}	6.26 ± 0.057^{aA}	6.16 ± 0.057^{aA}	6.23 ± 0.057^{aA}	6.20 ± 0.10^{aA}
1%	6.26 ± 0.057^{aA}	6.23 ± 0.057^{aA}	6.13 ± 0.057^{bA}	6.23 ± 0.057^{aA}	6.23 ± 0.057^{aA}
2%	6.26 ± 0.057^{aA}	6.23 ± 0.057^{aA}	6.23 ± 0.057^{aA}	6.20 ± 0.00^{aA}	6.20 ± 0.00^{aA}
4%	6.26 ± 0.057^{aA}	6.20 ± 0.10^{aA}	6.23 ± 0.057^{aA}	6.23 ± 0.057^{aA}	6.20 ± 0.10^{aA}
TVB-N§					
Control	14.36 ± 0.057^{eA}	14.46 ± 0.057^{dA}	16.70 ± 0.10^{cA}	19.10 ± 0.100^{bA}	35.10 ± 0.100^{aA}
1%	12.06 ± 0.057^{dB}	12.76 ± 1.069^{dB}	15.067 ± 0.06^{cB}	18.13 ± 0.057^{bB}	33.10 ± 0.100^{aB}
2%	10.76 ± 0.057^{dC}	10.86 ± 0.057^{dC}	14.23 ± 0.06^{cC}	16.23 ± 0.057^{bC}	30.16 ± 0.057^{aC}
4%	10.73 ± 0.057^{dC}	10.76 ± 0.057^{dC}	14.23 ± 0.15^{cC}	15.06 ± 0.152^{bC}	24.13 ± 0.057^{aD}
TBA¶					
Control	0.11 ± 0.001^{eA}	0.12 ± 0.001^{dA}	0.13 ± 0.001^{cA}	0.17 ± 0.002^{bA}	0.2 ± 0.001^{aA}
1%	0.10 ± 0.005^{dA}	0.11 ± 0.001^{cA}	0.12 ± 0.002^{cB}	0.14 ± 0.002^{bB}	0.15 ± 0.001^{aB}
2%	0.10 ± 0.005^{dA}	0.11 ± 0.002^{cB}	0.11 ± 0.001^{cC}	0.12 ± 0.002^{bC}	0.13 ± 0.003^{aC}
4%	0.10 ± 0.001^{dA}	0.11 ± 0.001^{cB}	0.11 ± 0.001^{cD}	0.11 ± 0.001^{bD}	0.12 ± 0.003^{aD}
FFA¶¶					
Control	0.61 ± 0.010^{eA}	0.80 ± 0.006^{dA}	1.31 ± 0.010^{cA}	1.71 ± 0.010^{bA}	2.16 ± 0.057^{aA}
1%	0.60 ± 0.006^{dA}	0.60 ± 0.006^{dB}	0.71 ± 0.015^{cB}	0.90 ± 0.005^{bB}	1.40 ± 0.100^{aB}
2%	0.60 ± 0.005^{dA}	0.59 ± 0.015^{dB}	0.70 ± 0.005^{cB}	0.81 ± 0.010^{bC}	1.06 ± 0.057^{aC}
4%	0.60 ± 0.000^{dA}	0.60 ± 0.010^{dB}	0.62 ± 0.005^{cC}	0.64 ± 0.011^{bD}	0.81 ± 0.010^{aD}

*The numbers (mean ± standard deviation) with the small letters in each row have not statistically significant differences ($p \leq 0.05$). The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p \leq 0.05$).

§Total volatile basic nitrogen, ¶ tiobarbituric acid, ¶¶ free fatty acid.

Table 3. Comparing the mean logarithm of the number of mesophilic bacteria in *Zataria multiflora* Boiss sample during refrigeration temperature storage for 12 days ($p \leq 0.05$)*

Sample / days	Control	1% w/w	2% w/w	4% w/w
0	4.47 ± 0.013^{eA}	$4.44\pm0.014_{eB}$	4.43 ± 0.010^{eC}	**4.41 ± 0.011^{eD}**
3	6.00 ± 0.132^{dA}	$4.69\pm0.056_{dB}$	4.60 ± 0.012^{dC}	**4.47 ± 0.018^{dD}**
6	8.47 ± 0.102^{cA}	$7.53\pm0.101_{cB}$	6.47 ± 0.302^{cC}	**5.3 ± 0.132^{cD}**
9	$13.91\pm0.132^{b}_{A}$	$12.90\pm0.021^{b}_{B}$	$10.85\pm0.012^{b}_{C}$	**8.6 ± 0.124^{bD}**
12	16.85 ± 0.235^{aA}	15.32 ± 0.015^{aB}	12.85 ± 0.245^{aC}	**$10.75\pm0.147^{a}_{D}$**

*The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p \leq 0.05$). The numbers (mean ± standard deviation) with the small letters in each row have not statistically significant differences ($p \leq 0.05$).

Table 4. Comparing the mean logarithm of the number of psychrotrophic bacteria in *Zataria multiflora* Boiss sample during refrigeration temperature storage for 12 days ($p \leq 0.05$)*

Sample / days	Control	1	2	4%
0	4.14 ± 0.186^{eA}	3.70 ± 0.014^{eB}	3.70 ± 0.010^{eB}	**3.69 ± 0.017^{eC}**
3	5.76 ± 0.203^{dA}	3.95 ± 0.046^{dB}	3.92 ± 0.013^{dC}	**3.90 ± 0.012^{dD}**
6	8.30 ± 0.165^{cA}	8.25 ± 0.023^{cB}	7.00 ± 0.042^{cC}	**6.98 ± 0.082^{cD}**
9	$15.34\pm0.152^{b}_{A}$	$14.32\pm0.108^{a}_{B}$	$14.14\pm0.013^{b}_{C}$	**$13.04\pm0.235^{b}_{D}$**
12	18.75 ± 0.298^{aA}	$17.98\pm0.138^{a}_{B}$	17.01 ± 0.172^{aC}	**16.45 ± 0.152^{aD}**

*The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p \leq 0.05$). The numbers (mean ± standard deviation) with the capital letters in each row have not statistically significant differences ($p \leq 0.05$).

According to the study of Angis and Uguzhan (2013) which addressed the effect of thyme essence and packing on chemical and microbiological properties of *Oncorhynchus mykiss* fillet during 18 days of storage in the refrigerator, initial content of TVB-N was reported 16.43 mf/100 g [43]. The results are in accordance with our data.

Erkan et al (2010) also investigated the effect of *Zataria multiflora* and *Laurus nobilis* essences on the durability of bluefish stored in ice for 13 days. The TVB-N content of the control sample exceeded acceptable range in 9 days and for the *Zataria multiflora*-treated samples; this index exceeded the acceptable range in 13 days[48]. The results of this study are also in agreement with ours. Comparing the mean logarithm of the number of chemical analyses in *Zataria multiflora* Boiss sample during refrigeration temperature storage for 12 days ($p \leq 0.05$)/

Microbiological analysis.

Mesophilic aerobic bacteria. The total mesophil aerobic bacteria count has been regarded as the index for determination of quality and durability of food products. The allowed rage of the total count of microorganisms has been determined as 10^7 cfu/ml for fresh and frozen fish by ICMSF (1978). According to the results of the present study about the effect of Zataria multiflora-containing nanoliposome on log of the mesophyll aerobic bacteria count in *Oncorhynchus mykiss* at refrigerator temperature during 12 days (Table 3) show that the total bacterial load was less 4.44 at the beginning of the period for all the samples indicating the suitable quality of the fish. Total bacterial load of the samples increased by the passing of time, however, this increase was more profound in the control sample in comparison with others. During the period, the control sample had higher levels in a way that the control sample and the one treated with 4% extract showed the highest and lowest bacterial count logarithm values, respectively. On general, control and 1% samples exceeded the allowed range in the 6th day and the samples treated with 2% and 4% extract remained in the allowed range. On the 9th day, all the samples exceeded the allowed range. The increase in the total bacterial load depends on numerous factors including storage method and the extent of compliance with hygiene principles during processing stage and the level of initial microbial load [53]. Lower total bacterial load due to antimicrobial effect of *Zataria multiflora* could be attributed to the presence of thymol and carvacrol. These two compounds are structurally similar and have hydroxyl groups in different positions of their phenolic ring [61].Thymol and carvacrol destroy the external membrane of the microorganisms and result in the discharge of liposaccharides and therefore increase of permeability of the cytoplasmic membrane to ATP. ATP discharge will then lead to the termination of cell energy storage and therefore cell death [61,62] Results of this study are in line with those of Kykkidou et al. (2009) which investigated the effect of thyme and packaging on the duration of Mediterranean Swordfish where the total bacterial load of fish fillet exceeded the allowed range in 6 days at 4℃ [63].

Results of the present study are in agreement with the work of Kostaki et al. (2009) who studied total bacterial load of seabass fish fillet during 7 days of storage at 4℃ which showed that the total load exceeded the allowed range in 7 days [51].

Comparing the mean logarithm of the number of mesophilic bacteria in *Zataria multiflora* Boiss sample during refrigeration temperature storage for 12 days ($p \leq 0.05$).

Psychrotrophic bacteria. Psychrotrophic bacteria are more effective in food spoilage. They change the taste and odor of the food by the production of ketones and aldehydes [64].Psychrotrophic bacteria, like Pseudomonas species, produce lipase and phospholipase enzymes which will increase FFA [63].The allowed range for aerobic psychrotrophic bacteria has been reported as 10^6 cfu/ml [65].

This study on the effect of Zataria multiflora-containing nanoliposome on the log of the psychrotrophic bacteria counts in *Oncorhynchus mykiss* fillet stored at refrigerator temperature for 12 days (Table 4) show that the number of bacteria increased by time and this increase was significant for all treatments during the storage period. At the end of the period, control and 4% sample has the highest and lowest log of bacteria numbers, respectively. This increase was more profound in the control sample. The highest value was for the control sample at the end of the period which was 18.75 (exceeded the allowed range) the lowest value was for 4% sample and equal to 3.69. In the 6th day, all the samples exceeded the allowed range. Low bacterial load of psychrotrophic bacteria is attributed to thymol and carvacrol compounds in thyme. Erkan et al (2010) also investigated the effect of *Zataria multiflora* and Laurus nobilis on the durability of bluefish in 13 days of storage in ice. They found that level of psychrotrophic bacteria remained in the allowed range during these 13 days (storage at 2℃) for *Zataria multiflora* incorporated samples[48].

Organoleptic evaluation.

Odor evaluation in raw fish. One of the fast and simple methods for evaluation of fish quality and freshness is an organoleptic test [5]. The results of the organoleptic evaluation are summarized in Table 5 to 9. The results on the effect of *Zataria multiflora* essence-containing nano-liposomes on odor score of *Oncorhynchus mykiss* fillet (Table 5) indicated that in the control sample, the odor score had a decreasing trend by passing of time. While for 1 and 2% treatments, a decrease of odor score occurred after 6 days. This decrease happened from day 9 for 4% treatment samples. This could be due to the formation of volatile materials from lipid oxidation such as aldehydes and ketones and degradation of proteins such as ammonia.

Organoleptic evaluation of taste, odor, color and overall acceptance in cooked fish. The results of an organoleptic study on the effect of *Zataria multiflora* essential oil-containing nano-liposomes on taste, odor, color and overall acceptance score of cooked *Oncorhynchus mykiss* fillet indicated during 12 days of storage in refrigerator indicated that 4% treatment managed to maintain acceptable taste score till day 9. Other samples had the acceptable state only until day 6.

Odor score of *Oncorhynchus mykiss* was acceptable only in 2% and 4% treatments up to day 9. By passing of time, odor score decreased in all the treatments in a way that they became unusable in day 12. By passing of time, a color score of *Oncorhynchus mykiss* decreased in all the samples as an increase of storage duration will result in pH increase and their water capacity will be enhanced, therefore they lose less water; hence the fish will be paler.

In terms of overall acceptance, control and treated samples had an equal score which decreased by passing of time. The studied samples had the highest and lowest overall acceptance in day 0 and12, respectively. In 9th day, 4% treatment had the highest

overall acceptance score among the samples. The variation trend of organoleptic features was in line with oxidation and bacterial spoilage changes in the studied samples as lipid oxidation will result in loss of organoleptic quality and a decrease of necessary unsaturated fatty acids and production of oxidation toxic products [66]. The decrease in protein destruction will lead to a reduction of TVB-N production and also decrease of samples oxidation and therefore improvement of odor score. Generally, organoleptic studies showed that application of essence up to the level of 4% not only has no undesirable impact on product's taste, but it will improve its taste, odor and overall acceptance.

Table 5. Odor organoleptic evaluation in *Zataria multiflora* Boiss in raw fish samples during refrigeration temperature storage for 12 days ($p\leq0.05$)*

sample / days	Control	1%	2%	4%
0	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}
3	4.1 ± 0.316^{bB}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}
6	1.8 ± 0.421^{cC}	3.9 ± 0.316^{bB}	3.9 ± 0.316^{bB}	4.9 ± 0.316^{aA}
9	1.00 ± 0.000^{dB}	1.9 ± 0.316^{cA}	2.00 ± 0.000^{cA}	2.8 ± 0.632^{bA}
12	1.00 ± 0.000^{dA}	1.00 ± 0.000^{dA}	1.00 ± 0.000^{dA}	1.00 ± 0.000^{cA}

*The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p\leq0.05$). The numbers (mean ± standard deviation) with the capital letters in each row have not statistically significant differences ($p\leq0.05$).

Table 6. Taste organoleptic evaluation in *Zataria multiflora* Boiss samples during refrigeration temperature storage for 12 days ($p\leq0.05$) *

Sample / days	Control	1%	2%	4%
0	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	**5.00 ± 0.000^{aA}**
6	1.8 ± 0.421^{bB}	$2.6\pm0.699^{b\,B}$	3.9 ± 0.316^{bB}	**4.00 ± 0.000^{bA}**
9	1.00 ± 0.000^{cC}	1.9 ± 0.316^{cB}	2.00 ± 0.000^{cB}	**2.9 ± 0.316^{cA}**
12	-	-	-	-

*The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p\leq0.05$). The numbers (mean ± standard deviation) with the capital letters in each row have not statistically significant differences ($p\leq0.05$).

Table 7. Odor organoleptic evaluation in *Zataria multiflora* Boiss samples during refrigeration temperature storage for 12 days ($p\leq0.05$)*

Sample / days	Control	1%	2%	4%
0	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}
6	3.9 ± 0.316^{bB}	4.00 ± 0.000^{bB}	4.9 ± 0.316^{aA}	5.00 ± 0.000^{aA}
9	1.9 ± 0.316^{cB}	2.00 ± 0.000^{cB}	3.00 ± 0.000^{bA}	2.9 ± 0.316^{aB}
12	1.00 ± 0.000^{eA}	1.00 ± 0.000^{eA}	1.00 ± 0.000^{cA}	1.00 ± 0.000^{cA}

*The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p\leq0.05$). The numbers (mean ± standard deviation) with the capital letters in each row have not statistically significant differences ($p\leq0.05$).

Table 8. Color organoleptic evaluation in *Zataria multiflora* Boiss samples during refrigeration temperature storage for 12 days ($p\leq0.05$)*

Sample / days	Control	1%	2%	4%
0	5.00 ± 000.0^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}
6	4.2 ± 421.0^{bB}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}
9	00.3 ± 000.0^{cB}	3.9 ± 0.316^{bA}	4.00 ± 0.000^{bA}	4.00 ± 0.000^{bA}
12	$1.8\pm421.0^{\ dA}$	1.9 ± 0.316^{dA}	2.00 ± 0.000^{dA}	2.00 ± 0.000^{dA}

*The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p\leq0.05$). The numbers (mean ± standard deviation) with the capital letters in each row have not statistically significant differences ($p\leq0.05$).

Table 9. Overall acceptance organoleptic evaluation in *Zataria multiflora* samples during refrigeration temperature storage for 12 days ($p\leq0.05$)*

Sample / days	Control	1%	2%	4%
0	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	5.00 ± 0.000^{aA}	**5.00 ± 0.000^{aA}**
6	2.9 ± 0.316^{bB}	2.8 ± 0.632^{bB}	3.9 ± 0.316^{bA}	**4.00 ± 0.000^{bA}**
9	1.9 ± 0.316^{cB}	2.00 ± 0.000^{cB}	2.00 ± 0.000^{cB}	**3.00 ± 0.000^{cA}**
12	-	-	-	-

*The numbers (mean ± standard deviation) with the small letters in each column have not statistically significant differences ($p\leq0.05$). The numbers (mean ± standard deviation) with the capital letters in each row have not statistically significant differences ($p\leq0.05$).

4. CONCLUSIONS

In spite of their high nutrition values fishes are among fast-spoiling foods due to having chemical compounds such as fatty acids with several double bonds, high protein content and pH. Bacterial and oxidative spoilage are two of the most important spoilage processes. Fish spoilage will cause undesirable changes in fish taste and odor and reduce its nutrition value. One of the methods to resolve this problem is to use biologic preservatives which in addition to having no side effects, will also improve taste, odor and quality of food and prolong its storage time. The present study showed that owing to its phenolic compounds such as thymol and carvacrol, *Zataria multiflora*-containing nano-liposomes will increase antimicrobial and anti-oxidative properties of *Oncorhynchus mykiss* fillet and improve the product taste. Results of chemical and microbial tests along with humidity, fat and protein assessment of nano-liposome-treated *Oncorhynchus mykiss* fillet showed that addition of *Zataria multiflora*-containing nano-liposomes will increase the quality and storage time of fillets during their cold storage. In this regard, a significant difference was observed in most of the parameters between the control sample and the one treated with 4% essential oil. Finally, as 4% treatment managed to enhance the fillet storage time chemically up to 12 days and up to 6 days in terms of microbial considerations, it could be concluded that this concentration can be applied for prolonging the storage life of the fillet. However, the practical application of this plant needs further investigations.

5. REFERENCES

[1] Rezaei M., Montazeri N., Langrudi H E., Mokhayer B,Parviz M., Nazarinia A., The biogenic amines and bacterial changes of farmed rainbow trout (Oncorhynchus mykiss) stored in ice, *Journal Food Chemistry*,103,1,150-154,**2007.**

[2] Billard R., Bry C., Gillet C., Stress,environment and reproduction in teleost fish, A.P.Pickering,ed), *N.Y.stress and fish*, 185-208, **1981.**

[3] Linccl., chung- saint., Enhancement of the storage quality of frozen bonito filled by glazing with tea, *extract food control*, 16, 169-175, **2005.**

[4] Vicetti R.,Ishitani T., Salas A., Ayala M., Use alfa – tochopherol combined with synergists and compared to other antioxidants on the oxidative stability of sardine skine lipids. *Journal of food composition and analysis*, 18, 2-3,131-137, **2005.**

[5] Sallam K H I., Ahmad A M., Elagazar M M., Eldaly E A., Chemical quality and sensory attributes of marinated pacific saury (Cololabis saira) during vacuum-packaged storage at 4°C, *Food chemistry*, 102,4,1061-1070, **2007.**

[6] Tmaino, A., Cimino, F., Zimbalatti V .,Venuti, V., Sulfaro, V., De Pasquale A., Saija,A., Influence of heating on antioxidant activity and the chemical composition of some spice essential oils, *Food Chemistry*, 89,4, 549-554, **2005.**

[7] Sherry M., Charcosset C., Fessi H., Greige-Gerges H., Essential oils encapsulated in liposomes, a review. *Journal of Liposome Research*, 23, 4, 268-275, **2013.**

[8] Khosravi-Darani K., Crit, Rev. *Food Science and Nurt,* 50,6,479,**2010**

[9] De Roos KB., Effect of texture and microstructure on flavour retention and release, *International Dairy Journal*, 13, 8, 593-605, **2003**

[10] Keller BC., Liposomes in nutrition. *Trends in Food Science & Technology*,12,1,25-31, **2001.**

[11] Mozafari M., Flanagan J., Matia-Merino L., Awati A., Omri A.,Suntres Z.,Singah H.,Recent trends in the lipid-based nanoencapsulotion of antioxidants and their role in foods, *Journal of the science of food and agriculture,* 86, 13, 2038-2045, **2006.**

[12] Khosravi-Darani K and Mozafari M R., Nanoliposome Potentials in Nanotherapy: a Concise Overview, *International Journal of Nanotechnology and Nanoscience*, 6, 1, 3-13, **2010.**

[13]Khosravi-Darani K., Mozafari M R., Rashidi L, Calcium Based Nonviral Gene Delivery: *An Overview of Methodology and Applications, Acta Medica Iranica, Acta Medica Iranica*, 48, 3,133-141, **2010.**

[14] Khosravi-Darani K., Pardakhty A., Honarpisheh H., Rao V S., Mozafari M R., The role of high-resolution imaging in the evaluation of nanosystems for bioactive encapsulation and *targeted nanotherapy, Micron*, 38, 8, 804-818, **2007.**

[15] Mortazavi, S.M., Mohammadabadi, M.R., Khosravi-Darani, K., Mozafari, M.R. Preparation of liposomal gene therapy vectors by a scalable method without using volatile solvents or detergents , *Journal of Biotechnology*, 129, 604-613, **2007.**

[16] Mozafari M.R., Khosravi-Darani K., An overview of liposome-derived nanocarrier technologies, Nanomaterials and Nanosystems for Biomedical Applications (Ed. Mozafari, M.R.), *Springer*, 113-123, **2007.**

[17] Vafabakhsh Z., Khosravi-Darani K., Khajeh KH., Mortazavian S A M., Jahadi M., Komeili R., Stability and catalytic kinetics of protease loaded liposomes, *Biochemical Engineering Journal*, 72, 11-17, **2013.**

[18] Mozafari M.R., Khosravi-Darani K, G. Gokce Borazan, J. Cui, A. Pardakhty., and S. Yurdugul, Encapsulation of food ingredients using nanoliposome technology, *International Journal of Food Properties*, 11 833–844, **2008.**

[19] Jahadi, M., Khosravi-Darani, K., Ehsani, M.R., Mozafari M R., Saboury AA., Seydahmadian, F., Vafabakhsh, Z, Evaluating the effects of process variables on protease-loaded nano-liposome production by Plackett-Burman design for utilizing in cheese ripening acceleration, *Asian Journal of Chemistry*, 24, 9, 3891-3894, **2012.**

[20] Jahadi M.,Khosravi-Darani K., Ehsani M R.,Saboury A A.,Sadat pourhosseini P.,The encapsulation of flavourzyme in nanoliposome by heating method, *Journal of Food Science and Technology*, 52, 4, 2063-2072, **2015.**

[21] Jahadi M., Khosravi-Darani K., Ehsani M R., Mozafari M R., SabouryA A., Zoghi A., Mohammadi M., Modeling of proteolysis in Iranian brined cheese using proteinase-loaded nanoliposome, International Journal of Dairy Technology, *International Journal of Dairy Technology*, 69, 1, 57-62, **2016.**

[22] Ebrahimi Khoosfi M., Khosravi-Darani K., Hosseini H., Arabi Sh., Komeili R., Koohi Kamali P., Production of Zataria multiflora Boiss Essential Oil Nanoliposomes by Response Surface Methodology, *Nanoscale*, 1, 1, 15-18, **2014.**

[23] Khanniri E., Bagheripoor Fallah N., Sohrabvandi S., Mortazavian, A. M., Khosravi-Darani K., Mohammad R., Application of Liposomes in Some Dairy Products, *Critical Reviews in Food Science and Nutrition* 0,1–10, **2016.**

[24] Mohammadi R., Mahmoudzade M., Atefi M., Khosravi-Darani K., Mozafari M.R., Applications of Nanoliposomes In Cheese Technology, *International Journal of Dairy Technology*, 68, 1, 11-23, **2015.**

[25] Khosravi darani K., Ebrahimi khoosfi M., Hosseini H., Encapsulation of Zataria multiflora boiss. Essential oil in liposome: Antibacterial activity against E.Coli O157:H7 in broth media and minced beef, *Journal of Food Safety*,36, 4, 515-523, **2016.**

[26] Mozafari M.R., and Mortazavi S M.., Nanoliposomes from fundamentals to recent developments, *Trafford Publishing*, UK, **2005.**

[27] Colas JC., Shi W., Rao V., Omri A., Mozafari MR., Singh H., Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting, *Micron*, 38, 8 ,841-847, **2007.**

[28] Rasti B., Jinap S., Mozafari MR., Yazid A., Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods, *Food Chemistry*, 135, 4, 2761-2770, **2012.**

[29] Association of Official Analytical Chemists (AOAC). Moisture in Meat and poultry product Method 985.14. Official Methods of Analysis (17th edn). Washington, DC*: Association of Official Analytical Chemists***, 2002.**

[30] Bligh E.G., Dyer W.J., A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 8, 911-917, **1959.**

[31] Folch J., Lees M., Sloanes – Stanley GH., A Simple method for the isolation and purification of total lipids from animal tissues, *Biological Chemistry*, 226, 1, 497-509, **1957.**

[32] Egan H., Kirk R.S., Sawyer R., Pearson's chemical Analysis of Foods. 9th edition. Churchill Livingtone, *Edingburgh, Scotland,* UK. 609-643, **1997.**

[33] AOCS cd., 19-90 – Animal and vegetable fats and oils-Determination 2-Thiobarbituric Acid Value Direct Method

[34] Jean Y J.,Kamali J Y., Shahidi F., Chitosan of an Edible Invisible film for Quality preservation of Herring and Atlantic Cod. *Agricultural and food chemistry*, 50, 18, 5167-5178, **2002.**

[35] Ojagh SM., Rezaei M., Razavi SH., Hosseini SMH., Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout, *Food Chemistry*, 120, 1, 193-198, **2010.**

[36] Shelef L A., Antimicrobial effects of spices, *Journal of Food Safety*, 6, 1, 29–44, **1984.**

[37] Zakipour Rahimabadi, E. and Divband, M., The Effects of Coating and Zataria multiflora Boiss Essential Oil on Chemical Attributes of Silver Carp Fillet Stored at 4ºC, International Food Research Journal ,19, 2, 685-690, **2012.**

[38] Sharififar F., Moshafi M.H., Mansouri S.H., Khodashenas M ., Khoshnoodi, M.,In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss,*Food Control*, 18, 7, 800-805, **2007.**

[39] Bagamboula C F., Uyttendaele M., Debevere J., Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigella sonnei and S. flexneri, *Food Microbiology*, 21, 1, 33-42, **2004.**

[40] Kyriakos V,Dimitrios K,Erini G,E katerini P,Sophia V.,Effectiveness of a natural Rosmary(Rosmarinus officinalis) extract on the stability of filleted and minced fish during frozen storage. *Zeitschrift für Lebensmitteluntersuchung und -Forschung* A, 205, 2, 93-96, **1997.**

[41] Ozyurt G., Polat,A., Tokur,B., Chemical and sensory changes in frozen(-18 C) wild sea bass(Dicentrarchus labrax)captured at different fishing seasons.*International journal of food science and Technology*, 42, 7, 887-893, **2007.**

[42]Grigorakis K., Taylor K.D.A., Alexis M.N.,Seasonal patterns of spoilage of ice-stored cultured gilthead sea bream(Sparus aurata), *food chemistry*.81, 2, 263-268, **2003**

[43] Angis S., and Oguzhan, P., Effect of thyme essential oil and packaging treatments on chemical and microbiological properties of fresh rainbow trout (Oncorhynchus mykiss) fillets during storage at refrigerator temperatures, *African Journal of Microbiology Research*, 7, 3, 1136-1143, **2013.**

[44] Laguerre M., Lecomte J., Villeneuve P., Evaluation of the ability of antioxidants to counreract lipid oxidation: Existing methods, new trends and challenges. *Progress in Lipid Research*, 46, 5, 244-282, **2007.**

[45] Bahar TS., Serhat ozkutu., Esin Atici., Gulsunn ozyurt., Caner,Enver Ozyurt., Chemical and sensory quality changes of fish fingers,made from

mirror carp(Cyprinus carpio L.,1758), during frozen storages(-18°C), *Food chemistry*, 99, 2, 335-341, **2006.**

[46] Nusrat Nm., Farah N,Talpur S., T H.Sherazi,M I,Bhanger., Impact of refrigerated strong on quality of oil from water jarko(wallago attu), *fish.pak j Anal Environ chem*, 11, 2, 37-43, **2010.**

[47] Aubourg S., Fluorescence study of the prooxidant activity of free fatty acids on marine lipids, *Journal of the Science of Food and Agriculture*, 81, 4, 385–390, **2001.**

[48] Erkan N,,Yasemin Tosun S., Ulusoy S., Uretener G.,The use of thyme and laurel essential oil treatments to extend the shelf life of bluefish (Pomatomus saltatrix) during storage in ice, *Journal für Verbraucherschutz und Lebensmittelsicherhei*t, 6,1, 39-48, **2011.**

[49] Shabanpoor B., Zolfaghari M., Falah Zade S., Alipoor GH., Effect of extract of Zararia multiflora boiss. on shelf-life of salted vacuum packaged rainbow trout fillet (Oncorhynchus mykiss) in refrigerator conditions: microbial, chemical and sensory attributes assessments, *Journal of Food Science and Technology*, 8, 33,1-11, **2011.**

[50] Guillen M D., Ruiz A., Study of the oxidative stability of salted and unsalted salmon fillets by Hnuclear magnetic resonance,*Food Chemistry*, 86, 2, 297-304, **2004.**

[51] Kostaki M., Giatrakou V., Savvaidis I N., Kontominas M G., Combined effect of MAP and thyme essential oil on the microbiological, chemical and sensory attributes of organically aquacultured sea bass (Dicentrarchus labrax) fillets, *Journal Food Microbiol*, 26, 5, 475- 482, **2009.**

[52] Lindsay RC.,Flavour of fish. Procceding of the 8th World Congress of Food Science and Technology; 29th September–4October, *Toronto, Canada*, **1991.**

[53] Chidanandaiah., Keshri RC., Sanyal MK., Effect of sodium alginate coating with preservatives on the quality of meat paties during refrigerated (4 ± 1°C) storage, *Journal of Muscle Foods*, 29, 3, 275-292, **2009.**

[54] Gomes H A., SEN,Nascimento M.R.L.,fukuma H.T., Evaluation of the 2-thiobarbituric acid method for the measurement of lipid oxidation in mechanically debond gamma irradiated chicken meat.*Food Chemistry*, 80, 3, 433-437, **2003.**

[55] P As., Interaction of malondialdehyde with biological molecules new trends about reactivity and significance, Food *Science and Technology*, 28, 4, 323-335, **1993.**

[56] Wenjiao fan., a Ycb., Shuo.,zhang., The use of a tea polyphenol dip to extend the shelf life of silver carp(Hypophthalmi cthys molitrix) during storage in ice.*food chemistry*, 108, 1, 148-153, **2008.**

[57] Mexis S.F., Chouliara E., KontominasM.G., Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4ºC, *Food Microbiology*, 26, 6, 598–605, **2009.**

[58] Razavi ShiraziH., Seafood technology: principles of handling and processing (2). 1st edn. Tehran: Naghsh-e- Mehr, **2001.**

[59] Erkan N., Özden Ö., Inuğur M ., The effects of modified atmosphere and vacuum packaging on quality of chub mackerel, *International Journal of Food Science Technology*, 42, 11, 1297-1304, **2007.**

[60] Razavi Shirzi H., Seafood technology, principles of handling and processing (1). *Pars negar Press*, 325, **2007.**

[61] Burt S., Essential oils: Their antibacterial Properties and potential applications in foods *–a review international journal of food microbiology*, 94, 223-253, **2004.**

[62] Hosseini MH., Razavi SH., Mousavi MA., Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils, *Journal of Food Processing and Preservation*, 33, 6, 727-743, **2009.**

[63] Kykkidou S., Giatrakou V., Papavergou A., Kontominas MG., Savvaidis INE., Effect of thyme essential oil and packaging treatments on fresh Mediterranean swordfish fillets during storage at 4°C, *Journal Food Chemistry*, 115, 1, 169-175, **2009.**

[64] Tsao S M ., Chin Yin M., In-vitro antimicrobial activity of 4 diallyl sulphides occurring naturally in garlic and chinese leek oils, *Journal of medical microbiology*, 50, 646-649, **2001.**

[65] Gimenze B.,Roncales P.,A Beltran J., Modified atmosphere packaging of filleted rainbow trout, *Journal of the Science of Food and Agriculture*, 82, 10, 1154-1159, **2002.**

[66] Kolakowska A., Domiszewski Z.,Bienkiewicz G.,Zienkowicz L., lipid changes and Qualityof whole of whole –and gutted Rainbow trout during storage in ice, *Acta Ichthyologica Et Piscatoria*, 36, 1, 39-47, **2006.**

6. ACKNOWLEDGEMENTS

Research reported in this publication was supported by Researcher Grant Committee of Research Vice chancellor under award number 13157, of Shahid Beheshti University of Medical Sciences, Tehran, Iran.