Journal of Food Biosciences and Technology,
Islamic Azad University, Science and Research Branch, Vol. 10, No. 1, 35-46, 2020

Nano-Encapsulation of Lemon Essential Oil Approach to Reducing the Oxidation Process in Fish Burger during Refrigerated Storage

Sh. Hasani [a], ***S. M. Ojagh*** [b *], ***M. Ghorbani*** [c], ***M. Hasani*** [d]

[a] PhD Graduated of the Department of Fisheries, Faculty of Fisheries and the Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[b] Associate Professor of the Department of Fisheries, Faculty of Fisheries and the Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[c] Associate Professor of the Department of Food Chemistry, Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
[d] Associate Professor of the Department of Food Science and Technology, Shahrood Branch, Islamic Azad University, Shahrood, Iran.

Received: 7 March 2019 Accepted: 12 September 2019

ABSTRACT: Lemon essential oils (LEOs) as a bioactive compound with antioxidative potential are used as safe additives in foods. However, this compound is sensitive to light, oxidation, and processing in order to solve this problem encapsulation could be a suitable technique to protect them from degradation. The aim of this study was to produce nano-encapsulated LEOs in Chitosan: Modified Starch (Hicap) to investigate the antioxidant effect of the addition of 0.5 and 1% (w/w) free and nano-encapsulated LEOs on the quality of fish burgers during storage and compare it to the control. Changes in chemical properties in treated samples at 0, 3, 6, 9, 12, 15 and 18 days of storage were investigated. Our results showed a nanocapsules particle size of about 339.2 nm with high encapsulation efficiency. The addition of nanocapsules prepared by a mixture of CS: Hicap (1.5: 8.5% w/v) in LEOs significantly ($P< 0.05$) improved the quality characteristics due to the reduction of PV, TBA and TVB-N values for all LEOs nanocapsules treated burgers in comparison to others during storage. Based on the sensory evaluation, the shelf life of burgers increased by incorporation with nano-encapsulated LEOs. According to the results, the application of encapsulated LEOs may be a successful technology for the protection of undesirable chemical, and sensory changes in seafood.

Keywords: *Chitosan, Fish Burger, Lemon Essential Oil, Nanoencapsulation, Quality Characteristics*.

Introduction

Fish burgers are considered as one of the secondary minced fish based products containing high concentrations of polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Oxidation of highly unsaturated lipids is an important factor which leads to the production of off-flavors and odors, rancid taste, texture changes, and discoloration (Orak & Kayisoglu, 2008). Refrigerated storage is well-known for the preservation of fish products (Kim *et al*., 2013), but many researchers reported that lipid oxidation and microbial spoilage can be efficaciously controlled or minimized by using natural antioxidants during cold storage (Aliakbarlu & Khalili Sadaghiani, 2015; Tajik *et al*., 2015). In recent years, consumers' tendency has been mainly on the application of essential oils (EOs) as the natural additives

*Corresponding Author: mahdi_ojagh@yahoo.com; ojagh@gau.ac.ir

to improve the safety of seafood due to the harmful effects of chemical preservatives on human health (Abdollahzadeh *et al*., 2014).

Essential oils (EOs) are composed of many valuable natural compounds that play important roles in human health and might be employed as additives in food, medicine and cosmetic industries (FDA, 2014). Lemon (*Citrus limon* L.) is one of the most important species of genus Citrus belonging to the large Rutaceae family, containing main valuable essential oil that is employed for several purposes throughout the world. Previous researches have shown that the incorporation of essential oils to fish and fish product can be an effective hurdle to reduce the rate of lipid oxidation and spoilage microorganisms (Karabagias *et al*., 2011; Ghaderi-Ghahfarokhi *et al*., 2016). Nevertheless, the use of EOs as the protective agents in foods still faces limitations such as sensitivity to oxygen and light, high temperature, and degradation during processing and storage. In order to overcome these challenges, EOs need to be encapsulated in an appropriate wall material to decrease the oxidative stability, controlled release and improve the shelf life of these ingredients (Jafari *et al*., 2008). Encapsulation could be a successful method to retain aroma from degradation and evaporation. Recently, a wide type of polysaccharide has been used for encapsulation of natural preservative with bioactive composition. Many studies have recently evaluated the application of chitosan (CS) as an encapsulating factor (Estevinho *et al*., 2013; Nuisin *et al*., 2013). The CS is a cationic polymer composed of linear beta-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine with antioxidant, antimicrobial, biodegradable and biocompatible characteristics (Liu, 2014). In this research, the CS was selected as a biopolymer because of its ability to control the release of active ingredient, readily available free amine groups for cross-linking, and suitable electro potential. Carbohydrates such as modified starch (Hicap100) due to the emulsifier properties and high stability are used as a wall material for encapsulation of a wide variety of aroma compounds. While chitosan and modified starch have been studied in the encapsulation of bioactive compounds, there are no publications on the direct addition of encapsulated essential oil in seafood. In this research, the potential of LEOs nanocapsules in CS: Hicap complex to reduce the lipid oxidation and sensorial changes of fish burgers during refrigerated storage has been investigated.

Materials and Methods

- *Chemicals*

LEOs were purchased from the Barij Essence Pharmaceutical Company (Iran). The coating materials were the CS with a low molecular weight (75–85% degree of deacetylation) (Sigma–Aldrich) and Hi-cap 100 (Saadatchemieazma Co., Tehran, Iran). Sodium tripolyphosphate (TPP) and Tween 80 were obtained from Sigma–Aldrich (St. Louis, MO, USA).

- *Preparation of emulsion*

Emulsions were prepared by modification of previous studies by Saloko *et al*., (2013). Briefly, the CS (1.5% w/v) and Hi-cap 100 (8.5% w/v) were dispersed in glacial acetic acid solution (1% v/v), and after it was completely dissolved, Tween 80 at 0.1% concentration was added as a surfactant and stirred for 2 hours until a completely uniform solution was obtained. In this study, all these combinations were prepared with 1 g tween 80 100 g^{-1} essential oil and tripolyphosphate (TPP) (1mg/ml) in the mixture. The total concentration of the dissolved solid (wall material + oil) was 30 % (w/w). The EOs were added in a 1:3 ratio (w/w) to the solution. The solution was incubated at room temperature with a magnetic stirrer and centrifuged for 30 min

in 50 ml tubes at 3000 rpm. Subsequently, the solution was homogenized with a 5200 rpm homogenizer for 2.5 min (Saloko *et al*. 2013). In order to obtain a smaller particle mean size of the capsules and increase the encapsulation efficiency of the nano-encapsulated EOs, the sonication was used. The emulsion was subjected to a sonication process in an ice bath for 7 min (1 sec on and 1 sec off) using a probe (200 UPS, Dr. Heischler, Germany) until the emulsion became completely clear. The emulsions were frozen at –70 °C overnight and dried in a freeze dryer for 72 h. When the freeze-drying process was performed, the obtained powder was kept in moisture–impermeable plastic bags and stored at –20 °C for further characterization of its properties.

- Characterization of nanocapsules

- Determination of encapsulation efficiency (EE%)

The EE% was performed according to Bringas-Lantigua *et al*. (2011) with slight modification. The surface oil of the nanocapsules was washed with 20 mL hexane and shaken for 30 min. The mixture was filtered by Whatman paper No.1. The remained powder was washed with hexane three times. The solvent was evaporated from the nanocapsules at room temperature until constant weight. The nanocapsules were weighted and dissolved in distilled water. The encapsulated oil content was measured by Clevenger distillation. The non-encapsulated oil was determined by the difference between the total essential oil and content of the encapsulated oil. EE % was calculated according to the following equation.

$$\text{EE } (\%) = \frac{T0-S0}{T0} \times 100$$

where T0 is the total LEOs content and S0 is the non–encapsulated LEOs content

-Particle size and polydispersity index (PDI)

A particle size analyzer Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, and Worcestershire, UK) was employed to determine the volume-weighted mean size (D [4,3]) of the nanocapsules.

- Application of LEOs nanocapsules on fish burger preservation

Common carps (*Cyprinus carpio*) were purchased from a freshwater culture pond (Gorgan, Iran). The fresh fish was washed in chilled water and dressed to remove scales, head, and viscera. They were washed once again in chilled water and filleted. Fish fillets were deboned manually. The deboned fish meat was later minced using a mincer (Moulinex, Germany). The minced meat obtained from common carp fish was applied for the preparation of fish burger. Formulation of fish burgers were composed of 80% fish mince, 5% potatoes, 1% sugar, 3% corn flour, 0.2% pepper powder, 5% onion, 1% green chili, 2.2% bread crumbs, 1.5% salt, 0.1% polyphosphate, and 1% egg white Produced fish burgers were separated into 5 different experimental samples: sample A: control (0% LEOs), sample B: 0.5 gr free LEOs/kg burger was added; sample C: 1 gr free LEOs/kg burger was added, sample D: 0.5 gr encapsulated LEOs/kg burger was added and sample E: 1 gr encapsulated LEOs/kg burger was added. The fish burgers were flash fried at 180^0C for 30 seconds in sunflower oil until the color turned slightly brown. The weight of each fish burger was less than 100g, and every 10 pieces were put in one package. Then products stored in the refrigerator for further analysis. The analysis was performed on every alternate day up to 18 days.

-Physicochemical analysis

Proximate composition of fish mince was determined as crude protein by Kjeldahl method. Total lipids, moisture and ash contents were determined according to

AOAC (2005). The pH value of fish burgers was measured by a pH meter (model Wagtech-cyber scan 510, Germany). Free fatty acid (FFA) was determined according to AOAC (2005). The peroxide value (PV) of the samples was determined according to the method described by Pearson (Egan *et al*., 1981). Thiobarbituric acid value (TBA, mg malonaldehyde/kg) was determined colorimetrically according to the method explained by Pearson (1981). Total volatile base nitrogen (TVB-N, mg N/100g) content of burgers was determined according to the method of Safari and Yosefian (2006). TVB-N was calculated in 100g of the fish burger according to the following equation.

TVB –N (mg N/100 g) = 1.4 × used Sulfuric acid×100 ×amount of sample/1000mg

- *Sensory evaluation*

Organoleptic properties of fried burger samples with added free LEOs and nanocapsules were performed by twenty semi-trained panelists using scores 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 for excellent, like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely respectively for each of the sensory characteristics (Watts *et al*., 1989).

- *Statistical analysis*

One-way analysis of variance (One way-ANOVA) was performed using SPSS (ver.15) software. Analytical data were obtained from analyses of three samples for each individual treatment in chemical assays. Differences among mean values were examined by Duncan's test ($p \leq 0.05$) significance level. Means and standard errors of the samples were also calculated.

Results and Discussion

- *Characterization of nanocapsules*

EE% is used to express the amount of LEOs incorporated into the nanocapsules and is normally determined by the percentage of LEOs retained in the capsules relative to the total amount of oil. The nano-encapsulation efficiency of LEOs was measured as 85.43 ± 2.35%. On the other hand, about less than 15% has remained as unencapsulated LEOs. EE% is an effective factor in physical characterization such as oxidative stability, physical stability, and structural properties. Results confirmed the high EE% in the nanocapsules with wall material CS: Hicap (1.5:8.5%). This may be related to the high capacity of the CS to encapsulate bioactive ingredient such as monoterpenes and also to the ability of the mixture of the CS and Hicap to increase EE%. High values of the EE are an appropriate feature in the encapsulation technique due to the acceptable protection of oil in nanocapsules. Previous studies reported that particle size and specific surface area of capsules are effective factors on encapsulation efficiency of the sensitive or valuable ingredients within the nanoencapsulation system (Hasani *et al*., 2018; Hosseini *et al*., 2017).

The size distribution of nanocapsules containing LEOs is shown in Figure 1. Mean diameter and Polydispersity index of LEOs nanocapsules after sonication was 339.3 nm and 0.42, respectively. As shown in Figure 1 LEOs capsules size is similar to other researches which found size range of nanocapsules (Hosseini *et al*., 2013). Polydispersity index is usually used for determining particles diameter distribution in suspension. According to the results, the polydispersity index of the nanocapsule in CS: Hicap was 0.42 indicating that the nanocapsules were monodisperse, stable and had a desirable uniform distribution. This result is in agreement with previous studies. They reported that the particle size of the encapsulated fish oil obtained by the same method was also in nano scale (Ojagh & Hasani, 2018).

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 339.2	Peak 1:	239.6	84.9	33.10
PdI: 0.426	Peak 2:	1142	15.1	170.5
Intercept: 0.568	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

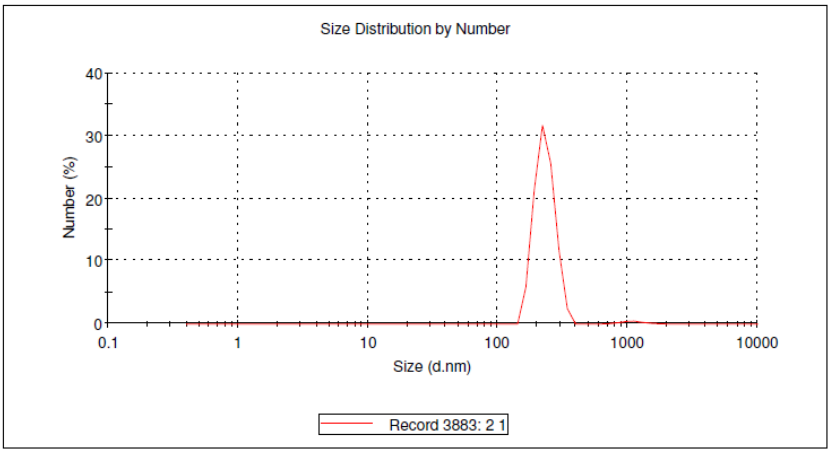

Fig.1. Particle size distribution of LEOs nanocapsules.

-Physicochemical analysis

The yield of mince was 59.70% from Common carp and the initial moisture, protein, fat and ash content of fish mince were determined 75.89 ±0.42 %, 16.824 ±0.17 %, 2.93 ±0.08 % and 1.21 ±0.02 % respectively. The raw mince yield and proximate composition values of Common carp are similar to the other freshwater fish (Khanipour *et al*., 2015).

Table 1 presents the effect of free and encapsulated LEOs on the pH value of burgers. The pH of fish burger decreased from 6.88 to 6.32 in control, whereas in samples incorporated with 0.5% LEOs, incorporated with 1% LEOs, incorporated with 0.5% and 1% Encapsulated LEOs the pH decreased from 6.87 to 6.54, 6.87 to 6.61, 6.86 to 6.63 and 6.90 to 6.67 respectively. Metin *et al*. (2002) found that the pH of fish burgers decreased from 6.5 to 5.6 which may be due to the fermentation of potato and bread ingredients of the burger. According to the results, samples containing encapsulated LEOs significantly showed a slower rate of decreasing in values than others over storage time. Our results illustrated that the addition of free and encapsulated LEOs to burger formulation present a significant effect on inhibition of pH reduction ($P< 0.05$) due to antibacterial effect of LEOs or synergistic effect of CS and LEOs in encapsulation system on bacteria. Our results displayed that the encapsulation process does not reduce the effect of LEO on the pH value during refrigerated storage.

The FFA content increased significantly ($P <0.05$) in control and treated samples with free and encapsulated LEOs as a function of increasing refrigerated storage. The results clearly showed that control sample had higher FFA 0.86 (% of oleic acid) than those of samples containing free and encapsulated LEOs. According to the results, FFA content did not exceed acceptable limits for freshness parameters in fish burger stored at refrigerated temperature (Vanitha *et al*., 2013). A similar trend was also reported by the Yerlikaya *et al*. (2005) during the refrigerated storage of fish patties from anchovy. The increase in FFA may be due to the cooking process of mince prior to preparation of product and flash frying after preparation of the product that might have deactivated the lipolytic enzymes. While the

formation of FFA does not decrease nutritional quality, its evaluation is considered important for studying the development of rancidity. Additionally, FFA has demonstrated to interact with proteins causing texture deterioration during storage. This research found that burgers incorporated with 1% LEOs nanocapsules had the lowest FFA (0.42 % as of oleic acid) after 18 days of storage. This result may be related to the presence of CS and Hicap in the nanocapsules as wall materialsdue to the hydroxyl groups (OH) in their chemical structures that contribute strongly to the antioxidant activity by donation of a free electron (hydrogen) (Younes & Rinaudo, 2015).

TVB-N values of Common carp burgers preserved using free and encapsulated LEOs during refrigerated storage are shown in Table 1. In all of the samples, the TVB-N value increased with prolonging the storage time. TVBN content increased in control, treated with free and encapsulated LEOs (0.5 and 1%) burger from an initial value of 3.44 to 13.63 mg /100g, 3.38 to 10.43 mg/100g, 3.33 to 10.68 mg/100g, 3.13 to 8.80 and 3.23 to 8.23 mg /100g samples, respectively, during 18 days storage at refrigerated temperature.

Freshness is the most important characteristic in evaluating the quality of fish. Total volatile basic nitrogen (TVB-N) is one of the biochemical methods used to recognize fish freshness. As observed in Table 1 TVB-N content increased with prolonging the storage time in all the samples. It was caused by the action of bacteria in the fish muscular tissue that produces ammonia, trimethylamine, and dimethylamine. Our results are in agreement with Metin *et al*. (2002) whom observed an increase in TVBN value from 10.98 to 19.66 mg/100 g in fish burgers stored at refrigerated temperature. The lowest TVB-N values in burgers incorporated with LEOs nanocapsules may be related to antioxidant and antimicrobial properties of essential oil and chitosan used as a coating material.

Peroxide value (PV) is one of the important indices of fish oxidation spoilage (Hasani *et al*., 2014). It illustrates the existence of concentrations of peroxides and hydroperoxides that are produced during the early stages of lipid oxidation. The PV values of control and treated samples are shown in Figure 2. There were no significant differences among all the samples on the first day; however, a significant difference in PV was noticed between the control and other treated samples with free and encapsulated LEOs with different concentration during storage ($P<0.05$). Lower PV values in the samples treated with free LEOs compared to control might be linked with phenolic content of natural essential oil. The PV values of the samples incorporated with 0.5 and 1% encapsulated LEOs were lower than the control and treated with free LEOs. It was found that the CS acted as antioxidants by scavenging oxygen radicals such as hydroxyl, superoxide, alkyl as well as highly stable DPPH radicals tested *in vitro* (Younes & Rinaudo 2015). Furthermore, it was reported that the radical scavenging properties of the CS depended on their MW as the low-MW CS were more active than those with higher MW. The PV value then decreased in control and treated sample with 0.5% free LEOs at the 12th and 18th days of storage, respectively. The decrease of the PV at the end of the storage may occur owing to decomposition of hydroperoxides into secondary oxidation products. These results were similar to Ojagh *et al*. (2010) reported that peroxide values increased in all treatments, but this increase was lower in the samples treated with chitosan and cinnamon due to their antioxidant activity. Lipid oxidation in seafood leads to rancid odour, discoloration, loss of nutrients, limiting in the shelf life of fish products (Mancini *et al*., 2015).

Table 1. Effect of free and encapsulated Lemon essential oil (LEOs) on the chemical quality of Common carp burgers during refrigerated storage

		Treatment				
	Time	**Control (0%)**	**Incorporated with 0.5% LEOs**	**Incorporated with 1% LEOs**	**Incorporated with 0.5% Encapsulated LEOs**	**Incorporated with 1% Encapsulated LEOs**
FFA (% of oleic acid)	0	0.19 ± 0.01 ^{Ae}	0.18 ± 0.00 ^{ABg}	0.18 ± 0.01 ^{ABCf}	0.17 ± 0.01 ^{BCg}	0.17 ± 0.01 ^{Cg}
	3	0.23 ± 0.01 ^{Ae}	0.22 ± 0.02 ^{Af}	0.22 ± 0.01 ^{Abe}	0.20 ± 0.01 ^{BCf}	0.20 ± 0.01 ^{Cf}
	6	0.32 ± 0.01 ^{Ad}	0.29 ± 0.02 ^{Be}	0.28 ± 0.01 ^{BCd}	0.26 ± 0.01 ^{Ce}	0.24 ± 0.02 ^{De}
	9	0.55 ± 0.02 ^{Ac}	0.42 ± 0.02 ^{Bd}	0.34 ± 0.02 ^{Cc}	0.30 ± 0.02 ^{Dd}	0.28 ± 0.01 ^{Dd}
	12	0.66 ± 0.03 ^{Ab}	0.57 ± 0.01 ^{Bc}	0.45 ± 0.01 ^{Cb}	0.38 ± 0.02 ^{Dc}	0.33 ± 0.02 ^{Ec}
	15	0.83 ± 0.06 ^{Aa}	0.65 ± 0.04 ^{Bb}	0.51 ± 0.02 ^{Ca}	0.42 ± 0.02 ^{Db}	0.38 ± 0.01 ^{Db}
	18	0.86 ± 0.03 ^{Aa}	0.68 ± 0.02 ^{Ba}	0.53 ± 0.02 ^{Ca}	0.46 ± 0.02 ^{Da}	0.42 ± 0.03 ^{Ea}
TVBN	0	3.44 ± 0.05 ^{Ag}	3.38 ± 0.07 ^{Ae}	3.33 ± 0.15 ^{Af}	3.13 ± 0.23 ^{Ag}	3.23 ± 0.21 ^{Ae}
	3	4.52 ± 0.05 ^{Af}	4.43 ± 0.25 ^{ABd}	4.27 ± 0.15 ^{ABCe}	4.17 ± 0.15 ^{BCf}	4.10 ± 0.10 ^{Cd}
	6	5.78 ± 0.04 ^{Ae}	5.43 ± 0.41 ^{ABc}	5.13 ± 0.32 ^{BCd}	4.63 ± 0.23 ^{Ce}	4.63 ± 0.33 ^{Ccd}
	9	7.93 ± 0.03 ^{Ad}	6.96 ± 0.61 ^{Ba}	6.93 ± 0.40 ^{Bc}	5.93 ± 0.12 ^{Cd}	5.17 ± 0.29 ^{Dc}
	12	9.80 ± 0.53 ^{Ac}	7.87 ± 0.35 ^{Ba}	7.60 ± 0.36 ^{Bc}	6.72 ± 0.28 ^{Cc}	6.17 ± 0.32 ^{Cb}
	15	12.80 ± 0.20 ^{Ab}	10.43 ± 0.78 ^{Ba}	9.33 ± 0.58 ^{Cb}	8.03 ± 0.25 ^{Db}	7.57 ± 0.74 ^{Da}
	18	13.63 ± 0.60 ^{Aa}	10.43 ± 0.78 ^{Ba}	10.68 ± 0.62 ^{Ba}	8.80 ± 0.36 ^{Ca}	8.23 ± 0.68 ^{Ca}
pH	0	6.88 ± 0.01 ^{ABa}	6.87 ± 0.02 ^{Ba}	6.87 ± 0.01 ^{Ba}	6.86 ± 0.01 ^{Ba}	6.90 ± 0.01 ^{Aa}
	3	6.78 ± 0.01 ^{Cb}	6.84 ± 0.01 ^{Bb}	6.84 ± 0.01 ^{Aa}	6.85 ± 0.01 ^{Aba}	6.86 ± 0.01 ^{Ab}
	6	6.70 ± 0.06 ^{Bc}	6.78 ± 0.02 ^{Ac}	6.80 ± 0.01 ^{Ab}	6.82 ± 0.01 ^{Ab}	6.81 ± 0.01 ^{Ac}
	9	6.60 ± 0.02 ^{Cd}	6.74 ± 0.02 ^{Bd}	6.78 ± 0.02 ^{Ac}	6.76 ± 0.01 ^{ABc}	6.78 ± 0.01 ^{Ad}
	12	6.56 ± 0.02 ^{Ce}	6.65 ± 0.03 ^{Be}	6.73 ± 0.03 ^{Ad}	6.73 ± 0.02 ^{Ad}	6.74 ± 0.01 ^{Ae}
	15	6.41 ± 0.01 ^{Df}	6.59 ± 0.01 ^{Cf}	6.66 ± 0.02 ^{Be}	6.69 ± 0.01 ^{Ae}	6.70 ± 0.01 ^{Af}
	18	6.32 ± 0.02 ^{Dg}	6.54 ± 0.01 ^{Cg}	6.61 ± 0.01 ^{Bf}	6.63 ± 0.01 ^{Bf}	6.67 ± 0.02 ^{Ag}

Values are expressed as Mean ± SD; n = 3; Means bearing different superscripts in the same row (upper case) and in the same column (lower case) are significantly different ($p<0.05$)

TBARS is most widely used as an indicator to measure the amount of the secondary lipid oxidation products that is closely related to the sensory quality of fish products, and a 0.55 mg MDA per kilogram sample has been considered as a threshold of rancidity acceptance by consumers (Hasani *et al*., 2014). The amount of TBARS increased in all samples, and significant differences were observed among treated samples as compared to the control ($P< 0.05$) (Figure. 3). Malondialdehyde (MDA)

is one of the most abundant aldehydes generated during secondary lipid oxidation, and it is probably the one most commonly used as an oxidation marker. The results demonstrated that burgers treated with 1% encapsulated LEOs had the lowest amount of MDA formation at 18th days of storage, and this index exceeds the permissible limits only in the control sample. The results of antioxidant potential of LEOs in the current work were in agreement with the results previously reported in the literature (Djenane *et al*., 2012) on the effects of essential oils on the protection of lipid oxidation in minced meat. Furthermore, many studies confirmed that γ-terpinene had the strongest antioxidant effect among the compounds tested in *Citrus* essential oil (Olatunya & Akintayo, 2017; Loizzo *et al*., 2016). As it is observed in Figure 3, it is worthwhile to note that not only encapsulation caused no negative impact on the antioxidant properties of the encapsulated LEOs, but also resulted in sustained EOs with higher antioxidant activity. The antioxidant activity of chitosan is related to the interactivity of amino groups with fats derivative and its potency in chelating ferrous ions, liberated by myoglobin degradation during storage (Kamil *et al*., 2002).

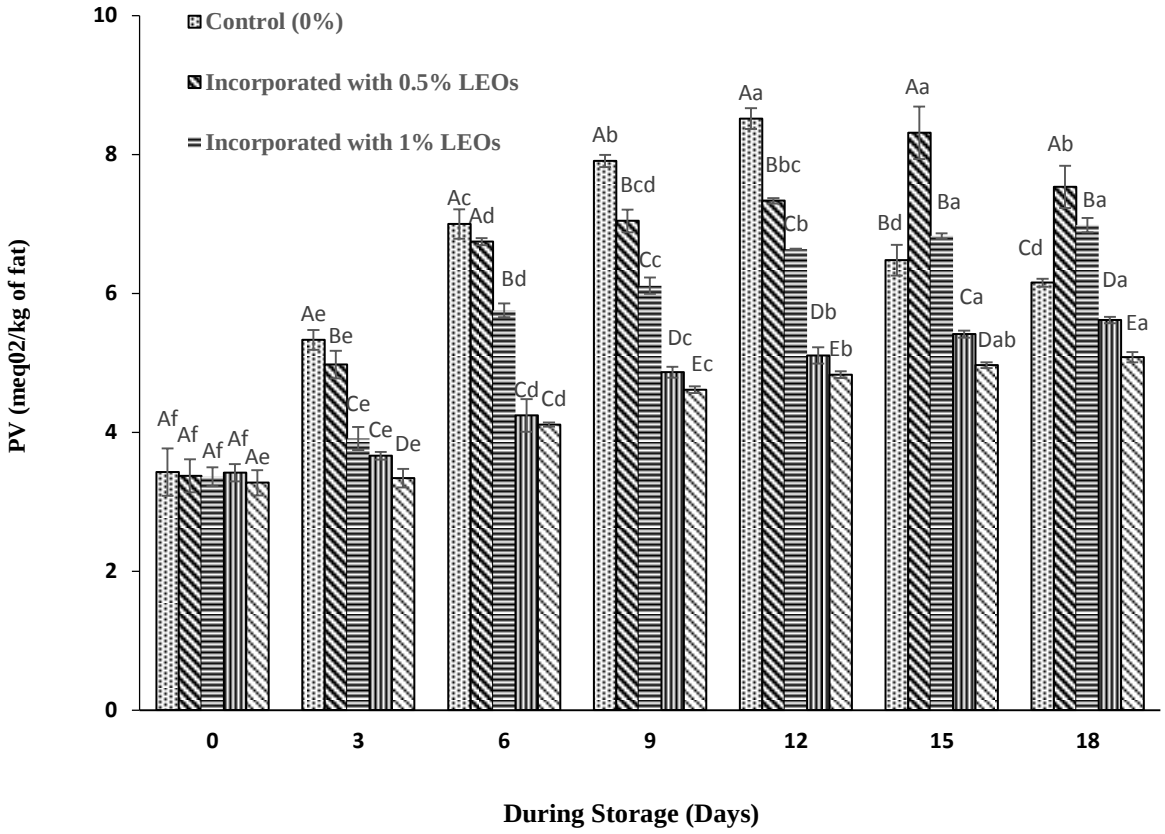

Fig. 2. Changes in Peroxide value (PV) of Common carp burgers with different formulations during 18 days of refrigerated storage
Capital letters (A-E) indicate significant differences (P<0.05) of treatment.
Small letters (a-f) indicate significant differences (P<0.05) of storage.

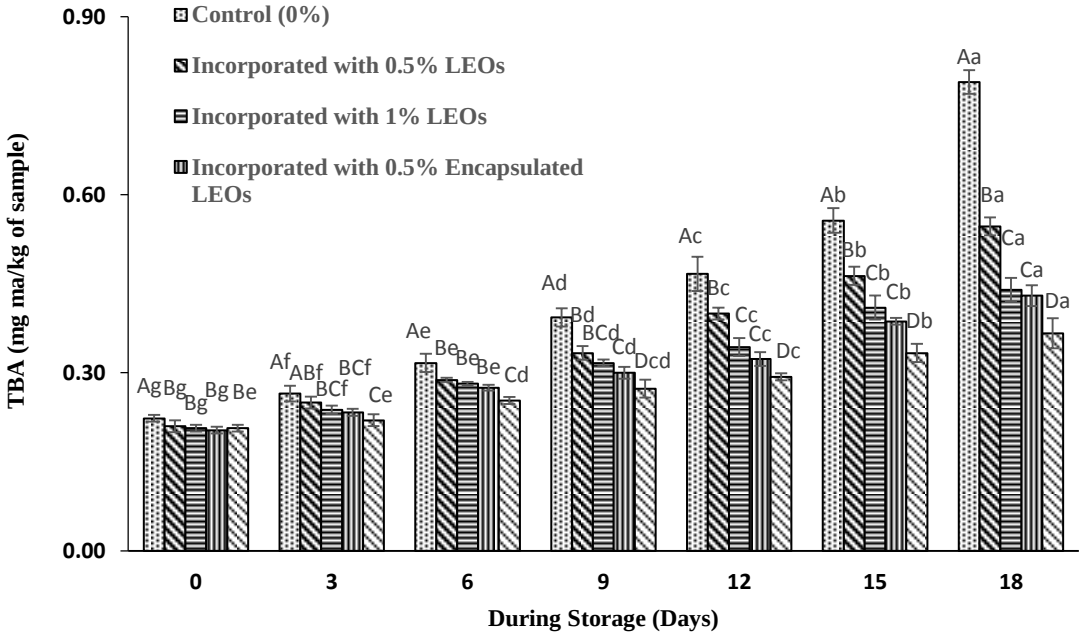

Fig. 3. Changes in TBARS value of Common carp burgers with different formulations during 18 days of refrigerated storage
Capital letters (A-D) in the same line indicate significant differences (P<0.05) of treatment.
Small letters (a-g) in the same column indicate significant differences (P<0.05) of storage.

The overall mean acceptability scores are given in Table 2. The results indicate that sensory scores showed a significant decrease in the all samples during the entire period of storage at refrigerated temperature, and the results also indicate that the sensory evolution values decreased significantly with increasing chemical spoilage (P<0.05). The overall scores of burgers incorporated with 1% encapsulated LEOs were higher than other samples. The sensory scores of burger containing free and encapsulated as compared to the control sample showed that the addition of pure LEOs and encapsulated LEOs could improve the shelf life of Common carp burgers. Thus, encapsulation of Lemon essential oil in suitable wall material can provide more prolonged shelf life by protecting the oils during processing and cover undesirable sensory characteristics.

Conclusion

This research work clearly revealed that nanoencapsulation is a successful technique due to nanocapsules characteristics such as particle size and encapsulation efficiency. Nanoencapsulation of LEOs in CS: Hicap complex presented several preferable advantages during storage of Common carp burgers. Encapsulation process extended the shelf life of LEOs and controlled the vaporization of active compounds at the beginning of storage. Encapsulation of LEOs in CS: Hicap could also maintain the antioxidant activity of it until the end of refrigerated storage. Both free and encapsulated LEOs showed the potential to inhibit lipid oxidation significantly. On the basis of our results, the addition of nanoencapsulated LEOs had no negative effect on burgers textural quality and sensory acceptability. Therefore, application of nanocapsules as an effective additive with antioxidant potential to improve the shelf life of burgers and its application is suggested for the production of other foods.

Table 2. Changes in the Overall acceptability scores of Common carp burgers with different formulations during 18 days of refrigerated storage

Time	Treatment				
	Control (0%)	**Incorporated with 0.5% LEOs**	**Incorporated with 1% LEOs**	**Incorporated with 0.5% Encapsulated LEOs**	**Incorporated with 1% Encapsulated LEOs**
0	9.33 ± 0.58^{Aa}	9.67 ± 0.58^{Aa}	9.00 ± 0.00^{Aa}	9.33 ± 0.58^{Aa}	9.67 ± 0.58^{Aa}
3	7.67 ± 0.58^{Ab}	7.67 ± 0.58^{Ab}	7.33 ± 0.58^{Ab}	8.00 ± 0.00^{Ab}	7.67 ± 0.58^{Ab}
6	5.33 ± 0.58^{Ac}	5.33 ± 0.58^{Ac}	5.67 ± 0.58^{Ac}	6.33 ± 0.58^{Ac}	6.67 ± 0.58^{Ab}
9	3.67 ± 0.58^{Cd}	4.67 ± 0.58^{BCc}	4.67 ± 0.58^{BCd}	5.67 ± 0.58^{ABc}	6.67 ± 0.58^{Ab}
12	2.67 ± 0.58^{De}	3.67 ± 0.58^{Cd}	4.00 ± 0.00^{BCde}	4.67 ± 0.58^{ABd}	5.33 ± 0.58^{Ac}
15	1.33 ± 0.58^{Df}	3.00 ± 0.00^{Cd}	3.67 ± 0.58^{BCe}	4.33 ± 0.58^{ABd}	4.67 ± 0.58^{Acd}
18	1.00 ± 0.00^{Df}	1.67 ± 0.58^{CDe}	2.33 ± 0.58^{BCf}	3.33 ± 0.58^{ABe}	4.00 ± 1.00^{Ad}

Values are the mean ± standard deviation
Capital letters (A-D) in the same line indicate significant differences ($P<0.05$) of treatment.
Small letters (a-f) in the same column indicate significant differences ($P<0.05$) of storage.

References

Abdollahzadeh, E., Rezaei, M. & Hosseini, H. (2014). Antibacterial activity of plant essential oils and extracts: the role of thyme essential oil, nisin, and their combination to control Listeria monocytogenes inoculated in minced fish meat. *Food Control*, 35(1), 177–183.

Aliakbarlu, J. & Khalili Sadaghiani, S. (2015). Effect of avishane Shirazi (Zataria multiflora) and clove (Syzygium aromaticum)essential oils on microbiological, chemical and sensory properties of ground sheep meat during refrigerated storage. *Journal of Food Quality*, 38(4), 240–247.

AOAC. (2005). Association of Official Analytical Chemists. 18th edition. Gaithersburg, Maryland, USA.

Bejrapha, P., Min, S.G., Surassmo, S. & Choi M. J. (2010). Physicothermal properties of freeze-dried fish oil nanocapsules frozen under different conditions. *Drying Technology*, 28, 481–489.

Bringas-Lantigua, M., Expósito-Molina, I., Reineccius, G. A., López-Hernández, O. & Pino, J. A. (2011). Influence of spray-dryer air temperatures on encapsulated mandarin oil. *Drying Technology*, 29, 520–526.

Djenane, D., Aïder, M., Yangüela, J., Idir, L., Gómez, D. & Roncalés, P. (2012). Antioxidant and antibacterial effects of Lavandula and Mentha essential oils in minced beef inoculated with E. coli O157: H7 and S. aureus during storage at abuse refrigeration temperature. *Meat Science*, 92(4), 667–674.

Egan, H., Kirk, R.S. & Sawyer, R. (1981). Pearson's Chemical Analysis of Foods, Churchill Livingstone, Edinburgh, UK.

Estevinho, B.M.A.N., Rocha, F.A.N., Santos, L.M.D.S. & Alves, M.A.C. (2013). Using water-soluble chitosan for flavor microencapsulation in food industry. *Journal of Microencapsulation*, 30: 571–579.

FDA. (2014). Aromatherapy. Retrieved from: http://www.fda.gov/Cosmetics/ProductsIngredients/Products/ucm127054.htm, Access: October 2016.

Ghaderi-Ghahfarokhi, M., Barzegar, M., Sahari, M.A. & Azizi, M.H. (2016). Nanoencapsulation approach to improve antimicrobial and antioxidant activity of thyme essential oil in beef burgers during refrigerated storage. *Food and Bioprocess Technology*, 9(7), 1187–1201.

Hasani, S.H., Ojagh, S.M. & Ghorbani, M. (2018). Nanoencapsulation of lemon essential oil

in Chitosan-Hicap system. Part 1: Study on its physical and structural characteristics. *International Journal of Biological Macromolecules*, 115, 143–151.

Hosseini, S.F., Ramezanzade, L. & Nikkhah, M. (2017). Nano-liposomal entrapment of bioactive peptidic fraction from fish gelatin hydrolysate. *International Journal of Biological Macromolecule*, 105, 1455–1463.

Hosseini, S.F., Zandi, M., Rezaei, M. & Farahmandghavi, F. (2013). Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and in vitro release study. *Carbohydrate Polymers*, 95, 50–56.

Jafari, S.M., Assadpoor, E., He Y. 7 Bhandari, B. (2008). Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology*, 26, 816–835.

Kamil, J.Y.V.A., Jeon, Y.J. & Shahidi, F. (2002). Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (Clupea harengus). *Food Chemistry*, 79(1), 69 77.

Karabagias, I., Badeka, A. & Kontominas, M.G. (2011). Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Science*, 88(1): 109–116. doi:10.1016/j.meatsci.2010.12.010.

Khanipour, A.A., Jorjani, S. & Soltani, M. (2014). Chemical, sensory and microbial quality changes of breaded kilka (*Clupeonella cultriventris*) with tempura batter in production stage and during frozen storage. *International Food Research Journal*, 21(6), 2421–2430.

Liu, D., Li, Z., Zhu, Y., Li Z. & Kumar, R. (2014). Recycled chitosan nanofibril as an effective Cu (II), Pb (II) and Cd (II) ionic chelating agent: adsorption and desorption performance. *Carbohydrate Polymer*, 111, 469–476.

Loizzo, M.R., Tundis, R., Bonesi, M., Sanzo, G.D., Verardi, A., Lopresto, C.G., Pugliese, A., Menichini, F., Balducchi, R. & Calabrò V. (2016). Chemical profile and antioxidant properties of extracts and essential oils from Citrus - limon (L.) burm. Cv. Femminello commune. *Chemitry & Biodiversity*, 13, 571–581.

Mancini, S., Preziuso, G., Dal Bosco, A., Roscini, V., Szendrő, Z., Fratini, F. & Paci, G. (2015). Effect of turmeric powder (Curcuma longa L.) and ascorbic acid on physical characteristics and oxidative status of fresh and stored rabbit burgers. *Meat Science*, 110, 93–100.

Metin, S., Erkan, N. & Varlik, C. (2002). The application of hypoxanthine activity as a quality indicator of cold stored fish burgers. *Turkish Journal of Fisheries and Aquatic Sciences*, 26, 363–367.

Nuisin, R., Krongsin, R., Noppakundilograt, J. & Kiatkamjornwong, S. (2013). Microencapsulation of menthol by crosslinked chitosan via porous glass membrane emulsification technique and their controlled release properties. *Journal of Microencapsulation*, 30, 498–509.

Ojagh, S.M. & Hasani, S.H. (2018). Characteristics and oxidative stability of fish oil nano-liposomes and its application in functional bread. *Food Measure Journal*, 12(2), 1–9.

Ojagh S.M., Rezaei, M., Razavi, S.H. & Hosseini, S.M.H. (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry*, 120, 193–198.

Olatunya, A.M. & Akintayo, E.T. (2017). Evaluation of the effect of drying on the chemical composition and antioxidant activity of the essential oil of peels from three species of citrus group. *International Food Research Journal*, 24(5), 1991–1997.

Orak, H.H. & Kayisoglu S. (2008). Quality changes in whole, gutted and filletd three fish species (*Gadus euxinus*, *Mugil cephalus*, *Engraulis encrasicholus*) at frozen storage period (–26°C). *Acta Scientiarum Polonorum Technologia Alimentaria*, 7, 15–28.

Pearson, D. (1981). The Chemical Analysis of Foods, 8th edn. London, UK.

Safari, R. & Yosefian, M. (2006). Changes in TVN (Total Volatile Nitrogen) and psycrotrophic bacteria in Persian sturgeon Caviar (*Acipenser persicus*) during processing and cold storage. *Journal of Applied Ichthyology*, 22, 416–418.

Saloko ,S., Darmadji, P., Setiaji, B., Pranoto, Y. & Anal, A.K. (2013). Encapsulation of coconut shell liquid smoke in chitosan-

maltodextrin based nanoparticles. *International Food Research Journal*, 20, 1269–1276.

Tajik, H., Aminzare, M., Mounesi Raad, T., Hashemi, M., Hassanzad Azar, H., Raeisi, M. & Naghili, H. (2015). Effect of Zataria multiflora Boiss essential oil and grape seed extract on the shelf life of raw buffalo patty and fate of inoculated Listeria monocytogenes. *Journal of Food Processing and Preservation*, 39, 3005–3013.

Vanitha, M., Dhanapal, K. & Reddy, G.V.S. (2015). Quality changes in fish burger from Catla (*Catla Catla*) during refrigerated storage. *Journal of Food Science and Technology*, 52(3), 1766–1771.

Yerlikaya, P., Gokoglu, N. & Uran, H. (2005). Quality changes of fish patties produced from anchovy during refrigerated storage. *European Food Research Technology*, 220, 287–291.

Younes, I. & Rinaudo, M. (2015). Chitin and Chitosan Preparation from Marine Sources. Structure, Properties and Applications. *Marine Drugs*, 13, 1133–1174.