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ANTIOXIDANT ACTIVITY OF *ZATARIA MULTIFLORA BOISS*. ESSENTIAL OIL ENCAPSULATED IN NANOLIPOSOME IN BROTH MEDIA AND MINCED BEEF

Running title: **Nanoliposomal Zataria as Antioxidant**

Kianoush Khosravi-Darani*[1], Omid Zahed[1] and Sheida Aarabi[2]

[1]Research Department of Food Technology, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, P.O.Box: 19395-4741, Tehran, Iran, [2]Master of Science, Food Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Yakhchal Ave., Shariati Ave., Tehran, Iran.
E-mail: k.khosravi@sbmu.ac.ir, ozahed@ut.ac.ir

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ABSTRACT

Natural herbal antioxidants as additives in food and biological systems are interesting but their application in food formulations is problematic due to low dispersion in aqueous phase and susceptibility to oxidation. Encapsulation is a solution to increase water solubility protection from environment as well as masking odor and taste of new additives. In this study, liposome-encapsulated *Zataria multiflora boiss* was produced by heating method and encapsulation efficiency was optimized. Then antioxidant activity of free and encapsulated essential oil was compared using radicals 2-'2-diphenyl-1-picrylhydrazy (DPPH) and thiobarbituric acid reactive substances (TBARS) in minced beef. Results showed that the most important parameter affecting the microencapsulation efficiency is phosphatidylcholine. The most suitable encapsulation was achieved in condition includes phosphatidylcholine (2.5% w/w), ratio of essential oil to phosphatidylcholine (0.81% w/w), temperature (35°C) and time (42 min). In these conditions, the encapsulation efficiency reached to 54.4 %. Antioxidant activity of liposomal essential oil was significantly higher than free oil. The sensory evaluation of minced meat containing encapsulated essential oil was significantly higher than control (including free essential oil) ($p \leq 0.01$) (p. Minced meat containing 0.05% and 0.1 % w/w encapsulated essential oils showed suitable overall acceptance.

Keywords: Antioxidant activity, Beef, Microencapsulation, Nanoliposome, *Zataria multiflora boiss*

1. INTRODUCTION

In recent century, food safety and quality during storage is not only the attention of the food industry experts and country's health organizations, but also consumers may not neglect it and know irreversible damage of safety ignorance to society (Burt 2004).

Meat and meat products are main parts of diet in developed societies, as an important source of high quality protein, vitamins (K, E, D, A) and minerals (iron, manganese, copper and zinc). So, seeking for new preservatives is gone a challenge for food industry (Bender 1992).

Fat oxidation as the most important causes of food spoilage effects on color, flavor, texture, and nutritional value of food (destruction of vitamins A, D, E and essential fatty acids and creation toxic compounds). Oxidation reactions, involving strong reactive molecules that called free radicals. Compounds resulting from the oxidation of lipids can interfere in the absorption of proteins or ferulic acid. Also, these compounds cause cardiovascular disease and cancer in human. So, oxidation of lipids in foods should be inhibited (Burt 2004). by incorporation of antioxidants in oils and fats (Pan *et al*., 2003; Shahavi *et al*., 2015, Tanizawa *et al*., 1984; Khoshtinat *et al*., 2016).

Incorporation of synthetic antioxidant lead to high performance oxidation control, but its accumulation in body tissues causes cancer. Phenolic compounds, which exist in extract of fruits, grasses, vegetables and grains have multiple biological effects e.g. antioxidant activity (Sahar *et al*., 2017).

Thyme is a perennial plant belonging to the Lamiaceae family (Labiatae) is one of the most medicinal plants (Wiseman *et al*., 1997). Thyme with the scientific name *Zataria multiflora* boiss of the species belonging to the *Zataria* genus was a native of Iran, Afghanistan and Pakistan. This plant is used as natural flavorings in foods. It shows also strong antioxidant and anti-microbial properties due to its phenolic compounds (Hamzeh and Rezaei, 2010; Ekhtiarzade *et al*., 2011; Rezaei *et al*., 2011). Volatile oils can be added to formulations of food for their performance characteristics. But problem is not suitable distribution in aqueous phase and their sensitivity to oxidation. Encapsulation is solution of increasing water-solubility and protection.

Liposomes are good candidate for this purpose in food applications due to its natural ingredients (Yoshida *et al*., 2010). Liposomes are spherical particles with one or more membrane bilayer which consist of the accumulation of phospholipid

lipid molecules and energy consumption in the aqueous medium. The advantages of liposomes compared to other methods encapsulated is the stability of water-soluble substances in the environment with high water activity (Khosravi and Mozafari; 2010, Mortazavi; *et al*., 2007, Mozafari, *et al*., 2008). Another advantage is their compounds which are natural and useful for human health. Recent research on biological functions phospholipid and sphingolipid, numerous health benefits, including protecting the liver, enhance memory and prevent the absorption of cholesterol have been mentioned (Mozafari *et al*., 2008). So these vesicles are very attractive carriers in medicine and several purpose in industries (Khosravi-Darani, *et al.*, 2007, Mortazavi, *et al.*, 2007; Mozafari, *et al.*, 2008; Khosravi-Darani *et al.*, 2010; Rashidi and Khosravi 2011; Jahadi, *et al.*, 2015; 2016; 2017; Khanninir *et al.*, 2016; Zoghi *et al*., 2018).

The purpose of this study, microencapsulation natural antioxidant extracts of *Zataria multiflora boiss*. Essential oil (ZEO) and evaluation of the antioxidant properties of the extract using DPPH before and after is encapsulated in nanoliposome.

MATERIALS AND METHODS

Procedure of research conduction: Extract of *Zataria multiflora boiss* was purchased from Barij-Essence company (Kashan, Iran) and was maintained in dark refrigerated storage (4°C). Also, to determine the number of phenolic compounds, Folin-Ciocalteu test was used. The beef, that 48 h had elapsed during the rigor, was purchased from the butcher and in terms of maintaining the cold chain; it was transferred to the laboratory. Sampling was done as a random sampling on the first day of eliciting the meat.

The condition for preparation of liposomal essential oil by Central: Composite design and relevant formulation was utilized for subsequent stages of experiment. All chemicals and solvents applied in this experiment were prepared by Merck (Germany). Free radical DPPH, standard essence and Folin-ciocalteu reagent were purchased from Sigma-Aldrich (US). Phosphatidylcholine of Across (Belgium), had been used with the highest purity.

Statistical design of research: Designing the statistical test in this section was performed in order to distinguish the optimum condition for producing Liposome through thermal treatment for the sake of acquiring the highest level of encapsulation (EE%). This method which is one of the widespread treatments of second order response, indeed, is created by adding focal points and one or some central points of a two factors design. In this study, initially, based on the pilot experiments, variation range of factors was selected on five distinct levels for each the percentage of phosphatidylcholine (1-3%), the ratio of essential oil to phosphatidylcholine (0.25:1) (%w/w), temperature (30-50°C) and the time (20-60 min). In this experiment, the phosphatidylcholine (X_1), ratio of essential oil to phosphatidylcholine (X_2), temperature (X_3) and time (X_4) are four variables and the encapsulation efficiency (EE) is the dependent variable (Table 1).

Table 1. Independent process variables and their levels for evaluation of impact on encapsulation efficiency

Independent Variables	Range and levels of variables				
	α-	-1	0	+1	α+
Phosphatidyl choline (%)	1	1.5	2	2.5	3
Ratio of phosphatidyl choline to essential oil (%)	0.25	0.44	0.63	0.81	1
Temperature (°C)	30	35	40	45	50
Time (min)	20	30	40	50	60

L_{12} array was designed to evaluate the impact of 11 variables (Table 2) Encapsulation efficiency of essential oil into liposomes was taken into account as a dependent variable or response. Expert Design Software (version 7) was used as statistical method. In RSM method, each dependent variable possesses an especial model that expressed separately main effects and interaction of factors on each variable state which is apparent in Eq. 1:

$$Y = b_0 + \Sigma\, bi\, xi + \Sigma\, bii\, xi^2 + \Sigma\, bij\, xixj \quad \text{Eq. (1)}$$

Here, Y is the predicted response, b_0 is the constant coefficient, b_i is the linear coefficient, b_{ii} is the squares coefficient, b_{ij} is the interaction coeficient, x_i and x_j are coded independent variables.

30 Liposomes containing essential oil of ZEO were prepared in order to optimize the process based on Central Composite Design (CCD) (Table 2) and heating method (or Mozafari method). Liposome components include phosphatidylcholine and essential oil of ZEO were hydrated through the addition of deionized water and glycerol (3% v/v). In the subsequent stage, they were mixed with each other in different temperatures and times via blender at the speed of 1000 rpm. In order for stability of the product, the resulted Liposomes solution were stored at first for 1 hour at room temperature and then in refrigerator at 4° C (Colas *et al*., 2007; Rasti *et al*., 2012).

Table 2: Central composite design for evaluation of process variables on response

1.	Phosphatidyl choline (PC) % w/w	PC/ oil % w/w	Temperature °C	Time min
2.	2	0.63	40	20
3.	2.5	0.81	45	50
4.	2	0.63	30	40
5.	1.5	0.44	45	50
6.	2	0.63	50	40
7.	2.5	0.44	35	50
8.	1.5	0.81	45	50
9.	2.5	0.44	45	50
10.	2.5	0.81	45	30
11.	3	0.63	40	40
12.	2	0.63	40	40
13.	2	0.63	40	40
14.	1.5	0.44	45	30
15.	2.5	0.44	35	30
16.	2	1.00	40	40
17.	2.5	0.81	35	50
18.	2	0.63	40	60
19.	2	0.63	40	40
20.	1.5	0.81	35	50
21.	1.5	0.81	35	30
22.	2	0.63	40	40
23.	1.5	0.44	35	50
24.	1	0.63	40	40
25.	2.5	0.81	35	30
26.	2	0.63	40	40
27.	2.5	0.44	45	30
28.	2	0.25	40	40
29.	1.5	0.81	45	30
30.	1.5	0.44	35	30

Encapsulation of *Zataria multiflora* essential oil in Liposome: Preparation of nanoliposome from phosphatidylcholine and glycerol were carried out according to the method of Colas *et al*., (2017), Rasti, *et al*., (2012), and Vafabakhsh *et al*., (2013) with the necessary improvement. Nanoliposomes containing essential oil of ZEO were evaluated at the concentrations of 0.8, 0.4, 0.1, and 0.05 % w/w.

Determination of encapsulation efficiency of the Liposomes containing essential oil of ZEO (EE%): At first, by using a solution of Tween 80 (0.5% v/v), distinct dilutions of essential oil of ZEO were prepared and then, calibration curve was obtained based on the concentration of essential oil versus optical absorption at the wavelength of 274 nm (the maximum wavelength of essence) by spectrophotometer (Secomam XTD5, France). Vesicles containing the essential oil were separated by using centrifuge at 2000 × g for 30 min and the supernatant and pellet were transferred via deionized water. Correspondingly, by the usage of 1 ml of 0.02% Triton X-100, the nanoliposome was disrupted and its essential oil was released. 1 ml of 0.02% Triton was added to 1 ml supernatant and the amount of essential oil in supernatant was calculated. Encapsulation efficiency was calculated based on Eq. 2 (Vafabakhsh *et al*., 2013).

$$\% EE = P/(S+P) \times 100 \quad \text{Eq. (2)}$$

In which P is the measure of essential oil in residuum and S is the measure of essential oil in supernatant.

Essential oil analysis by GC/MS: Analysis of the extract had been carried out by GC/MS (Agilent 5975C) and GC/FID (Agilent 6890N) systems both with a HP-5MS (30 m X 0.25 mm X 0.25 µm film thickness) capillary column. Helium was used as carrier gas of these systems with a flow rate of 0.5 mL/ min. Oven temperature was raised from 40 to 140 °C at 5 °C/ min. it was remained for 1 hour and then with the speed of 30° C/min reached to 280° C and were kept 18 min. Similarly, the ionization energy of 70 eV was consumed and injection volume was 2 µl. The percentage composition was obtained by normalization from FID The identification of the components of the natural and modified essential oil were carried out by comparison of mass spectra data with NIST 14 and Willey 275 libraries, and also, by the calculated and literature linear retention indices. The number of compounds was calculated by usage of its specific standard injection amount.

Determination of Anti-oxidant activity of essential oil in both free and encapsulated by DPPH: To assess the strength of essential oil (in free and liposomal encapsulated) on trapping free radical DPPH, 50 µl of sample was mixed with 2 ml of 0.004% methanol DPPH. After 5 min of incubation at room temperature and dark environment, the absorption of the control and sample and eliminating proportions of free radicals were determined at a wavelength of 517 nm. Anti-oxidant activity of essential oil was compared and evaluated via DPPH method in concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 (%) one time in free mode and on the other time in the liposomal encapsulated form. Scavenging proportions of free radicals was calculated by using the Eq. 3:

$$\% \text{ Inhibition} = (\text{Control absorbance} - \text{Sample absorbance})/\text{Control absorbance} \times 100 \quad \text{Eq. (3)}$$

To sum up, measuring anti-oxidant activity of the Liposomal solution containing the essential oil of ZEO and its free form in minced meat was done by utilizing DPPH (Vida Mortes *et al*., 2009) With modification, and TBA (AOAC 1990).

Distinct amounts of essential oil (in free and encapsulated form) were added to both raw and cooked minced meat at different content (0, 0.05, 0.1, 0.4 and 0.8 %) and homogenized. Sensory

evaluation was carried out by a group of 10 people after training the test to them. Investigation of sensory properties was based on taste, odor, color, texture and overall acceptability in five points hedonic scale as: 1- very poor, 2- poor, 3- average, 4- good, 5- very good. In order to prevent distortion of the results, samples were randomly coded.

Statistical design of research: In this study, the effect of ZEO in different amount in both free and encapsulated form on the minced meat was discussed and investigated for discovering the antioxidant properties on days 0, 3, 6 with 3 times repetition in a random. One-way ANOVA was performed, and average different treatments were compared by Duncan's multiple range test. In addition, Wilcoxon test was used as a nonparametric one to evaluate sensory characteristics and just in the case of being significant, Mann-Whitney test was performed.

RESULTS AND DISCUSSION

The average size of particles and dispensation of liposomes size were studied by using dynamic light scattering technology (DLS) in our previous investigating (Khosravi *et al*., 2016). Liposomes containing appropriate essential oil of ZEO showed a polydispersity coefficient in the range of 0 for monodispers systems and 1 for dispersions contain polydispers particles (Colas *et al*., 2007).

The main components of the essential oil of ZEO were thymol (48.2%) and carvacrol (13.8%). The total amount of phenolic compounds had been measured in our previous study (Khosravi *et al*., 2016) according to Dorman (Dorman *et al*., 2003) and absorption at 765 nm was read through spectrophotometric ultraviolet-visible method.

Determination antioxidant activity of essential oils in both free and encapsulated using DPPH: As shown in Table 3 and Figure 1, in all samples free radicals in liposomal essential oil was higher than free form. There was a significant difference between trials, which represents correct selection of variables and levels showing positive impact of encapsulation on increased anti-oxidant properties of essential oil of ZEO.

Table 3. Free radical scavenging of essential oil of *Zataria multiflora*[§] in free and liposome encapsulated form

Sample	Free radical Scavenging %
Sample 1	92.00±1.67
Sample 2	92.9±1.02
Sample 3	93.3±1.14
Sample 4	94.2±0.88
Sample 5	93.9±2.1
Sample 6	95.1±1.01
Sample 7	95.5±1.16
Sample 8	96.9±0.81
Sample 9	97.1±1.98
Sample 10	97.9±1.21

[§]Sample 1: containing 0.05% essential oil in free form. Sample 2: containing 0.05% encapsulated essential oil. Sample 3: containing 0.1% essential oil in free form. Sample 4: containing 0.1 % encapsulated Essential oil, sample 5: containing 0.2% essential oil in free form. Sample 6: containing 0.2% encapsulated essential oil. Sample 7: cont-aining 0.4% essential oil in free form. Sample 8: containing 0.4% encapsulated essential oil. Sample 9: containing 0.8% essential oil in the free form. Sample 10: containing 0.8% encapsulated essential oil

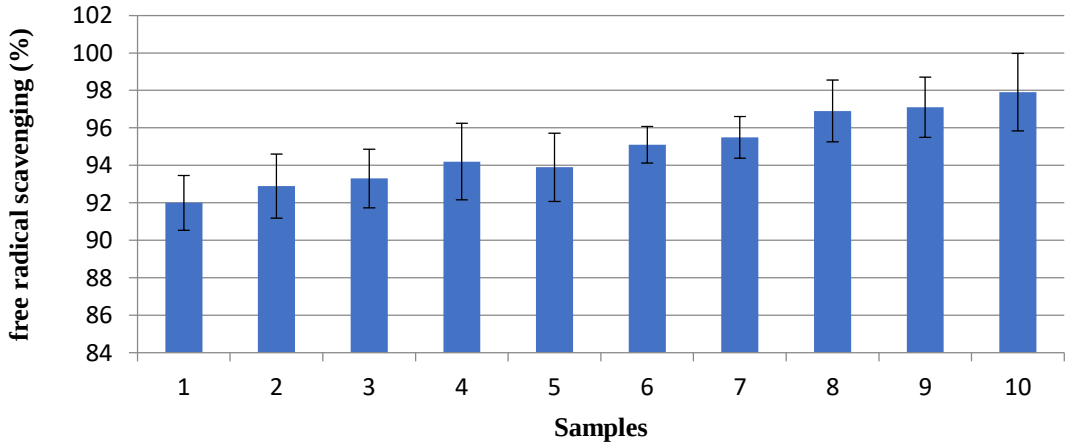

Fig. 1: Free radical scavenging by the *Zataria* essential oil before and after micro-coating in nanoliposome

Measuring the antioxidant activity of liposomal solution contains essential oils of ZEO and free form in minced beef: Scavenging of free radicals by ZEO before and after microencapsulation in minced beef is shown in Table 4. As can be seen, free radical scavenging of the samples is reduced during 6 days of refrigerated storage.

Table 4: Free radical scavenging of liposomal essential oil of *Zataria multiflora* in minced meat within 6 days refrigerated storage

Time (day) Sample	0	3	6
Sample 1	36.28± 0.07 [a]	25.62 ± 0.03 [a]	13.57 ± 0.09[a]
Sample 2	53.84± 0.12 [b]	48.40± 0.16 [b]	44.42± 0.14[b]
Sample 3	54.80 ±0.11[c]	53.27 ± 0.19[c]	50.39 ±0.13[c]
Sample 4	55.43 ± 0.06 [c]	52.42 ± 0.09 [c]	48.43 ± 0.14[d]
Sample 5	57.68 ± 0.06 [e]	56.11 ±0.19 [d]	54.73 ± 0.13[e]
Sample 6	56.89 ±0.19 [f]	53.48 ± 0.14 [e]	50.39 ± 0.14[f]
Sample 7	60.38 ± 0.04 [g]	58.85 ± 0.11 [f]	57.71 ± 0.09[g]
Sample 8	60.91 ±0.02[h]	57.28 ±0.09[g]	54.33 ± 0.19[g]
Sample 9	64.93± 0.12[i]	62.79 ± 0.06[g]	60.98 ±0.11[g]
Sample 10	62.44 ±0.16[j]	58.89 ± 0.12[i]	56.68 ± 0.04[i]
Sample 11	66.35 ±0.09 [k]	64.64 ± 0.03 [j]	62.44 ± 0.13[j]

In the same column, means followed by different small letters (a-k) differ significantly (P≤0.05).

Also, according to Table 5, in the process of changing the TBA, samples are quite different, and by the end of the 6th day, all the results were increased. At the beginning of the study (day zero) was related to the sample 1. The lowest and highest amounts of TBA at 6th day were observed for sample NO.1 and 11, respectively.

Table 5: Antioxidant activity[§] of liposomal essential oil of *Zataria multiflora* in minced meat within 6 days refrigerated storage (×100)[§§]

Time (day) Sample	0	3	6
Sample 1	1.17±0.08[g]	6.58±0.36[a]	84.17.0.50[a]
Sample 2	1.58±0.08[fg]	46.08±0.30[b]	78.25±0.29[b]
Sample 3	2.25±0.14[ef]	38.67±0.22[c]	69.08±0.16[c]
Sample 4	2.42±0.16[e]	42.17±0.22[d]	70.00±0.38[d]
Sample 5	34.2±0.22[d]	35.42±0.30[e]	54.42±0.22[e]
Sample 6	3.83±0.08[d]	36.50±0.25[f]	66.08±0.22[f]
Sample 7	5.67±0.22[c]	30.75±0.29[g]	50.00±0.14[g]
Sample 8	5.83±0.16[c]	29.58±0.30[h]	58.83±0.22[h]
Sample 9	8.25±0.14[b]	26.67±0.16[i]	46.08±0.16[i]
Sample 10	8.83±0.68[b]	26.25±0.25[ij]	50.92±0.16[j]
Sample 11	14.72±0.29[a]	40.55±0.36[j]	42.17±0.22[k]

[§] Sample 1: control. Sample 2: containing 0.05% essential oil in free form. Sample 3: containing 0.05% encapsulated essential oil. Sample 4: containing 0.1% essential oil in free form. Sample 5: containing 0.1 % encapsulated Essential oil, sample 6: containing 0.2% essential oil in free form. Sample 7: containing 0.2% encapsulated essential oil. Sample 8: containing 0.4% essential oil in free form. Sample 9: containing 0.4% encapsulated essential oil. Sample 10: containing 0.8% essential oil in the free form. Sample 11: containing 0.8% encapsulated essential oil

[§§] In the same column, means followed by different small letters (a-k) differ significantly (P≤0.05).

Sensory analyses: The use of essential oils as natural preservatives will be practical if and only if it does not cause any negative impact on sensory properties of meat. It is clear that the essential oil like any other food seasoning may be considered undesirable by some consumers, anyway, others who like herbal flavor of seasoning (or those who do not like the taste of meat), incorporation of essential could be desirable (Michalczyk *et al*., 2012). So, it was necessary for sensory evaluation on samples of minced meat with and without oil in both raw and cooked done.

The results of sensory analyses are provided in figure 2 to 6 for raw meat samples, and figure 7 for cooked meat. Figure show that a significant difference in terms of taste, color, texture, odor and overall acceptability between samples containing free form of essential and samples containing encapsulated essential oil. According to these results, it can be concluded that the essence of liposomes improved the sensory characteristics of products in both raw and cooked.

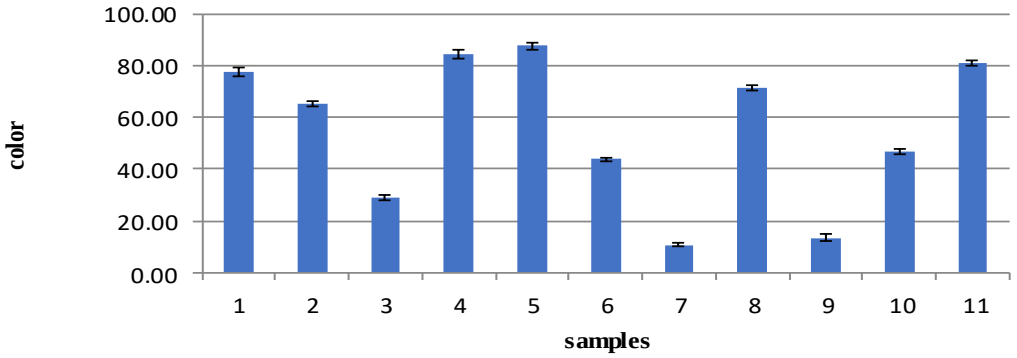

Fig. 2 color evaluation of the raw minced beef containing free and encapsulated forms of essential (Sample 1: control. Sample 2: containing 0.05% essential oil in free form. Sample 3: containing 0.05% encapsulated essential oil. Sample 4: containing 0.1% essential oil in free form. Sample 5: containing 0.1 % encapsulated Essential oil, sample 6: containing 0.2% essential oil in free form. Sample 7: containing 0.2% encapsulated essential oil. Sample 8: containing 0.4% essential oil in free form. Sample 9: containing 0.4% encapsulated essential oil. Sample 10: containing 0.8% essential oil in the free form. Sample 11: containing 0.8% encapsulated essential oil

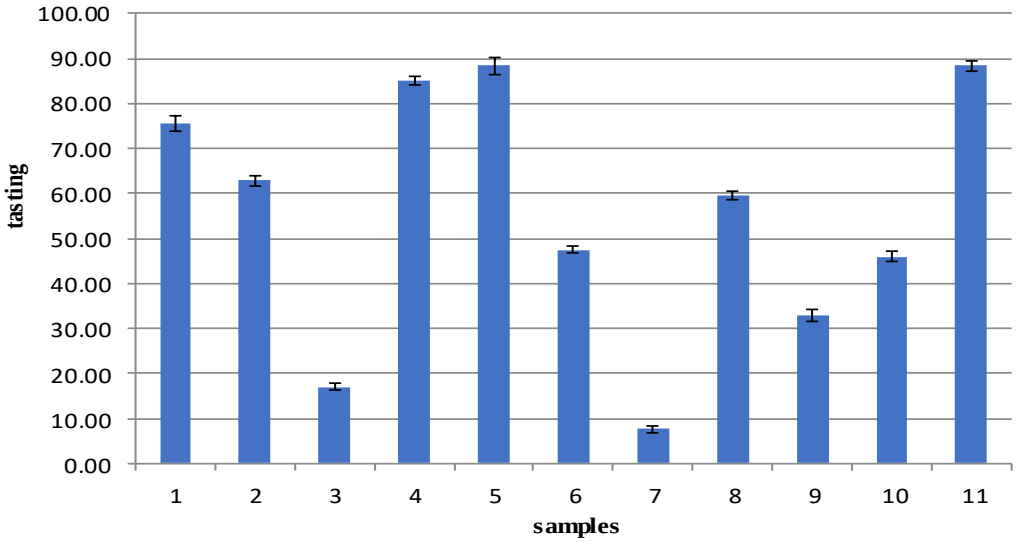

Fig. 3. taste evaluation of the raw minced beef containing free and encapsulated forms of essential (Sample 1: control. Sample 2: containing 0.05% essential oil in free form. Sample 3: containing 0.05% encapsulated essential oil. Sample 4: containing 0.1% essential oil in free form. Sample 5: containing 0.1 % encapsulated Essential oil, sample 6: containing 0.2% essential oil in free form. Sample 7: containing 0.2% encapsulated essential oil. Sample 8: containing 0.4% essential oil in free form. Sample 9: containing 0.4% encapsulated essential oil. Sample 10: containing 0.8% essential oil in the free form. Sample 11: containing 0.8% encapsulated essential oil)

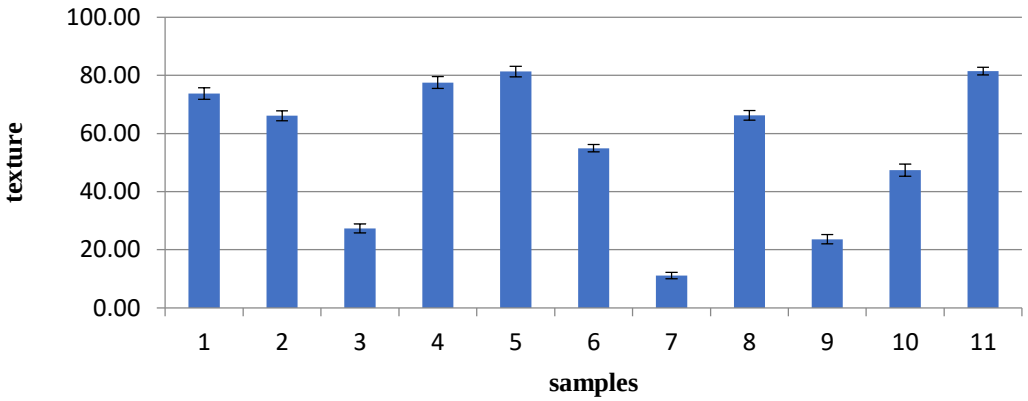

Fig. 4. Tissue evaluation of the raw minced beef containing free and encapsulated forms of essential (Sample 1: control. Sample 2: containing 0.05% essential oil in free form. Sample 3: containing 0.05% encapsulated essential oil. Sample 4: containing 0.1% essential oil in free form. Sample 5: containing 0.1 % encapsulated Essential oil, sample 6: containing 0.2% essential oil in free form. Sample 7: containing 0.2% encapsulated essential oil. Sample 8: containing 0.4% essential oil in free form. Sample 9: containing 0.4% encapsulated essential oil. Sample 10: containing 0.8% essential oil in the free form. Sample 11: containing 0.8% encapsulated essential oil

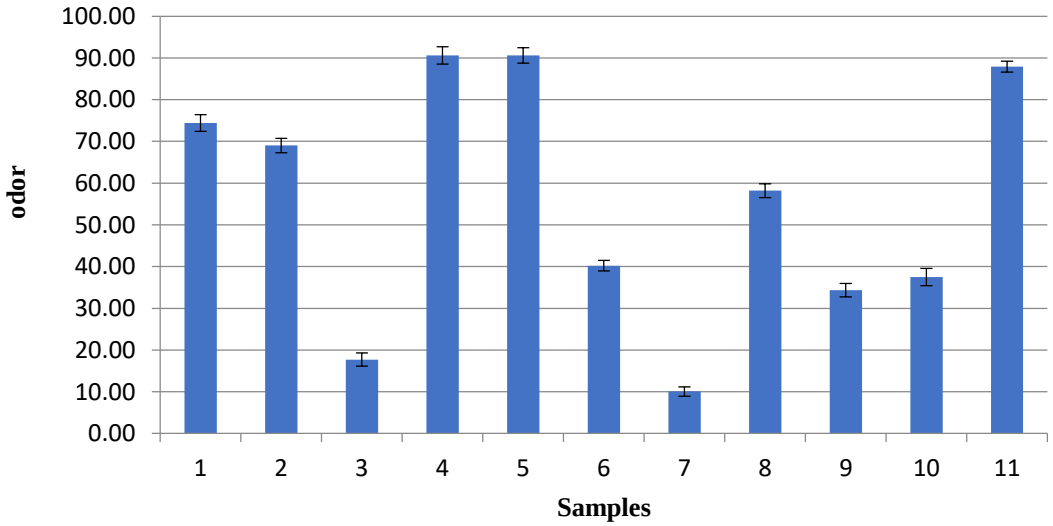

Fig. 5. odor evaluation of the raw minced beef containing free and encapsulated forms of essential (Sample 1: control. Sample 2: containing 0.05% essential oil in free form. Sample 3: containing 0.05% encapsulated essential oil. Sample 4: containing 0.1% essential oil in free form. Sample 5: containing 0.1 % encapsulated Essential oil, sample 6: containing 0.2% essential oil in free form. Sample 7: containing 0.2% encapsulated essential oil. Sample 8: containing 0.4% essential oil in free form. Sample 9: containing 0.4% encapsulated essential oil. Sample 10: containing 0.8% essential oil in the free form. Sample 11: containing 0.8% encapsulated essential oil

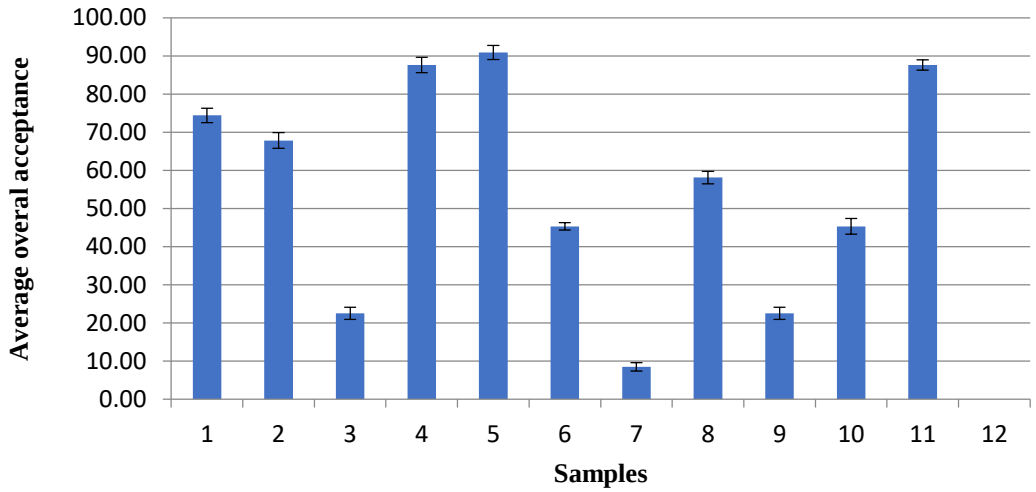

Fig. 6. Overall acceptance of the raw minced beef containing free and encapsulated forms of essential (Sample 1: control. Sample 2: containing 0.05% essential oil in free form. Sample 3: containing 0.05% encapsulated essential oil. Sample 4: containing 0.1% essential oil in free form. Sample 5: containing 0.1 % encapsulated Essential oil, sample 6: containing 0.2% essential oil in free form. Sample 7: containing 0.2% encapsulated essential oil. Sample 8: containing 0.4% essential oil in free form. Sample 9: containing 0.4% encapsulated essential oil. Sample 10: containing 0.8% essential oil in the free form. Sample 11: containing 0.8% encapsulated essential oil

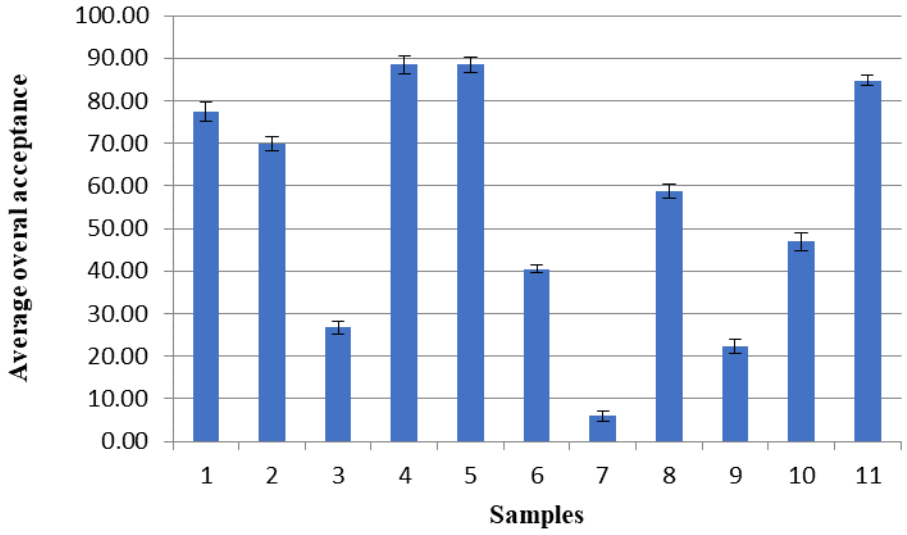

Fig. 7: Overall acceptance of the cooked minced beef containing free and encapsulated forms of essential (Sample 1: control. Sample 2: containing 0.05% essential oil in free form. Sample 3: containing 0.05% encapsulated essential oil. Sample 4: containing 0.1% essential oil in free form. Sample 5: containing 0.1 % encapsulated Essential oil, sample 6: containing 0.2% essential oil in free form. Sample 7: containing 0.2% encapsulated essential oil. Sample 8: containing 0.4% essential oil in free form. Sample 9: containing 0.4% encapsulated essential oil. Sample 10: containing 0.8% essential oil in the free form. Sample 11: containing 0.8% encapsulated essential oil

As the results of the Kruskal-Wallis follows, there is a difference between raw samples based on taste. So according to Figure 8, samples containing encapsulated essential oils at concentrations of 0.05%, 0.1%, and control sample, had the highest acceptance tasting, respectively, which is indicative of the positive effect of microencapsulation. Table 6 shows other quality parameters of meat, effective concentrations of free and encapsulated essential oils. Data represents all the positive effect of microencapsulation.

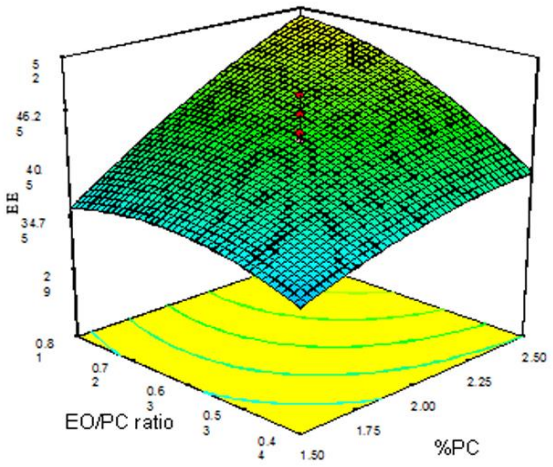

Fig. 8: Three-dimensional diagram of interaction effect of phosphatidylcoline and ratio of essential oil to phosphatidylcholine

Table 6. Sensory properties of free and liposomal essential oil of *Zataria multiflora* in minced meat in first day of production

Influencing factor	Order of panel scores for encapsulated essential oil	Order of panel scores for free essential oil
Taste	Control<0.1<0.05	0.2<0.1<0.05
Color	Control>0.1>0.05	0.2<0.1<0.05
Texture	Control<0.1<0.05	0.2<0.1<0.05
Odor	Control>0.1>0.05	0.2<0.1<0.05
Total acceptance	Control<0.1<0.05	0.2<0.1<0.05

Optimization of microencapsulation efficiency by Mozafari method using a central composite design: According to what was mentioned earlier, the experiments were designed and conducted in accordance with Table 2. Responses obtained were analyzed using the software Expert Design v.7. After quadratic regression, Eq. 4 was obtained based on coded values:

$$\%\ EE = +43.61 + 5.21\ X_1 + 3.26\ X_2 - 3.41X_3 + 2.41X_1X_2 - 1.1X_{12} - 2.23X_{22} \quad \text{Eq. 4}$$

The model was adapted on experimental data and the coefficient of R^2 and adjusted-R^2 was obtained at. $R^2 = 0.90$ and adjusted $R^2 = 0.87$,

respectively. These values show a suitable fitness between experimented and predicted results. It can be seen that proposed model fit on real data (Figure 9).

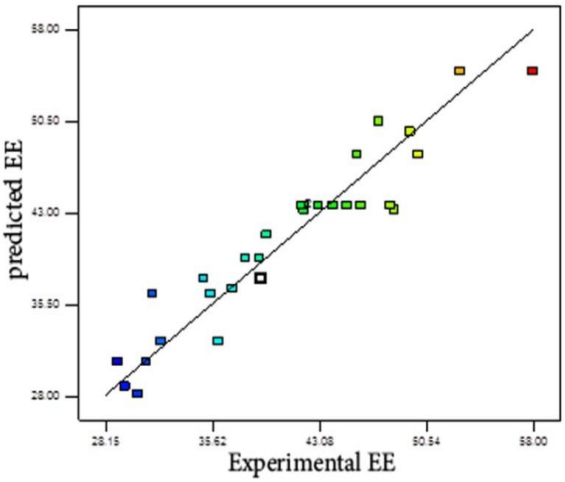

Fig. 9. Regression of predicted versus the experimental encapsulation percentage Y=1.057X – 5.4531; R^2= 0.9982

Analysis of variances of results for encapsulated essential oil produced by heating method using central composite design are shown in Table 7.

Table 7: Analysis of variance for central composite design of liposomal encapsulation of *Zataria multiflora* essential oil

Source	Degree of Freedom	Sum of Square	P value	F value
Model	6	1438.43	0.000	35.19
X_1	1	648.96	0.000	95.25
X_2	1	254.8	0.000	37.40
X_3	1	278.8	0.000	40.92
X_1X_2	1	93.12	0.001	13.67
X_1^2	1	34.22	0.035	5.02
X_2^2	1	142.01	0.000	20.84
Lack of Fit	18	126.97	0.463	1.19

As shown in Table 7, the lack of fit error is not significant, and the value is 0.46 and 1.19, respectively for P and F. Three independent variables of phosphatidylcholine percent (X_1), the ratio of essential oil to phosphatidylcholine (X_2) and the temperature (X_3) are effective linearly on the percentage of liposomes containing encapsulated essential oils. Among them phosphatidylcholine by a coefficient of 5.21, has the highest impact on the encapsulation efficiency of liposomes containing essential oil. Followed by variable ratio of the essential oil to phosphatidylcholine by a factor of 3.26 directly and changing the process temperature by a factor of -3.41, inversely, correlated with the percentage of encapsulated essential oil. Interaction effects of "ratio of the essential oil to phosphatidylcholine" and "phosphatidylcholine" was significant (P≤0.0012).

Figure 8 shows the interaction effect of variables X_1 and X_2 is significant. As figure shows, with simultaneous increase of phosphatidylcholine and the ratio of the essential oil to phosphatidylcholine, the response increases. So that the interaction of these two variables was significant and most response was obtained at the point of phosphatidylcholine 2.5 % and the ratio of essential oil to phosphatidylcholine was 0.81

Finally, the results of process optimization showed that the best condition can be achieved when the variables of phosphatidylcholine percent, the of ratio essential oil to lecithin, temperature and time were adjusted at 2.5 %, 0.81, 35°C, and 42 min, respictively. In this condition the best predicted encapsulation efficiency was obtained at 54.4%. A reconfirmation test was conducted to compare this predicted value calculated value with real experimented response in optimal conditions. Predicted encapsulation efficiency was 54.4 ± 0.15 while the experimented amount was 53.5 ± 0.20.

Conclusion

This study showed the effect of process variables on the encapsulation efficiency of liposomal containing thyme essential oil produced by Mozafari. Three investigated variables including phosphatidylcholine percent, the amount of ratio of essential oil to phosphatidylcholine, and temperature, have a significant impact on liposomal encapsulation efficiency. The results of statistical analysis showed that encapsulated phosphatidylcholine is the most important parameter affecting on the microencapsulation percent. The optimal preparation of liposomes include: phosphatidylcholine percent (2.5), the amount of essential oil to phosphatidylcholine (0.81), temperature (35°C) and time (42 min). In the optimum conditions, encapsulation efficiency was estimated 54.4%, which was confirmed by extra test. Essential oil microcapsules showed more anti-oxidant properties in compared to free oil in minced meat. Increased the activity of antioxidant compounds encapsulated in liposomes can be usable as a strong support, not only in the food industry, but also in the cosmetics and pharmaceutical industries as well as promote.

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