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Optimizing Production of *Mentha longifolia* Essential Oil Emulsion Loaded with Omega 3 Fatty Acids by Nano-Fiber/Gas Chromatography

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Abstract

The main aim of this study is providing of *Mentha longifolia* L essential oil emulsion loaded with omega 3 fatty acids and optimization by polyaniline (PANI) nano-fiber/gas chromatography (PANI-F/GC). The head space solid phase microextraction (HS-SPME) method by polyaniline nanofibers was used to extract released essential oils of emulsion. The effects of four factors, including omega 3 percent, surfactant-to-oil ratio (SOR%), surfactant type (TWEEN 20, 80 and caseinate sodium) and storage time on the encapsulation efficiency, and essential oil chromatographic characteristics including total peak number, total peak area and total peak height were studied. The Central Composite Design (CCD) was used to design experiments and study the effects of four factors on the responses and P-value of <0.05 was considered statistically significant. Desirability function (D) was used for determination of optimum condition. Results showed that all four factors affected GC profile, essential oil release and encapsulation efficiency. According to the results the emulsion has the best release power on the condition: Omega 3 (%); 25, Storage Time (day); 60, SOR (%); 193.33 and Surfactant type; T80 where the total peak number was 3, total peak area: 407, total peak height: 77 and encapsulation efficiency was 75%.

Keywords: *Mentha longifolia* L; Essential oil; Omega 3 fatty acids; Emulsion; Nano-fiber; Gas chromatography

Introduction

An emulsion is defined as a mixture of two or more liquids that are unmixable or unblendable. Emulsions are part of a more general class of two-phase systems of matter called colloids [1,2]. Oil and water are the most liquids that are used to form emulsions in the food and pharmaceutical industry. Two types of emulsion are commonly used. Oil/Water (O/W) emulsion is formed by dispersing of oil phase in the water phase, but Water/Oil (W/O) emulsion is formed by dispersing of water phase in the oil phase. Conventional emulsions, nano-emulsions, or micro-emulsions are types of emulsions that are categorized based on their particle diameter and thermodynamic stability [1,2]. The emulsions with mean droplet diameters <200 nm have been defined as nano-emulsions [3,4]. The small particle size of nano-emulsions can lead to good kinetic stability and delivery systems with high optical clarity, so these nano particles are suitable candidates for using in pharmaceutical industries, supplement and food products [3,4]. There are two common methods for providing emulsions, including low energy and high energy method. In the high energy method some instrument and techniques like microfluidizers, high-pressure homogenizers and sonicators are used. These techniques rely on specialized equipment to disrupt and intermingle the oil and water phases. The high energy methods can form small droplets of emulsions [5,6]. Omega-3 fatty acids are important for normal metabolism in the human body. Mammals are unable to synthesize omega-3 fatty acids, so using of foods, including omega-3 is necessary for human health. Now days the food industry is trying to provide and develop new functional foods contain omega-3 fatty acid. Functional foods enriched with omega-3 provide health benefits above their basic nutritional aspects. Some Omega-3 enriched foods like beverages are quite popular, and there are large areas of growth for omega-3 products in countries with both small and large existing omega-3 markets [7-9].

Encapsulation is a useful method for protection, facilitating the use and preserving of some food materials and food ingredients that are sensitive. In fact, by encapsulation method the sensitive ingredients of food are covered and are protected from undesirable reaction like oxidation. Encapsulation can also mask undesirable flavors or odors, control the release rate and location of a compound, and impact bioavailability of the encapsulated material. Hydrophobic liquid containing volatile aroma compounds of plants is called essential oil. Essential oils are also known as volatile oils, ethereal oils, or simply as the oil of the plant from which they were extracted, such as oil of *Mentha longifolia*. These oils have some interesting properties like antioxidant, antimicrobial, antifungal, and insecticidal. Essential oils can be used to further decrease the particle size of emulsions. A variety of oils can be used, including medium chain triglyceride oil, citrus oils, and herb oils [10].

Mentha longifolia plant has some benefit properties like digestive, antispasmodic, and carminative that in Iran is used as a stomach pain relieving agent in traditional medicine. The essential oils of Aerial part of the *Mentha longifolia have* medicinal effects. *The Mentha longifolia* essential oils have antimicrobial, antioxidant, anti-inflammatory, and fungicidal activity that was reported in the previous works. The essential oil content of this medicinal plant is depending on the climatic and geographical factors [11,12].

Solid-phase microextraction (SPME) is a solid phase extraction sampling technique that involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (Sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media, that can be in liquid phase like water and wastewater samples, food samples and so, or gas phase.

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The quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation [13].

In this work, new emulsion based on *Mentha longifolia* essential oil loaded with omega-3 fatty acids was provided. The effects of omega 3 percent, surfactant-to-oil ratio (SOR%), surfactant type (TWEEN 20, 80 and caseinate sodium) and storage time on the encapsulation efficiency and gas chromatography characteristic were studied. The gas chromatography characteristics, including total peak number, total peak area and total peak height were used to study essential oil release from an emulsion. The optimum condition of emulsion production function was obtained based on desirability function.

Experimental

Reagents and chemicals

Fish oil was kindly donated by a zahravi company (Perimondo, New York, NY, USA). Tween 80 and 20 (Sigma- Aldrich, St. Louis, MO, USA) caseinate sodium (5.2 wt% moisture, 0.05 wt% calcium) were obtained from Demelkindustrie (Veghel, The Netherlands). Distilled and deionized water obtained from a water purification system (Milli-Q, Millipore, Darmstadt, Germany) was used to prepare all emulsions. All other chemicals used in this study were purchased from either Sigma-Aldrich (St. Louis, MO)

Mentha longifolia oil (penny royal oil) was kindly donated by the magnolia company (Iran). The supplier reported the chemical composition as determined by gas chromatography-mass spectroscopy instrument (GCMS-QP2010 SE, GAS, SHIMADZU, Japan).

Emulsion preparation

Emulsions were prepared by spontaneous emulsification (according to the reported process) by titrating an organic phase into an aqueous phase [14]. The organic phase consisted of a mixture of fish oil and essential oil (*menthe longifolia* essential oil) and types of surfactants (tween 20, 80, caseinate sodium) by varying the surfactant-to-oil ratio (SOR) (according to the Table 1) were first mixed together for 15 min at 25°C and then the mixture was slowly poured into aqueous phase for 8 hours with continuous stirring at 800 rpm.

A set of standard conditions was used to prepare the emulsions: composition = 20% wt oil, and 80 wt% deionized water. Nevertheless, a number of parameters were systematically varied to determine their influences on the emulsion formation [15,16]. Four main variables were tested (according to the Table 1): surfactant-to-oil ratio, surfactant type, storage time, and fish oil concentration. A series of non-ionic surfactants (TWEEN 20, 80, 20:80 and caseinate sodium) was tested to establish the influence of surfactant type on emulsion formation. The standard conditions mentioned above were utilized to prepare the emulsions in these experiments using fish oil. The surfactant-to-oil ratio was varied to determine the most suitable surfactant concentration to prepare the emulsions. The total oil content was fixed at 20 wt%, while the surfactant-to-oil ratio was varied according to the Table 1 and the following equation:

$$SOR=M_S/M_o\times100$$

Where, M_s and M_o are the masses of the surfactant and oil respectively. The influence of time during storage was determined by varying the time (0-60 days).

Run order	Factors			
	A: Omega 3 (%)	B: Storage Time (day)	C: SOR (%)	D: Surfactant type
1	75	24.6	300	SC
2	52.75	9.2	91	T80
3	50	38.1	155	T20
4	65	1.0	10	SC
5	50	38.1	155	T20
6	75	60.0	300	T80
7	25	60.0	276	T80:20
8	75	30.5	10	T80
9	25	60.0	10	T80
10	75	60.0	68	SC
11	25	1.0	10	T20
12	75	1.0	134	T80:20
13	52.75	9.2	91	T80
14	25	1.0	10	T80
15	25	36.4	10	SC
16	75	1.0	300	T80
17	71.25	60.0	10	T80:20
18	25	30.5	300	T80
19	25	1.0	242	SC
20	25	1.0	10	T80:20
21	71.75	37.8	161	T80:20
22	25	60.0	300	T20
23	43.60	58.8	10	T20
24	25	1.0	10	T80:20
25	75	60.0	10	T20
26	50	38.1	155	T20
27	40.95	1.0	174	T20
28	75	1.0	300	T20
29	25	30.5	300	T80
30	47	1.0	300	T80:20
31	56.07	54.1	300	T80:20
32	35.25	60.0	300	SC

Table 1: List of experiments in the CCD.

Analysis methods

Encapsulation efficiency: Total oil and free oil should be calculated for calculation of encapsulation efficiency so the following processes were done:

a) Extraction of free oil: Extraction of the free oil was made with petroleum ether (b.p.60–90°C) following the method as described by Anna Millqvist-Fureby [17,18], which was modified to suit the sample volume. One gram of emulsion was added to 10 ml dried petroleum ether, and shaken for 2 min. The solvent was then separated by filtration. The solid residue was washed two times in 2 ml petroleum ether. The combined filtrate was evaporated using a rotary evaporator. Fat residue was then dried at 105°C until a constant weight was reached.

b) Extraction of total oil: The extraction of total oil was based on the method of Utai Klinkesorn et al. with some modification [19]. Five-milliliters of distilled water (60°C) were added to 0.5 g emulsion and shaken for 15 min using thermostat, Water Bath Vibrator (CD3192, Xutemp, Hangzhou, China). The resulting solution was then extracted with 25 ml hexane/isopropanol (3:1, v/v). The tubes were then vortexed for 15 min, and centrifuged for another 15 min at 8000 × g. The clear organic phase was collected while the aqueous phase was re-extracted

with the solvent mixture [20,21]. After filtration through anhydrous Na_2SO_4, the solvent was evaporated in a rotary evaporator (RE-52AA, Shanghai Biochemical Instrument Company, China) at 70°C. The solvent-free extract was dried at 105°C. The amount of encapsulated oil was determined gravimetrically Microencapsulation efficiency (MEE)

The encapsulation efficiency (EE) was calculated as follows:

$$EE(\%) = \frac{Total\ oil - Free\ oil}{Total\ oil} \times 100 \quad (1)$$

Chromatographic Conditions: A gas chromatography instrument (Agilent 7890 A, Wilmington, DE, USA) with flame ionization detector (GC-FID) at the following condition was used for separation, detection, and analysis of released essential oils: capillary column, silica, 30 m length, 0.25 µm phase thickness and 320 µm i.d; N_2 used as carrier gas with a flow rate of 2 ml/min; the column pressure was set at 8.8913 psi. Splitless mode injection was 50 ml/min splitting ratio in 0.75 min. The initial column temperature was 50°C and then the temperature was increased to 80°C at the 2°C/min and kept in 80°C for 5 min. The detector temperature was 250°C. Heater temperature 200°C, H_2 flow 27 mL/min and air flow 20 ml/min [23,24].

Headspace extraction procedure: The PANI fiber was synthesized by chemical polymerization on the polyester at room temperature according to the our previous work [22]. HS-SPME extraction procedure was done like previously reported works [23,24].

The emulsion sample (2 ml) was extracted with PANI fiber using headspace solid phase microextraction (HS-SPME). PANI fiber connected to the needle of designed syringe was used. To the condition of provided PANI fiber, it was injected into GC injection port for 1 h at 100°C prior to use. A glass (10 ml) with a polytetrafluoroethylene silicon septum containing a magnetic stir bar and 2 ml of emulsion sample was provided. An aluminium cap was used to seal the vial to prevent sample loss due to evaporation. A hot plate was used to heat vials contained some emulsion samples during the extraction process. When the stirring liquid sample (600 rpm) in the sealed vial is heated on the hotplate, the PANI fiber by designing syringe was exposed to the headspace of it. After completing of extraction of the analyte to PANI fiber, the fiber was withdrawn into the designed syringe needle and removed from the vial, then immediately inserted into the injection port of the GC [23,24]. Some parameters that affect extraction efficiency like, extraction time (20 min) and temperature (80°C) were optimized experimentally. The chromatographic separation is shown in Figure 1.

Statistical analysis: Response surface method (RSM) was used to study the effects of the independent variables, including omega 3 (A), storage time (B), SOR% (C) and type of surfactant (D). The experiments were designed according to the central composite design (CCD). Thirty two Runs were formed. In Table 1, the 4 processing variables as factors, levels and experimental design are given. Statistical analysis was performed through subjection on the data for analysis of variance (ANOVA) using commercial statistics software (Design Expert-7). Multiple range test and Polynomial equations ($p \leq 0.05$) was used to detect different factors effect on the emulsion properties.

Results and Discussion

Morphology

The morphology of P-ANI synthesized on the surface of polyester fiber analyzed by scanning electron microscopy. Figure 2 shows the morphology of P-ANI coated on the surface of polyester fiber. The shape of the particles is typically seed like (≈ 50–130 nm).

Design expert and polynomial equations

Table 2 presents the evaluated responses for each run in Table 1 including, total peak number, total peak area, total peak height and

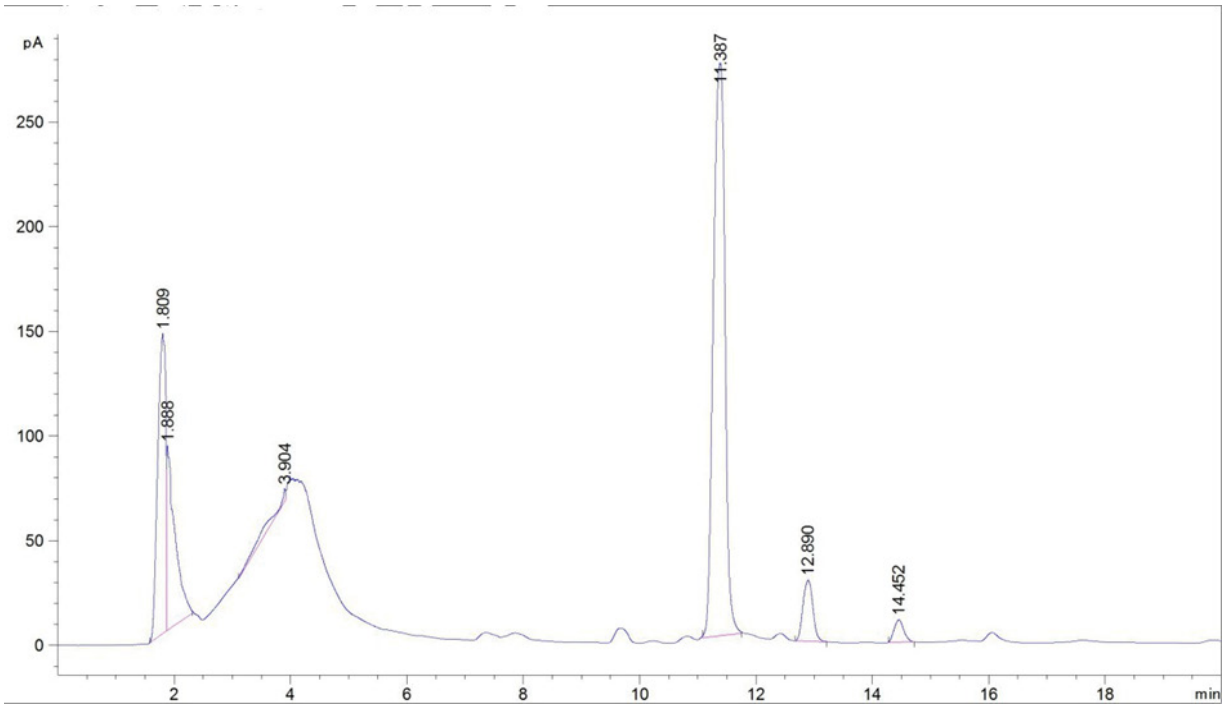


Figure 1: GC-FID chromatogram for volatile organic compounds of emulsion extracted by PANI fiber.

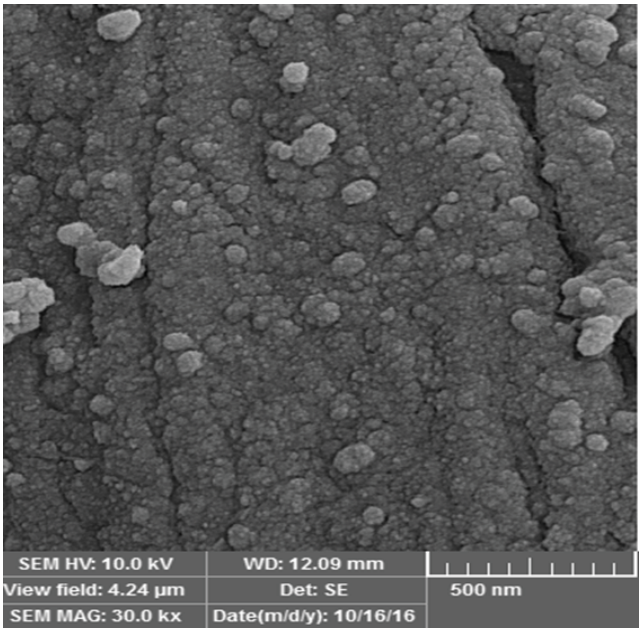


Figure 2: Morphology of P-ANI synthesized on the surface of polyester fiber.

encapsulation efficiency. The Design-Expert software (version 7) was used to perform statistical analysis. Initially, the full term second order polynomial response surface models were fitted to each of the response variables, according to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j + \sum_{i=1}^{3} \beta_{ij} x_{ii}^2 \quad (2)$$

Where Y is the responses (total peak number, total peak area, total peak height and encapsulation efficiency); Xi and Xj are variables and β values are the coefficient values obtained through multiple linear regressions. The quadratic polynomial models for all response functions accompanied by F values and corresponding R2 was used, the estimated regression coefficients summarized in Table 3.

Response surface method

Response Surface Method (RSM) was used to study the effects of four factors on the encapsulation efficiency and chromatographic characteristics of provided emulsions. In this study the contour plots and multi regression plots were used to study factors effects on the responses and interactions of different factors.

Chromatographic responses: The volatile compounds GC profile, including total peak number, total peak area and total peak height were used for studying the effects of four factors, including omega 3 percent, surfactant-to-oil ratio (SOR%), surfactant type (TWEEN 20, 80 and caseinate sodium) and storage time on the volatile compound releasing from emulsion.

Figure 3 shows interaction curves of total peak number versus omega 3% in the different type of surfactant at the storage time (30.5 day) and SOR (155%) and total peak number versus SOR% in the different type of surfactant at the storage time (30.5 day) and omega 3 (50%) and total peak number versus storage time in the different type of surfactant at the omega 3 (50%) and SOR (155%).

According to the Figure 3 increasing of omega 3 from 25 to 75% in the presence of SC, T20 and T80 slightly increases the total peak number, but in the presence of T20:80 decreases the total peak number. Increasing of SOR% from 10 to 160% decreases the total peak number, but after 160% to 300% the total peak number almost is constant for all types of surfactants. The total peak number is increased for all surfactants by increasing of storage time.

Run order	Responses			
	Total peak number	Total peak area	Total peak height	Encapsulation efficiency (%)
1	8	820	146	63.8
2	6	235	83	81.4
3	6	295	74	72
4	10	934	134	55.7
5	5	311	85	81.4
6	8	1178	91	80.4
7	9	627	141	66.0
8	7	1367	260	65.1
9	6	1278	271	72.3
10	11	1131	96	50.2
11	9	1087	159	50.8
12	3	201	98	77.0
13	4	315	75	71.5
14	8	340	88	54.4
15	11	1589	383	59.7
16	5	946	98	85.2
17	13	2024	269	64.5
18	5	431	124	60.5
19	6	465	102	58.8
20	11	1378	261	60.8
21	5	341	125	75.5
22	8	379.6	84	70.7
23	9	1260	268	67.4
24	10	1366	188	71.3
25	11	1489	248	52.5
26	6	353	90	76.0
27	4	418	42	83.5
28	5	334	67	79.1
29	6	759	155	69.4
30	5	536	102	80.0
31	7	587	111	70.4
32	7	769	234	66.3

Table 2: List of responses in the CCD for the each run.

The SPME is a method for extraction of volatile compounds from a headspace of sample and considering that encapsulation technique protect bioactive material (essential oil) against chemical and environmental degradation factors, so it is expected that by encapsulation of essential oil and omega-3, the low volatile compound accumulate in the head space of sample. The volatile compounds in the head space are caused by the essential oil and oxidation of the omega-3. So decreasing of volatile compound in the head space of samples leads to reduction of total peak number in the gas chromatography [25]. Increasing of storage time cause to release of essential oil to the head space, so the total peak number is increased.

It should be noted that all the emulsions showed a lower peak number in comparison with the control sample (the sample of the essential oil without encapsulation). Dias et al. used the SPME method to investigate the products of oxidation of beta-caryophyllene. They reported that beta-caryophyllene emulsion compared to the beta-

Response	Regression equation	Model Summary
Total peak number	=5.556955609 -0.093711304×A+ 1.025317552 × B -1.343693072× C +1.192336915× D_1-1.477937133× D_2 -0.179733966× D_3 +0.909056462× AB+0.671995845× AD_1 +0.425125788× AD_2 -0.070508785× AD_3 -0.259597245× BD_1 -0.883104921× BD_2 +0.192514328× BD_3 -0.582122122× CD_1 +0.73769481× CD_2 +0.644606816× CD_3 +2.569014589× C^2	R-sq= 0.953 R-sq(adj)= 0.895
Total peak area	=436.4087911- 8.093946564× A+ 191.7448448× B -349.2842413× C+95.21633027× D_1 -41.56635817× D_2 -155.0526183× D_3 -35.02569084× AD_1 +256.8420021× AD_2 -87.67357641× AD_3 -184.5255613× BC+2.871472696 × BD_1 +111.216313 × BD_2 -162.0832887× BD_3+46.72043076× CD_1+229.4298495× CD_2-58.97056477× CD_3+549.6992994× C^2	R-sq=0.970 R-sq(adj)= 0.933
Total peak height	Total height =119.4461879-21.50204977× A+28.26674797× B-64.76314278 × C+25.62928665× D_1-25.20132568× D_2-26.59405465 × D_3-27.67634566× AB-48.2282646× AD_1 +29.26286617× AD_2 +16.57379467× AD_3-19.62097401× BC+28.41806686 × CD_1 +15.16039544 × CD_2-21.72498266× CD_3-53.8909451× B^2+ 99.23627192× C^2	R-sq= 0.951 R-sq(adj)= 0.90
Encapsulation Efficiency (%)	=77.96555489+0.487290136× A+0.286941484× B+4.299373327× C-7.443909122× D_1+4.265741504× D_2-0.286371369× D_3-3.516814174× AB+3.67351858× AC-2.460457422× AD_1+4.280249425× AD_2-1.170527672× AD_3-2.605453954× BC-0.300114916× CD_1+0.810430638× CD_2+4.160500924× CD_3-8.203480592× A^2-5.523429726× C^2	R-sq=0.886 R-sq(adj)= 0.75
A=Omega-3 (%) B=storage time (day) C= SOR (%) D= Surfactant type (D1=SC, D2=T80, D3=T20, D4=T80:T20)		

Table 3: Some characteristics of the constructed models for responses.

caryophyllene control solution had a lower total peak area, indicating the protective effect of the nanocomposite structure against oxidation [26].

Figure 4 shows interaction curves of total peak area versus omega 3% in the different type of surfactant at the storage time (30.5 day) and SOR (155%) and total peak area versus SOR% in the different type of surfactant at the storage time (30.5 day) and omega 3 (50%) and total peak area versus storage time in the different type of surfactant at the omega 3 (50%) and SOR (155%).

According to the results (Figure 4), increasing of omega 3 from 25 to 75% (in the presence of SC, T20 and T20:80) increases the total peak number, but in the presence of T80 decreases the total peak area. Increasing of SOR% from 10 to 160% decreases the total peak area, but after 160% to 300% the total peak area is almost constant for all type of surfactants. The total peak area is increased for all surfactants by increasing of storage time.

Increasing the concentration of surfactant increases the absorption of surfactant molecules to the surface of oil/water and decreases surface tension. The decrease in surface tension cause to increase surface motility and superficial disturbances and smaller particles are produced (nanometer) [27]. In the reported works, it had been mentioned that the particle size had the important influence on the volatile compounds protection and big particles can protect the volatile compound lower than small particles [28]. The particle size and encapsulation efficiency were decreased by Increasing of omega-3 percent. In the previous similar works, it has been reported that using of protein compounds for encapsulation of volatile compounds cause to interact between protein and volatile compound and inhibit release of volatile compounds [29].

Encapsulation efficiency: The encapsulation efficiency was used for studying the effects of four factors, including omega 3 percent, surfactant-to-oil ratio (SOR%), surfactant type (TWEEN 20, 80 and caseinate sodium) and storage time on the nano-encapsulation production.

Figure 5 shows Contour plots of encapsulation efficiency versus omega-3 (%), SOR (%) and storage time. According to the Figure 5 and other Contour plots of encapsulation efficiency, increasing of omega 3 from 25 to 62% in the presence of all types of surfactants increases the encapsulation efficiency but from 62% to 75% decreases the encapsulation efficiency. Increasing of SOR% from 10 to 160% increases the encapsulation efficiency but from 160% to 300% the encapsulation efficiency is decreased for all type of surfactants. The encapsulation efficiency is increased for all surfactants by increasing of storage time.

According to the results T80 has the best encapsulation efficiency. The complete coating of the interlayer section and the connection between the hydrophilic sections of the surfactant with the hydrophobic core cause to protect bioactive material in the emulsion structure, but in the case of sodium caseinate because the caseinate is a heavy molecule, molecules interact together and cannot be packed, and the protein cannot reduce surface tension, so the sodium caseinate has the lowest encapsulation efficiency [30]. Ton and coworkers (2015) in the similar study on the microencapsulation of *Rambutan* oil loaded with unsaturated fatty acids by sodium caseinate, isolated whey protein and isolated soybean protein reported that the best encapsulation efficiency was obtained by isolated whey Protein and isolated soybean protein and the worst encapsulation efficiency was obtained by sodium caseinate that confirm our results Figure 5 [31].

Relation between encapsulation efficiency and chromatographic responses: Figure 6 shows main affect plot of encapsulation efficiency versus total peak number and total peak height. According to the Figure 6 there are good relations between encapsulation efficiency and the total peak number and total peak height. Results showed that in the chromatographic data, the high encapsulation efficiency showed the low peak area and peak height.

The SPME is a method for extraction of volatile compounds from a headspace of sample and considering that encapsulation technique protect bioactive material (essential oil) against chemical and environmental degradation factors, so it is expected that by encapsulation of essential oil and omega-3, the low volatile compound accumulate in the head space of sample. The volatile compounds in the head space are caused by the essential oil and oxidation of the omega-3. So decreasing of volatile compound in the head space of samples leads to the reduction of the total peak number in the gas chromatography.

Combined desirability function analysis

The combined desirability function (D) is a parameter that calculated by geometric mean and used for estimate the best condition in the experimental analysis that several factors affect the responses. In the combined desirability function D, each response can be assigned an importance relative to the other responses. The following equation is used to compute the combined desirability function:

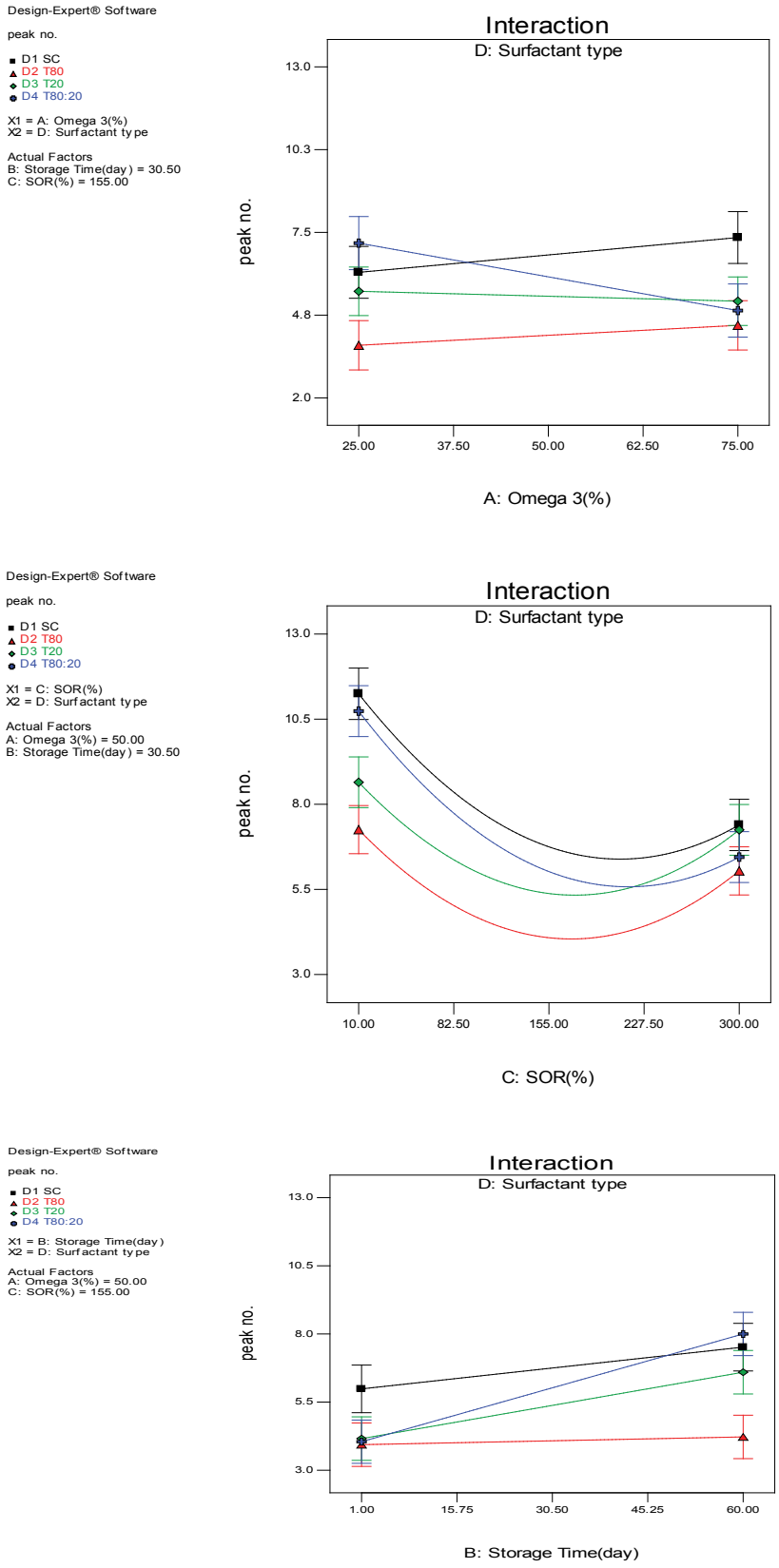
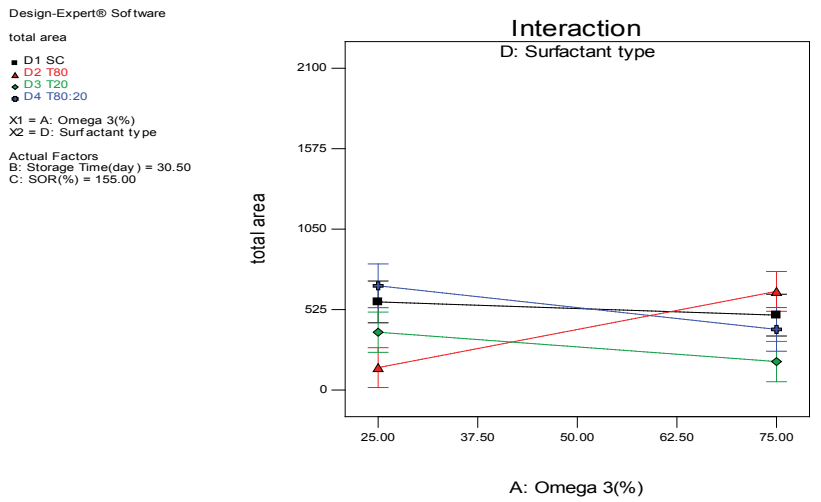
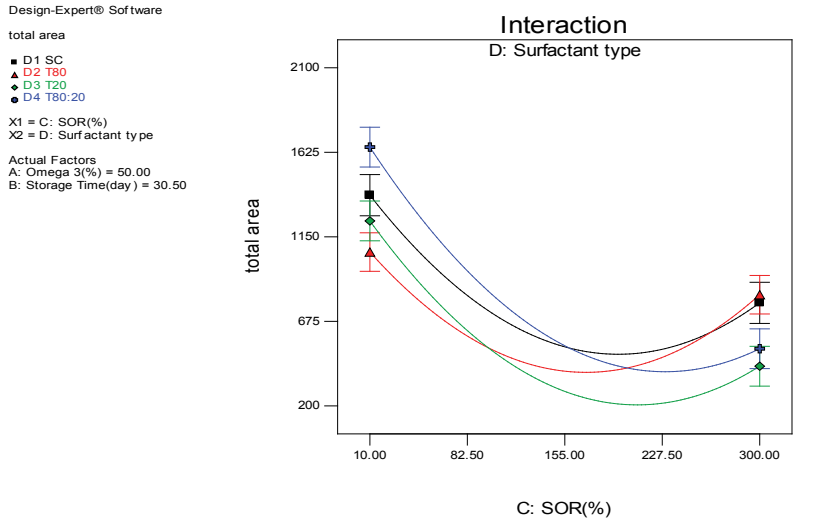


Figure 3: Interaction curve of total peak number versus omega3 (%), SOR (%) and storage time.





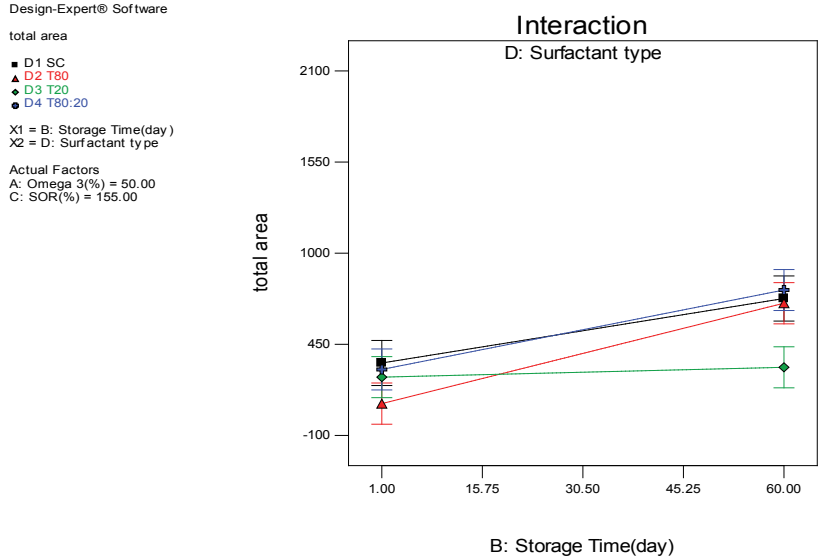


Figure 4: Interaction curve of total peak area versus omega-3 (%), SOR (%) and storage time.

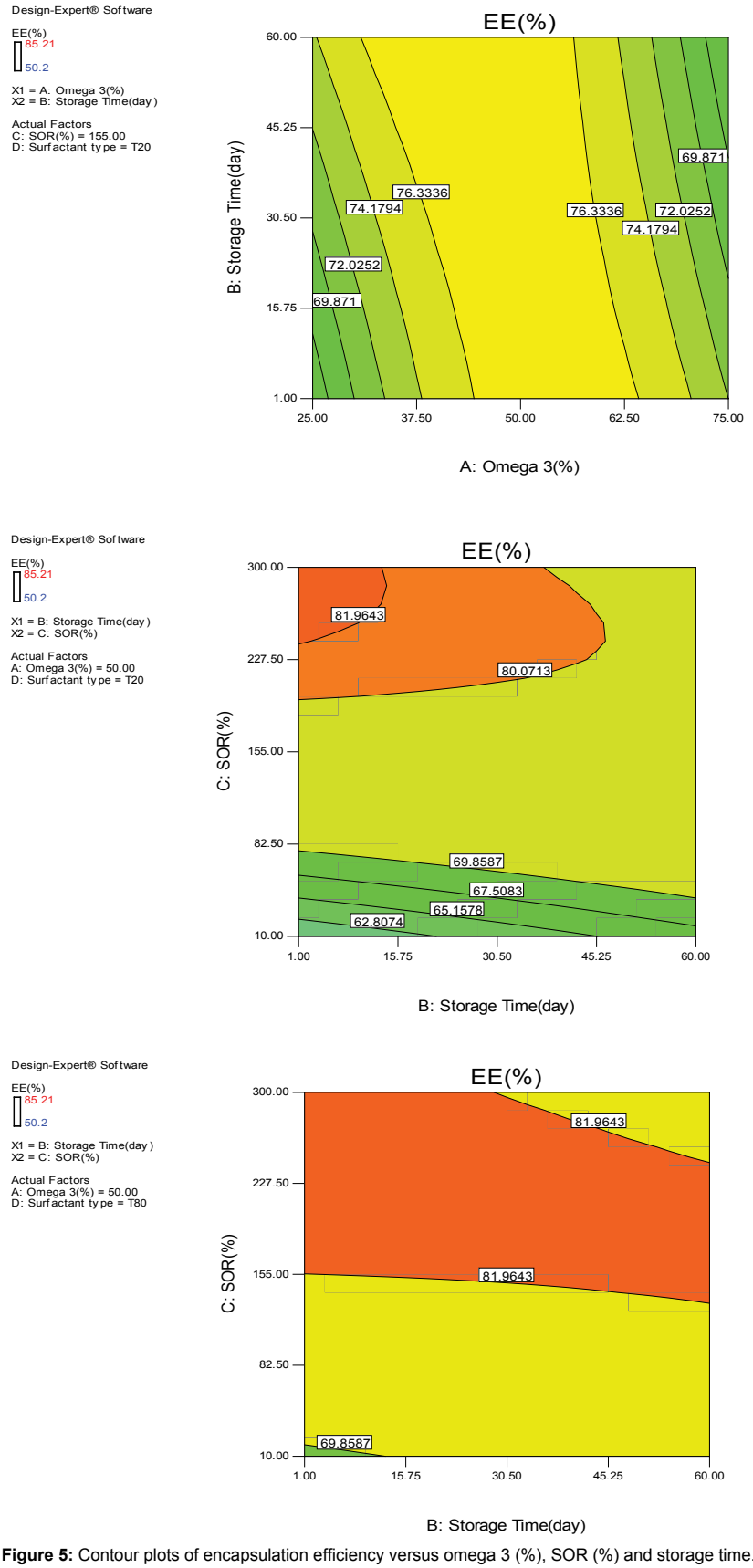


Figure 5: Contour plots of encapsulation efficiency versus omega 3 (%), SOR (%) and storage time.

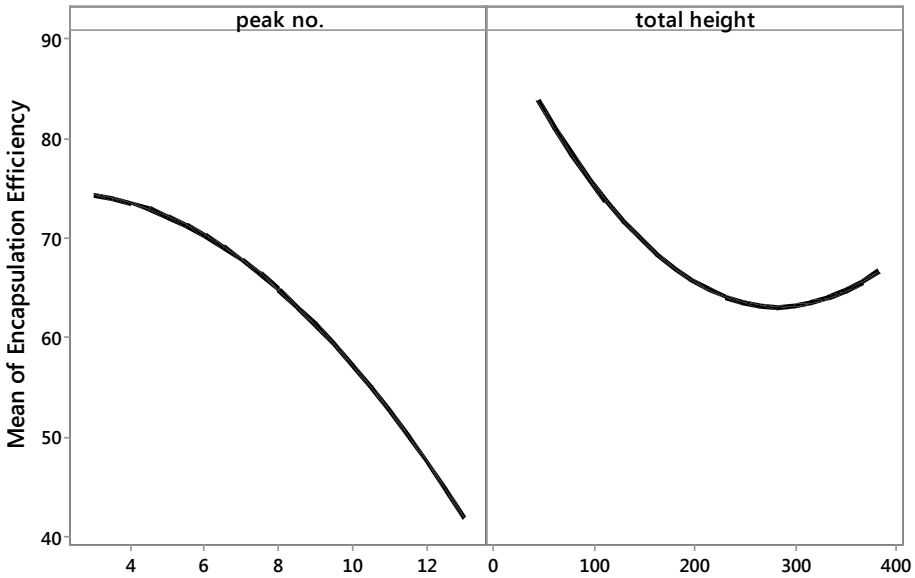


Figure 6: Main affect plot of encapsulation efficiency versus total peak number and total peak height.

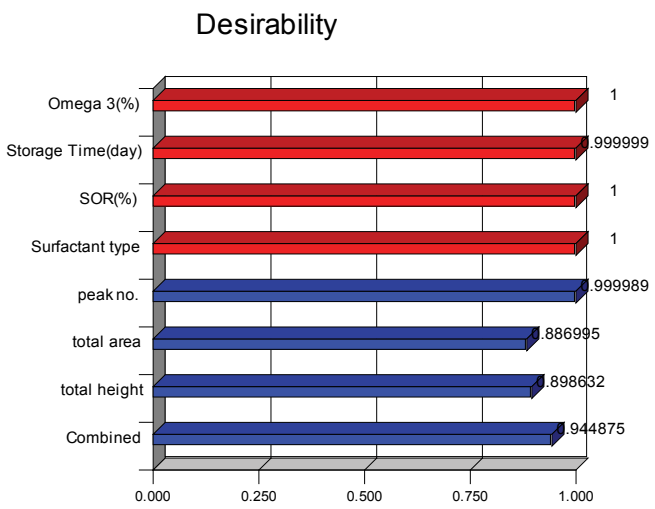


Figure 7: Desirability functions for all factors and resposes.

$$D = \left(d_1^{r1} \times d_2^{r2} \times \ldots d_n^{rn}\right)^{\frac{1}{\sum ri}} = \left(\prod_{i=1}^{n} d_i^{ri}\right)^{\frac{1}{\sum ri}} \quad (3)$$

Where n is the number of responses in the measure.

In this study the combined desirability function was used to estimate the condition that emulsion had the best release of volatile compounds (essential oils) and best encapsulation efficiency and the best omega 3 percent, surfactant-to-oil ratio (SOR%), surfactant type and storage time.

Figure 7 show the desirability functions for factors and responses. According to the Figure 7 combined desirability function was calculated (0.944) and the optimum condition was calculated as: Omega 3(%); 25, Storage Time (day); 60, SOR (%); 193.33 and Surfactant type; T80 where the total peak number is 3, total peak area is 407, total peak height is 77 and encapsulation efficiency is 75%. The mentioned values were selected as optimum values for providing emmulsion that has the best release.

Conclusions

New emulsion based on *Mentha longifolia* Essential oil loaded with omega 3 fatty acids was provided in the present work. The effects of omega 3 percent, surfactant-to-oil ratio (SOR%), surfactant type (TWEEN 20, 80 and caseinate sodium) and storage time (as independent variables) on the encapsulation efficiency and chromatographic characteristics (power of release) were studied. Extraction and determination of released essential oils from emulsion were done by HS-SPME/GC-FID method. The PANI nano fiber was used as an extraction solid phase. The effect of studied factors on the encapsulation efficiency and power of release was done based on the CCD. Desirability function was used for determination of optimum condition. Results showed that all studied four factors affected the

encapsulation efficiency and gas chromatography characteristic. There is a relation between encapsulation efficiency and total peak area. The higher encapsulation efficiency showed the lower total peak number and area. The optimum condition for the best power of release was obtained as: Omega 3(%); 25, Storage Time (day); 60, SOR (%); 193.33 and Surfactant type; T80.

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