Int. J. Nano Dimens., 8 (4): 351-360, Autumn 2017

ORIGINAL ARTICLE

Impact of process parameters in the generation of nanoemulsions containing omega 3 fatty acids and α-tocopherol

***Sepideh Hosseini*[1]*; *Somaye Hosseini*[2]**

[1]*Department of Food Science and Technology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran*

[2]*Plasma Physics Research Centre, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran*

Received 10 July 2017; revised 02 September 2017; accepted 09 September 2017; available online 11 September 2017

Abstract

Nanoemulsion is an alternative and promising approach to overcome insolubility and bioavailability problems of bioactive compounds. In this study, the sonication parameter for preparing nanoemulsions with high α- tocopherol and linolenic acid content were optimized by using response surface methodology and their stability were evaluated during 2 months' storage. The response surface analysis results indicated that the variability of four responses could be depicted as a quadratic function of ultrasonic processing variables. This investigation revealed that, ultrasound cavitation is a powerful promising approach for controlled production of nanoemulsion with small average droplet size in a range of 94.5 ± 1.14 nm with a polydispersity index of 0.1 ± 0.022 with high α- tocopherol and linolenic acid content which are 851 ± 11.62 mg/L and 43 ± 1.27 mg/L, respectively. This study showed that applying appropriate time and intensity of low frequency ultrasound is considerable importance to maintain the nutritional value of nanoemulsions in bioactive delivery system.

Keywords: *a-Tocopherol; Droplet size; Linolenic acid; Nanoemulsion; Polydispersity index.*

How to cite this article

Hosseini S, Hosseini S. Impact of process parameters in the generation of nanoemulsions containing omega 3 fatty acids and α-tocopherol. Int. J. Nano Dimens., 2017; 8 (4): 353-360.

INTRODUCTION

Natural antioxidants such as α, β, γ, δ-tocopherol are commonly used as vitamins or antioxidants in the food and pharmaceutical products [1]. The poor water solubility of these compounds complicate their formulations. Most of antioxidants are usually soluble in organic solvents that lead to insufficient bioavailability after consumption [2]. Consequently, the solubility of these ingredients has become one of the major issues of concern in food industry.

Applying nanoemulsions as an alternative approach contributes to the development of nanometric delivery systems in a considerable manner is capable of encapsulating the antioxidants by protecting them from physicochemical reactions and controlling the changes in organoleptic properties of products [3, 4]. Unique features of nanoemulsions, such as low turbidity, high physical stability and bioavailability make them appropriate for their application in commercial products [5]. Nanoemulsions act as delivery agents of lipophilic bioactive compounds such as curcumin [4, 6], essential oils and antimicrobial agents [7, 8] in the food and pharmaceutical industries [6]. They solubilize water-insoluble pesticides [9] in agrochemical industry and are used in skincare and cosmetics [10, 11].

There are a number of high and low energy emulsification methods for the production of nanoemulsions. The applied shear energy, type and quantity of surfactant and nature of the ingredients are the main parameters of high energy methods while the low-energy methods controlled

* Corresponding Author Email: *Sepidehpaeizan@yahoo.com*

by physicochemical properties of the components [12, 13]. Homogenization valves are one of the traditional high energy methods employed for the formation of nanometric scaled droplets [12]. This process has high energy consumption and only a small fraction of operating energy is used to reduce the size of droplet [14]. Other high-throughput method for producing nanoemulsions is microfluidizer [15].

Expensive production and chambers contamination are the major disadvantages for microfluidization method [16]. Applying low frequency ultrasound for generating emulsion has been established in the recent years [17, 18]. Not all traditional methods are capable of having appropriate control over the droplet size distribution, hence, lead to a poor dispersion stability. High intensity ultrasound outperforms other techniques in generating nanoemulsions without over processing [19], accordingly. It can be claimed that ultrasound processor has capable of producing nanoemulsion at big scale to meet industrial demands [20]. Linseed oil is a low expensive vegetable oil with pleasant odor (especially in relation to other oils high in omega-3 fatty acids) that can be used as the organic phase of emulsions. Encapsulation of this unsaturated oil is made in different manners to prevent chemical degradation and to enhance its incorporation in a product [8, 21].

The objectives of this study are to assess the influence of sonication technique on droplet size, polydispersity index (PDI) and bioactive compound in nanoemulsions; to optimize the processing parameter for preparing nanoemulsions with smallest droplet size and highest amount of α-tocopherol and linolenic acid through response surface methodology (RSM) and also evaluate the stability of optimized nanoemulsion during the storage.

EXPERIMENTAL

Chemicals

The α-tocopherol, linolenic acid, tween80 and span60 are purchased from Sigma Aldrich Chemicals Company (USA). Unrefined cold pressed linseed oil whit contains 51.8% linolenic acid (C18:3 -3) and 11 ppm - tocopherol is purchased from Verjen Company, Gorgan, Iran and consumed as the nonpolar phase, high in omega-3 fatty acid. Fresh deionized water is produced by Milli-Q Plus apparatus (Millipore, Billerica, USA) and is consumed throughout the experiments.

Preparation of emulsion

Linseed oil and deionized water are used as dispersed and continues phase, respectively in order to obtain about 100 mL of emulsion. The organic phase is prepared by dispersing α-tocopherol into the oily phase and then mixture is added to aqueous phase which containing tween 80 and span 60 (HLB = 11.7) as emulsifiers. Then initial emulsions are homogenized by high speed homogenizer (Silverson Model L5M-A, USA) at 9300 rpm for 30 min at room temperature.

Emulsification through ultrasound wave

Sonication is done in the continuous sonication mode at 24 kHz frequency and power of 400 W by an ultrasonic processor (model UP400S, Hielscher Ultrasonics GmbH, Teltow, Germany). This simple ultrasonic device is constructed by an electric ultrasound wave generator and a titanium probe. The intensity of ultrasound wave could be diverse with the amplitude, which is adjustable from 20% to 100% of the maximum amplitude delivered by the sonicator at different time. The power intensities at these operating amplitudes are measured according to method described by Margulis [22].

Experimental design

The RSM and central composite design (CCD) applied to studying the effects of the independent variables: ultrasound ampilitude (X1) and sonicating time (X2) on the droplet size, polydispersity index (PDI), α-tocopherol and linolenic acid content of nanoemulsions. The treatments design and results of the particle size, PDI, α-tocopherol and linolenic acid content are tabulated in Table 1.

Emulsion droplet size and polydispersity index analysis

The nanoemulsions' droplet size is obtained through dynamic laser scattering, were a Master Sizer 2000 (Malvern, Worcestershire, UK) is applied. The obtained droplet size is the average of three measurements taken by He–Ne laser at a 633 nm wavelength and a fixed scattering angle of 173° at 25 °C [23].

Measuring the linolenic acid content

For determination of linolenic acid content, aliquots of nanoemulsion (1 mL) and 5 mL of a mixture of isooctane /2-propanol (3 : 1, v/v) are

placed in 10-mL eppendorf centrifuge tubes and vortexed 3 times, then centrifuged (Xingke model XTA16-3, China) at 18000 × g for 30 minutes. The supernatant is removed, dried under nitrogen gas at room temperature. Subsequently, 1mL of isooctane is added as solvent of oil [24].

The fatty acid methyl esters are prepared according to AOCS method Ce 1-62 [25] and analyzed through gas chromatography (Shimadzu model 14A equipped with a mass spectrometer EI (HP- 6890)) in order to measuring the ω-3 fatty acids content according to the method described by Hosseini *et al*. [26]. The quantification is performed by applying the linolenic standard solution (0.2–1.0 $mgmL^{-1}$).

Measuring the α-tocopherol content

HPLC separation is carried out through liquid chromatography system (CT-10A VP, Shimadzu, Kyoto, Japan) equipped with UV-Vis detector (SPD-20AV) and 3 m silica gel column (Purospher STAR RP-18 encapped, Merck, Germany). Mixture of methanol: water (99: 1 v/v) at 1.0 mL/min is applied as mobile phase and α-tocopherol content is measured at 295 nm. An external calibration is made by applying external calibration functions established by injecting 10 and 20 µL of α-tocopherol solutions (0.2-1 $mgmL^{-1}$) on the subject column [27].

Stability of nanoemulsion during storage

Droplet size and size distribution are among the most important features for assessing the stability of emulsion. Consequently, the effect of storage time on the optimum nanoemulsion is studied. The droplet size, size distribution, α-tocopherol and linolenic acid content are assessed immediately after nanoemulsion production and during 2 months of storage at 4 °C [6].

RESULTS AND DISCUSSION

Selection of independent variables

Exposing food products to ultrasound is advantageous in many processes. In sonication equipment, the power or energy transmitted per unit medium area is known as the intensity that is proportional to the square of the amplitude expressed as $I \propto A^2$. The amplitude of ultrasound waves influences the intensity of cavitation by determining the number of bubbles that implode per unit of time. Thus, during intense cavitation, extreme temperatures and pressures can be produced inside the collapsing bubbles and the molecules present within the bubbles, decompose severely and this can generate several highly reactive radicals in the sonicated medium. This phenomenon indicates that the amplitude must be carefully controlled and maintained during sonication.

Table 1: Scheme of central composite design (CCD): independent (X) and response variables (Y).

Run	X1 Ultrasonic amplitude		X2 Irradiation time	Y1 Droplet size	Y2 Polydispersity index	Y3 α-Tocopherol	Y4 Linolenic acid
	(%)	(Wcm^{-2})*	(min)	(nm)	(%)	(mg/L)	(mg/L)
1	20	40.53	2.0	1106.4	0.93	985.15	49.31
2	100	88.71	2.0	683.1	0.61	934.35	43.72
3	20	40.53	7.0	755.4	0.50	979.14	46.10
4	100	88.71	7.0	84.4	0.18	866.81	40.92
5	20	40.53	4.5	846.3	0.77	983.32	47.91
6	100	88.71	4.5	96.7	0.29	870.06	42.33
7	60	63.05	1.0	998.2	0.83	952.53	46.84
8	60	63.05	8.0	389.7	0.75	922.61	44.19
9	60	63.05	4.5	411.5	0.53	936.80	46.02
10	40	52.84	4.5	745.3	0.48	971.74	47.05
11	80	75.95	4.5	253.2	0.22	919.59	44.61
12	60	63.05	4.5	468.4	0.48	925.46	45.96
13	60	63.05	4.5	419.8	0.44	930.72	46.72

*Acoustic intensity of samples at related operating amplitude.

Selecting an appropriate model

The results of each dependent variable under different processing design conditions are given in Table 2. There is a significant regression correlation (p < 0.05) between the processing variables and response variables according to response surface analysis. The analysis of the droplet size model shows a higher coefficient determination (R^2 = 0.9686) as compared to the PDI model (R^2 = 0.7985). Therefore, more than 95% of the droplet size variations could be appropriately described as the quadratic function of the preparation variables while second-order polynomial model of PDI couldn't explain the systems behaviour completely (the percentage of accurate predictions is 79% of the found response variations).

It is obvious that, the smaller droplet size and narrow PDI is preferable. Moreover, according to the fitted second-order and 2FI models of the experimental data, the coefficients of determination (R^2) for linolenic acid and α- tocopherol values are 0.9821 and 0.9390, respectively. Therefore, obtained models would suffice to explain the effects of the processing variables on linolenic acid and α- tocopherol levels of the nanoemulsions.

Nanoemulsion droplet size

In a general sense, the emulsion systems containing smaller droplet size (< 1 m) is highly recommended in food industry since the nano-sized droplets improve functional properties of poorly water-soluble bioactive compounds [28]. As shown in Table 2, the ultrasound amplitude influences the droplet size of nanoemulsion severely (p < 0.0001) which is then followed by processing time (p < 0.05), while the interaction effects between independent variables have no significant effect on droplet size (p > 0.05).

During emulsification by ultrasound, the size of droplets is controlled by the interaction between breakup and coalescence of droplet [28]. In this process, the droplet breakup rate is restrained by induced shear forces and Laplace pressure of droplets which is affected by the surfactant [2]. Fig. 1(a) clearly illustrates that increasing ultrasound amplitude can reduce the droplet size of nanoemulsion. Ultrasound amplitude results are in agreement with previous findings in the high energy systems [12, 19]. In general, by increase time of sonication droplet size reduces. The data reported here demonstrate that higher treatment time can increase the droplet size. The "over-processing" which is caused by an increase in emulsion droplet coalescence at the higher processing time may be contributed to the above mentioned facts [29, 30]. There is a direct relation between, Bjerknes forces [31] and the applied ultrasound intensity. When emulsions droplets exposed to a standing mechanical wave, not only affect by primary acoustic force, but also experience secondary (Bjerknes) forces that are created by scattered waves by other droplets. These secondary forces can have destructive effect and correlate neighbour droplets and cause recoil back to their equilibrium position [32].

Therefore, there is an optimum power level to obtain the maximum effect of ultrasound emulsification which means the maximum acoustic power has been induced in medium couldn't reduce size of droplet anymore. Another possible explanation is that high level of shear force would increase the temperature which disrupts the interfacial layer and influences the organic phase.

According to Eq. 1, the low density and velocity of sound wave in oil system leads to the total amount of energy received per unit area in oil

Table 2: Analysis of variance (ANOVA) of regression coefficients of the fitted quadratic equations by using F-ratio and p-value in the final reduced models.

variables	Droplet size (nm)			PDI			α-Tocopherol(mg/L)			Linolenic acid(mg/L)		
	F Value	*P* Value	R.C.*	*F* Value	*P* Value	R.C.*	*F* Value	*P* Value	R.C.*	*F* Value	*P* Value	R.C.*
Model	43.26	< 0.0001	Q**	5.55	0.0221	Q**	46.15	< 0.0001	2FI	54.82	< 0.001	Q**
X1	117.75	< 0.0001	-321.53	12.82	0.0090	-0.19	116.69	< 0.0001	-46.53	202.46	< 0.001	-2.7
X2	71.78	0.0205	-226.28	6.31	0.0402	-0.12	13.91	0.0047	-14.48	55.2	0.001	-1.22
X1X2	2.69	0.1451	-61.92	0.00	1.0000	0.00	7.85	0.0201	-15.38	0.33	0.5825	0.1
X_1^2	2.26	0.1761	67.03	0.05	0.8356	0.02	0.00	0.0000	0.00	13.05	0.0086	-0.92
X_2^2	21.8	0.0023	132.60	8.57	0.0221	0.15	0.00	0.0000	0.00	3.07	0.1233	-0.28
R^2		0.9686			0.7985			0.9390			0.9821	

*Regression Coefficient, **Quadratic, X1: ultrasonic amplitude, X2: irradiation time.

become higher than aqueous medium.

$$I = \frac{P_{max}}{2\rho V} \quad (1)$$

where, P_{max} is the wave maximum pressure amplitude, ρ is the medium density and V is the sound velocity in the medium [33].

Our previous study [26] has confirmed that some changes in the physicochemical parameters or structures of oil components had occurred. These changes depended on the sources and initial conditions of the oils as well as the time and intensity of the applied ultrasound. These changes can be controlled by choosing appropriate processing parameters. Although most of the high energy methods are effective and efficient for producing nanoscale droplet but they affect bioactive ingredient [12]. Low energy methods can be used to overcome this problem which also requires high amounts of surfactants that are not very suitable for food systems [16].

The Polydispersity index

Polydispersity index is a measure of the heterogeneity of droplet size in an emulsion which is one of the main parameters for evaluation of nanoemulsion. As expressed in Table 2, time and ultrasound intensity exhibit the significant effects on PDI (p < 0.05). The association of the average PDI with ultrasound amplitude and irradiation time

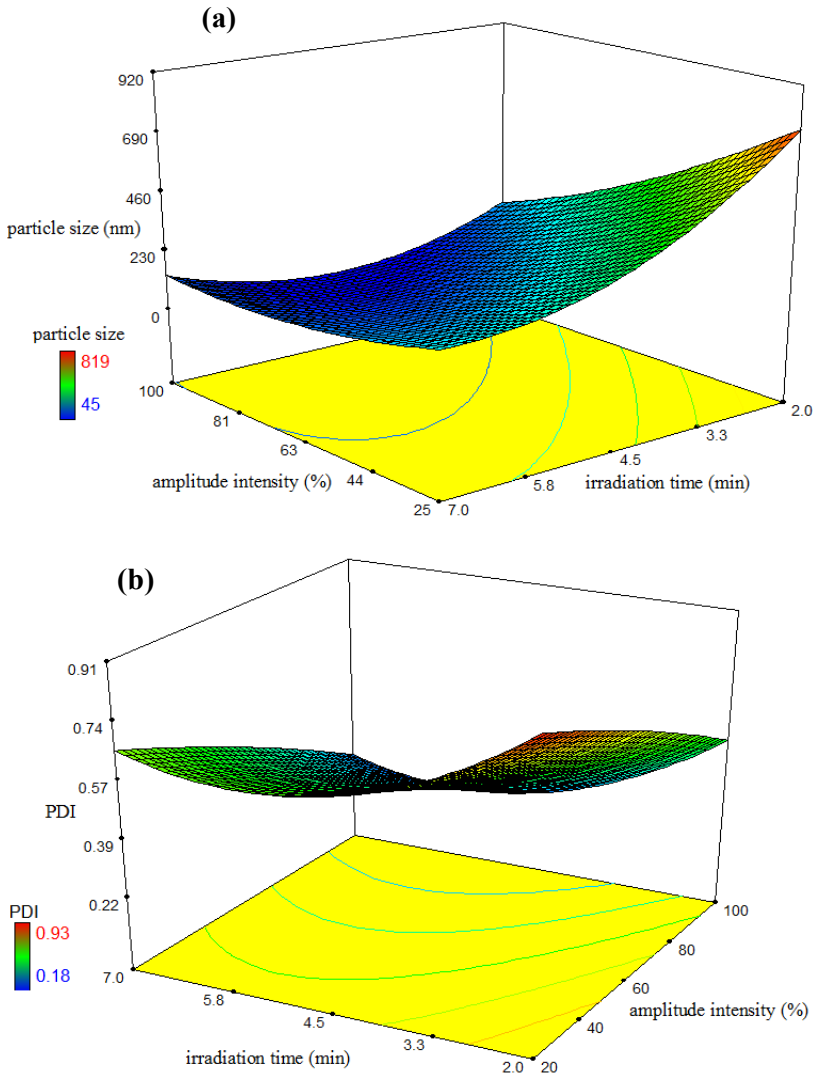

Fig. 1: Response surface plot showing the significant (p < 0.05) effect for droplet size (a) and PDI (b) as a function of ultrasonic amplitude and irradiation time.

is presented in Fig. 1(b). The excessive rise in both sonication parameters may lead to an increase in heterogeneity of droplet size. These findings are in agreement with some previous studies, Tang *et al.* [13], Cheong *et al.* [2], Chu *et al.* [29] and Li and Chiang [7] who observed that an increase in the shear force leads to an increase in the PDI of the final emulsions. It is obvious that over processing would led to raise the possibility of hydrolysis and the oxidation of emulsifier and oil through the free radicals produced in the bubbles cavitation. Thus the optimum level of amplitude, time and temperature sonication procedure should be considered otherwise physicochemical changes are favoured [34, 35].

Effect of process conditions on the α-tocopherol content

Fig. 2(a) shows the tocopherol degradation of nanoemulsion systems. It can be observed that tocopherol remained in linseed oil during nanoemulsion preparation.

After 7 minutes' irradiation of high intensity ultrasound (88.71 W/cm^2) about 86% of total tocopherol was remained. The variables having the largest effect on the degradation of α-tocopherol were the linear term of ultrasound amplitude, followed by the linear term of irradiation time and interactive terms ($p < 0.01$). The α-tocopherol showed a lower degradation rate when it was treated by amplitude and time lower than 60%

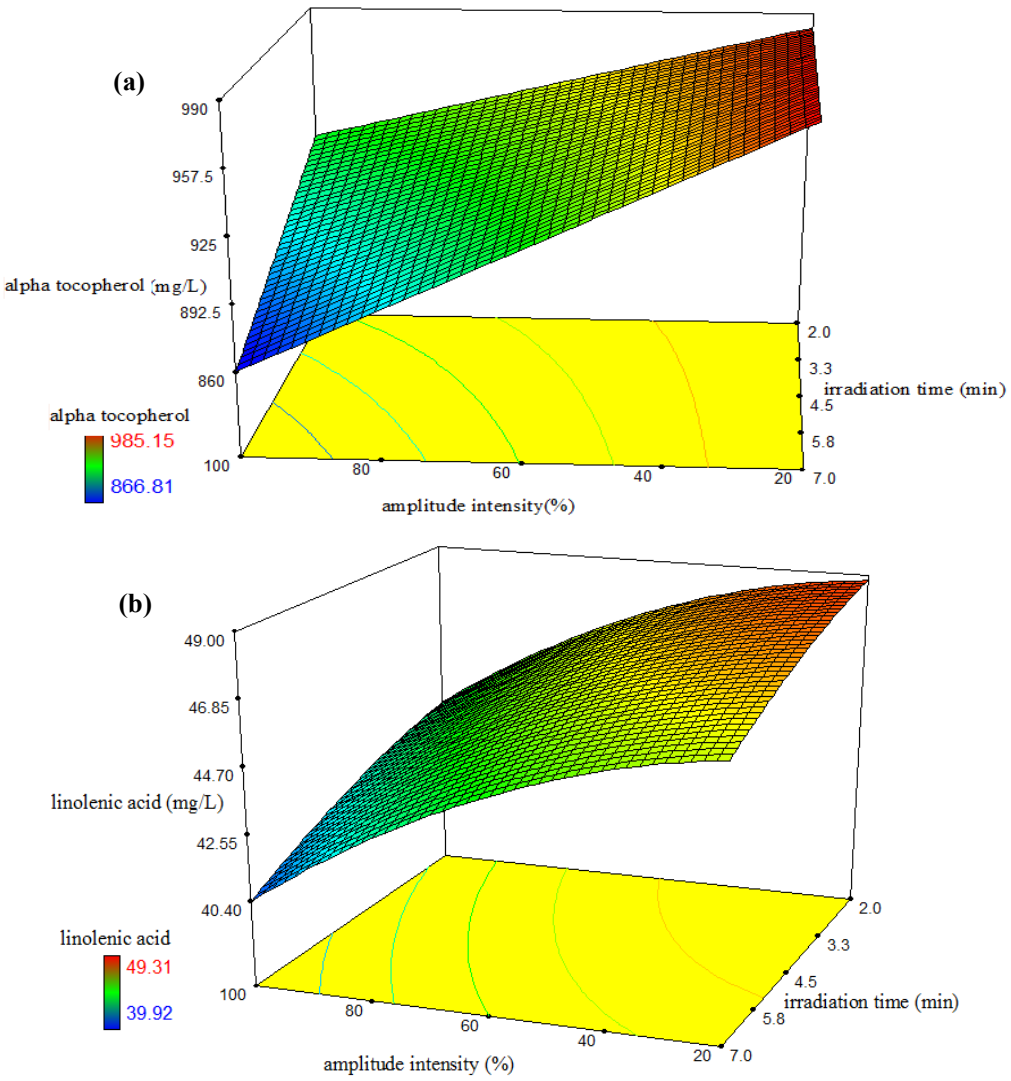

Fig. 2: Response surface plot showing the significant ($p < 0.05$) effect for α-Tocopherol (a) and (b) linolenic acid content as a function of ultrasonic amplitude and irradiation time.

and 4.5 min, respectively. However, any increase in amplitude from 20 % to 60 % in less than 4.5 minutes is not effective in reducing the droplet size. In a sense applying this time and amplitude range may not prepare enough energy to break the droplet and form the desired nano-size droplet [28]. Also at low shear rates, there is not enough disruptive forces to overcome interfacial tension. By applying the severe sonication condition, droplet size and distribution reduce favourably but simultaneously α-tocopherol contents decrease significantly ($p < 0.05$). The two reasons for this phenomenon might be: the high percentage of primary oxidation in polyunsaturated fatty acids of linseed oil and the polymerization of α-tocopherol which accelerates due to sonication as the absorbed ultrasound power increases.

Effect of process conditions on the linolenic acid content

The loss of linolenic acid during preparation of linseed oil nanoemulsion is presented in Fig. 2(b). The linolenic acid content of a coarse emulsion immediately after preparation is approximately 51.8 mg/L as can be seen in Fig. 2(b) after the lowest time and intensity of ultrasound treatment linolenic acid content is about 49 mg/L which is decreased significantly ($p < 0.05$) by increasing in the intensity and time of sonication procedure. The ultrasound amplitude has the most significant ($p < 0.0001$) effect on the linolenic acid contents with F-value of (292.95). In a high-energy emulsification system, like ultrasound technique, a temperature rise during the cavitation is expected, which might be caused a loss in the linolenic acid content. Also the free radicals generated by sonolysis may induce poly unsaturated fatty acids oxidation [28]; consequently, after sonication a loss in linolenic acid content is normal.

Optimization of nanoemulsion preparation

The nanoemulsion preparation is considered as optimal if obtained droplet size and PDI of the nanoemulsion attained the smallest and α-tocopherol and linolenic acid contents have reached their greatest possible values. The optimization of nanoemulsion processing parameter indicated that the highest desirability could be reached by ultrasound amplitude and irradiation time in a ratio of 90% and 6 minutes, respectively. Under the mentioned conditions it is predicted that the droplet size, PDI, α-tocopherol and linolenic acid contents are 98.2 nm, 0.085, 880.99 mg/L and 44.19 mg/L, respectively. The optimal condition is evaluated by three parallel experiments and their results are compared with the predicted values (Design Expert 7.1.6). These finding reveal the actual values of 94.5 ± 1.14 nm, 0.10 ± 0.022, 851.43 ± 11.62 mg/L and 43.02 ± 1.27 mg/L for particle size, PDI, α-tocopherol and linolenic acid contents, respectively that are very close to the predicted results.

The effect of storage on the nanoemulsion stability

Some of the most important features applied to assessing the stability of emulsion system are the droplets size and PDI. Therefore, both the droplet size and PDI parameters are assessed immediately after the production of nanoemulsion and during 2 months' storage of at 4 °C. As observed in Fig. 3, during the first 30 days the nanoemulsion have nano-size droplet (< 100 nm), and the PDI remained in the acceptable magnitude as compared to the initial values.

After this period a slight increase is observed in the droplet size and PDI. But emulsions still display satisfactory long term stability (PDI < 0.300) without any visible separation. The droplet coalescence might be possible reason for increasing in PDI of nanoemulsion which often assumed as an important factor responsible for the destabilization of nanoemulsions [33]. This phenomenon could be controlled by either applying sufficient surfactant, decreasing the storage temperature or through hydrodynamic stabilizer in continues phase [31]. Nevertheless, during storage at 4 °C, there is a decrease in the α-tocopherol and linolenic acid concentration. The degradation profile of α-tocopherol and linolenic acid as a function of storage time is shown in Fig. 4. It is worth to mention that after 2 months' storage (4 °C) about 90% α-tocopherol and 70% of linoleic acid level have been already remained. Emulsion droplets may less coalescing since they are surrounded by emulsifier. Oxidant and other oxidation products are relatively hydrophilic and readily disperse into aqueous phase [35]. So, surfactant membranes protect lipids from oxidation by acting as a shield against penetration and diffusion of molecular species [34]. Tween molecules which are surrounding a droplet might capable to scavenge free radicals, hence, a retarding lipid oxidation process. Emulsifier is likely to be effective when they form a part

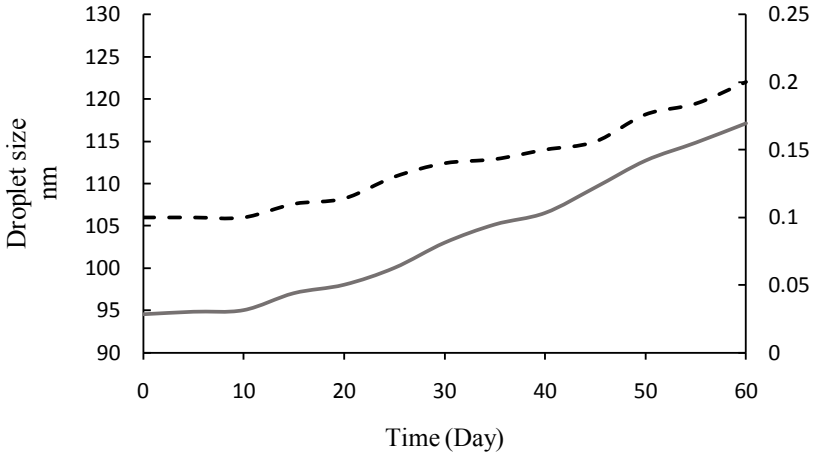

Fig. 3: Polydispersity Index (PDI) and Droplet size measurement of nanoemulsion duration at 4 °C storage for 60 days.

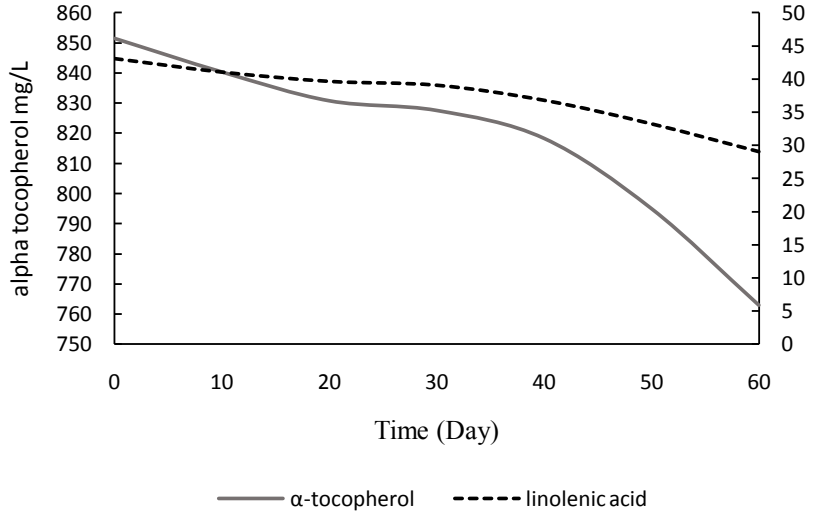

Fig. 4: α-tocopherol and linolenic acid contents of nanoemulsion after production and subjected to 60 days of storage at 4 °C.

of a nanodroplet membrane, since their local concentration is relatively high in compare to the conventional emulsion.

Fig. 4 shows by increasing droplet diameters during the storage, the degradation of bioactive ingredients accelerates. When the droplet size increase, surfactant thickness decrease and diffusion area increase which both cause bioactive compound exposure to free radicals and oxidation may promote the degradation of this sensitive bioactive compound during storage.

CONCLUSION

At this stage it could be deduced that assigning an appropriate and accurate ultrasound amplitude and irradiation time would contribute to nanoemulsion process in a significant manner. The optimal conditions for ultrasonic preparation of α-tocopherol nanoemulsions are at applied 90% amplitude and 6 min ultrasonic time. Nanoemulsion is a probable solution in solving the oxidation and low bioavailability of α-tocopherol problems; hence, it might be applied as one of the nano-encapsulated antioxidant systems in food industry. Applying an appropriate organic phase, like linseed oil that is contains unsaturated fatty acids, could improve nutritional value of the nanoemulsion. The linolenic acid and α-tocopherol contents strongly depend on the parameters of their preparation. The results of this study also confirmed that the sonication is relatively a simple method that needs lower amount of

surfactant and lower percentage of input energy for producing more stable and desired droplet size in emulsion which could be applied in cosmetic, pharmaceutical and food grade nano-delivery systems.

ACKNOWLEDGMENT

The study was funded by Department of Food Science and Technology, Islamic Azad University.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

[1] Rolewski P., Siger A., Nogala-Kałucka M., Polewski, K., (2009), Evaluation of antioxidant activity of alpha-topcopherol and quercetin during oxidation of phosphatidiylcholine using chemiluminescntection of lipid hydroperoxides. *J. Food Nutr*. 59: 123-127.

[2] Cheong J. N., Tan C. P., Che Man Y. B., Misran, M., (2008). A-tocopherol nanodispersion: Preparation, characterization and stability evaluation. *J. Food Eng.* 89: 204-209.

[3] Jo Y. J., Kwon Y., (2014), Characterization of β-carotene nanoemulsions prepared by microfluidization technique. *J. Food Sci. Biotechnol*. 23: 107-113.

[4] Yang D., Wang X. Y., Lee J. H., (2015), Effects of flavonoids on physical and oxidative stability of soybean oil O/W emulsions. *Food Sci. Biotechnol*. 24*:* 851-858.

[5] Sivakumar M., Towata A., Yasui K., Tuziuti T., Kozuka T., Iida Y., Maiorov M. M., (2012), Ultrasonic cavitation induced water in vegetable oil emulsion droplets–A simple and easy technique to synthesize manganese zinc ferrite nanocrystals with improved magnetization. *Ultrason. Sonochem*. 19*:* 652-658.

[6] Tan C. P., Nakajima M., (2005), β-carotene nanodispersions: Preparation, characterization and stability evaluation. *Food Chem*. 92**:** 661-671.

[7] Li P. H., Chiang B. H., (2012), Process optimization and stability of D-limonene-in-water nanoemulsions prepared by ultrasonic emulsification using response surface methodology. *Ultrason. Sonochem*. 19**:** 92-107.

[8] Hosseini S., Ghiassi Tarzi B., Gharachorloo M., Ghavami M., Bakhoda H., (2015), Optimization on the stability of linseed oil-in-water nanoemulsions generated by ultrasonic emulsification using response surface methodology (RSM). *Orient. J. Chem.* 31**:** 1223- 1230.

[9] Wang L., Li X., Zhang G., Dong J., Eastoe, J. J., (2007), Oil-in-water nanoemulsions for pesticide formulations. *Colloid Interface Sci*. 314**:** 230-235.

[10] Sonneville-Aubrun O., Simonnet J. T., L'Alloret F., (2004), Nanoemulsions: a new vehicle for skincare products. *Adv. Colloid. Interface Sci.* 108: 145-149.

[11] Konga M., Chenb X. G., Kweonc D. K., Park H., (2011), Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. *J. Carbohydr. Polym*. 86*:* 837-843.

[12] McClements D. J., (2011), Edible nanoemulsions: fabrication, properties, and functional performance. *Soft. Mater*. 7*:* 2297-2316.

[13] Tang S. Y., Sivakumar M., Tan K. W., Billa N., (2012), Formulation development and optimization of a novel cremophore EL-based nanoemulsion using ultrasound cavitation. *Ultrason. Sonochem.* 19*:* 330-345.

[14] Tadros T., Izquierdo P., Esquena J., Solans C., (2004), Formation and stability of nano-emulsions. *Adv. Colloid Interface Sci.* 108: 303−318.

[15] Strawbridge K. B., Ray E., Hallett F. R., Tosh S. M., Dalgleish D. G., (1995), Measurement of particle size distributions in milk homogenized by a microfluidizer: Estimation of populations of particles with radii less than 100 nm. *Adv. Colloid Interface Sci*. 171: 392−398.

[16] Kentish S., Wooster T. J., Ashokkumar M., Balachandran S., Mawson R., Simons L., (2008), The use of ultrasonics for nanoemulsion preparation. *Innov. Food Sci. Emerg. Technol*. 9: 170-175.

[17] Jafari S. M., He Y., Bhandari B., (2007), Effectiveness of encapsulating biopolymers to produce sub-micron emulsions by high energy emulsification techniques. *Food Res. Int*. 40*:* 862-873.

[18] Tang S. Y., Shridharan P., Sivakumar M., (2013), Impact of process parameters in the generation of novel aspirin nanoemulsions–comparative studies between ultrasound cavitation and microfluidizer. *Ultrason. Sonochem*. 20*:* 485-497.

[19] Tang S. Y., Sivakumar M., Nashiru B., (2013), Ultrasound cavitation as a green processing technique in the design and manufacture of pharmaceutical nanoemulsions in drug delivery system, in green chemistry for environmental remediation. *Colloids Surf. B*. 102*:* 653-658.

[20] Loong N. C., Basria M., Fui Fanga L., Fard Masoumia H. R., Tripathy M., Abedi R., (2014), Comparison of Box–Behnken and central composite designs inoptimization of fullerene loaded palm-based nano-emulsions forcosmeceutical application. *Ind. Crops Prod*. *59:* 309-317.

[21] Gambuś H., Borowiec F., Zając T., (2003), Chemical composition of linseed with different colour of bran layer. *Pol. J. Food Nutr*. 12*:* 67–70.

[22] Margulis M. A., Margulis I. M., (2003), Calorimetric method for measurement of acoustic power absorbed in a volume of a liquid. *Ultrason. Sonochem*. 10*:* 343-345.

[23] Choi A. J., Kim C. J., Cho Y. J., Hwang J. K., Kim C. T., (2009), Effects of surfactants on the formation and stability of capsaicin-loaded nanoemulsions. *Food Sci. Biotechnol*. 18*:* 1161-1172.

[24] Gambuś H., Mikulec A., Gambuś F., Pisulewski P., (2004), Perspectives of linseed utilization in baking. *Pol. J. Food Nutr*. 13**:** 21–27.

[25] AOCS., (1996), Official methods and recommended practices of the AOCS, 4thed. Method Ce 1-62. *American Oil Chemistry Society, Champaign*, USA.

[26] Hosseini S., Gharachorloo M., Ghiassi Tarzi B., Ghavami M., Bakhoda H., (2015), Effects of ultrasound amplitude on the physicochemical properties of some edible oils. *J. Am. Oil Chem. Soc.* 92*:* 1717-1724.

[27] Pegg R. B., Amarowicz R., (2009), Content of tocopherol isomers in oilseed radish cultivars-a short report. *Pol. J. Food Nutr.* 59: 129–132.

[28] Floury J., Desrumaux A., Axelos M. A. V., Legrand J., (2003), Effect of high pressure homogenization on methylcellulose as food emulsifier. *J. Food Eng.* 58: 227-238.

[29] Chu B. S., Ichikawa S., Kanafusa S., Nakajima M.,

(2007), Preparation of protein stabilized b-carotene nanodispersions by emulsification-evaporation method. *J. Am. Oil Chem. Soc*. 84: 1053-1062.

[30] Perrier-Cornet J. M., Marie P., Gervais P., (2005), Comparison of emulsification efficiency of protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill homogenization. *J. Food Eng*. 66**:** 211-217.

[31] Leighton T. G., (1994), The acoustic bubble. Academic Press, Oval Road London NWI 7DX, pp. 126-133.

[32] Laurell T., Petersson F., Nilsson A., (2006), Chip integrated strategies for acoustic separation and manipulation of cells and particles. *Chem. Soc. Rev*. 3*:* 492- 506.

[33] Almong R., Forward R., Samsonoff C., (1991), Stability of sonicated aqueous suspensions of phospholipids under air. *Chem. Phys. Lipids*. 60*:* 93-100.

[34] Capek I., (2004), Degradation of kinetically-stable oil-in-water emulsions. *Adv. Colloid Interface Sci.* 107: 102–110.

[35] Patrick M, Blindt R, Janssen J., (2004), The effect of ultrasonic intensity on the crystal structure of palm oil. *Ultrason. Sonochem*. 11: 251–255.