# Changes in the surface tension and viscosity of fish oil nanoemulsions developed by sonication during storage

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## Abstract

Adequate consumption of ω-3 essential fatty acids (EFAs) has a positive impact on human health. EFAs-enriched functional foods may be used for this purpose. Nanoemulsion is a promising delivery system for incorporating EFAs into a variety of foods and beverages. In this work, fish oil nanoemulsions developed by sonication method were subjected to various analyses as a function of hydrophilic lipophilic balance (HLB) and surfactant to oil ratio (SOR). Analyses were performed upon production and during 1-month storage at two temperatures (4 and 25 °C) in the presence (100 ppm) or absence of α-tocopherol. Increasing in HLB and SOR decreased the particle size and surface tension; while, increased the refractive index and viscosity. During storage, the particle size of α-tocopherol-loaded nanoemulsions decreased; whereas, that of α-tocopherol-free nanoemulsions increased in a temperature-dependent manner. Irrespective of the storage temperature, surface tension values of antioxidant-loaded nanoemulsions remained constant. However, their viscosity values increased. Antioxidant incorporation fairly increased the nanoemulsions stability likely due to partitioning at the interface. TEM micrographs confirmed the results obtained by static light scattering. The results of this study may help the rational design of functional foods using nanoemulsion-based delivery systems.

**Keywords:** Fish Oil, High Intensity Ultrasound, Viscosity, Surface Tension, Nanoemulsion

## Introduction

Recently, nanoemulsions, as a sub-group of emulsion-based systems, have gained particular attention due to simple fabrication method, high kinetic stability and bioavailability (McClements, 2011; Walker *et al*., 2015). The droplet radii of nanoemulsions (<100 nm) results in the formation of semi-turbid or even optically transparent systems particularly at sufficiently small particle sizes (<50 nm) (Mason *et al*., 2006; Tadros et al; 2004). High- or low-energy methods can be used to fabricate nanoemulsions. In high-energy approaches, mechanical devices such as microfluidizer, high pressure valve homogenizer and high intensity horn sonicator are usually applied to create fine droplets

(McClements, 2011; McClements & Rao, 2011). These methods do not have any limitations on the types of the oils and surfactants. Moreover, low surfactant to oil ratio (SOR) is typically needed. As determined in previous works (Nejadmansouri *et al*., 2016; Kumar Dey *et al*., 2012; Ghosh *et al.,* 2013), the application of sonication for the formation of nanoemulsions may lead to promising results without any requirement for coarse emulsion.

The increasing awareness about the biological roles of ω-3 polyunsaturated fatty acids (PUFAs) in human health has prompted significant researches to find appropriate methods for incorporating fish oil (or its essential fatty acids) into functional foods and beverages, while preventing them from oxidation and off-flavor development. Natural antioxidants, such as α-tocopherol, are used for this purpose. They act as chain-breaking electron-donor antioxidants (Shimajiri *et al.,* 2013). The antioxidant activity depends on the chemical structure of antioxidant molecules and interactions in the emulsions. The "polar paradox" theory describes that lipophilic

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antioxidants are more effective than hydrophilic ones to retard the oxidation in O/W emulsions (Porter, 1993). This hypothesis cannot be extended to all antioxidant molecules. Therefore, the application of antioxidants in real food emulsions generally leads to unpredictable results (Shahidi & Zhong, 2011; Asnaashari *et al.*, 2014).

In this work, we studied the effects of hydrophilic lipophilic balance (HLB), surfactant to oil ratio (SOR) and α-tocopherol incorporation on the particle size, dynamic viscosity and surface tension of nanoemulsions during storage. Research in this area is important to find appropriate strategies for incorporating lipophilic active ingredients into aqueous-based foods or beverages (such as fortified waters, soft drinks and sauces) using emulsion-based delivery systems.

### Materials and methods

Fish oil (containing 25% EPA+DHA of total fatty acids) was purchased from Pars Kilka Co. (Babolsar, Iran). Tween 80 (HLB≈15), and Span 80 (HLB≈4.3) were purchased from Merck Co. (Darmstadt, Germany). α-tocopherol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Double distilled water (DDW) was utilized to prepare all emulsions.

### Nanoemulsion preparation

O/W nanoemulsions containing 5 wt% dispersed phase were prepared using a mixture of non-ionic surfactants at various SORs (0.5-1.5) and HLBs (9-15). Briefly, the dispersed phase containing the required amount of Span 80 was gradually added into the aqueous phase (containing Tween 80) under magnetic stirring (700 rpm for 15 min) at room temperature. After that, a coarse emulsion was prepared by a disperser/homogenizer (Heidolph Silent Crusher, Schwabach, Germany) at 20000 rpm (715 xg) for 5 min. The coarse emulsion was then treated (amplitude 80%, cycle 0.7 s, duration 10 min) by a horn sonicator (Hielscher, UP 200H, Teltow, Germany) to develop nanoemulsions. Sonication was performed in a glass beaker placed in an ice bath. The temperature did not exceed 25-30 °C during the process. In some batches, α-tocopherol (100 ppm) was incorporated into the dispersed phase prior to emulsification.

### Calculation of HLB

The required amounts of non-ionic surfactants to obtain a given HLB (denoted as $HLB_x$) were calculated using the Pearson's square:

$$(A)\% = \left(\frac{HLB_X - HLB_B}{HLB_A - HLB_B}\right) \times 100 \tag{1}$$

$$(B)\% = 100 - (A) \tag{2}$$

where, $HLB_A$ and $HLB_B$ are the HLB values of Tween 80 and Span 80, respectively. (A) and (B)% represent the required amounts of surfactants in the emulsifier mixture (Peshkovsky *et al.*, 2013). The final weight of the surfactant mixture was determined based on the required SOR.

### Particle size measurement

The volume-weighted mean droplet size ($D_{4,3}$) and Span of 10-times diluted nanoemulsions were determined using static light scattering (SLS, laser diffraction, SALD-2101, Shimadzu, Japan) at room temperature (Gulotta *et al.*, 2014).

### Refractive index measurement

The refractive indices of nanoemulsion, fish oil and aqueous phase were measured at 20 °C using a refractometer (RX7000α; Atago, Japan) (Rao *et al.*, 2013).

### Surface tension measurement

The surface tension (m$N$ $m^{-1}$) of nanoemulsions was measured at 20 °C using a tensiometer (Sigma 703D; KSV, USA) equipped with a Du Nouy ring (Joe *et al.*, 2012). The dimensions of ring and wire were 9.545 and 0.185 mm, respectively.

### Viscosity measurement

The viscosity of nanoemulsions was

measured using a U-tube capillary viscometer (type 51810, Schott Geräte, Germany) at 25±0.1°C. Dynamic viscosity was calculated according to the following equation (Secco *et al.*, 2014):

$$Dynamic\ Viscosity\ (mpa.s) = Density\ (kg/m^3) \times Kinematic\ Viscosity\ (mm^2/s) \times 0.001 \qquad (3)$$

## Density measurement

The density of some selected samples was measured using a 25-ml pycnometer at 25 °C (Qian *et al.*, 2011).

## Storage stability

α-tocopherol–free and α-tocopherol–incorporated (100 ppm) nanoemulsions (HLB 12, SOR 1.5) were subjected to various physical stability tests (namely changes in droplet size, viscosity and surface tension) during storage at 4 and/or 25 °C for 1 month. The experiments were carried out in 7-day intervals (Li *et al.*, 2013).

## Droplet morphology

Morphological studies were performed using transmission electron microscopy (TEM). A nanoemulsion drop was placed on a carbon-coated copper grid followed by staining with uranyl acetate. After 2 min, the grid was dried by forced air and then imaged by CM10 transmission electron microscope (Philips, Netherlands) (Salvia-Trujillo *et al.*, 2013).

## Statistical Analysis

A completely randomized design was used in this study. All experiments were performed at least three times. The results were reported as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) with Duncan's multiple comparison post hoc tests at a significance level of 0.05. The analysis was conducted using SPSS software (version 21, IBM Corp. USA) (Chang *et al.*, 2013).

## Results and discussion

### Effects of SOR and HLB on the particle size and Span

As a preliminary experiment, coarse emulsions were subjected to various sonication times. The smallest droplet size (82 nm) was obtained after 10-min sonication. Particle size reached to a plateau at higher sonication times. The average particle size and Span values of nanoemulsions prepared at various SOR and HLB values are reported in Table 1.

The main reason for choosing Span 80 was to obtain different HLB values. Using various combinations of Span 80 and Tween 80. HLB is an important factor during the release process. An increase in HLB value increases the lag time in the jejunum and decreases the rate of lipolysis and hence the bioaccessibility of FFAs in the small intestine (Walker et al., 2015). Mixed-emulsifier systems are more effective than a single-type emulsifier in retarding the particle aggregation (McClements *et al.*, 2011). Simultaneous application of lipophilic and hydrophilic surfactants may facilitate the formation of small particles using high-energy approaches. Both emulsifiers used in this study have an oleic acid residue which may facilitate the bending of surfactant molecule. Previous studies have also shown that the presence of double bonds (unsaturation) along the non-polar chains of non-ionic surfactants favors the formation of nanoemulsions with smaller droplet sizes (Wang *et al.,* 2009). At the same SOR, the effects of HLB values (from 11 to 14) on the particle size were not significant; confirming that the hydrophilicity of the emulsifier mixture was adequate to develop nanoemulsions. Molecular geometry and HLB value of surfactant molecules are of paramount importance (Wang *et al.*, 2009). The effects of HLB value on the particle size can be explained in terms of partition coefficient of surfactant molecules in lipid and aqueous phases. In addition, deposition of surfactant molecules onto the interfacial layer has an important role on particle size (i.e., heterogeneous emulsifier molecules present

within an emulsifier mixture show a preference to be arranged beside the molecules

of their own type to decrease the free surface energy) (Nejadmansouri *et al.*, 2016).

**Table 1. Effect of SOR and HLB on particle size (nm) and refractive index of nanoemulsions**

| HLB | SOR | Particle size (nm) | Span | Refractive index |
|-----|-----|--------------------|------|------------------|
| 9 | 0.5 | 460±3[b] | 0.403±0.016[b] | 1.3441±0.0004[d] |
|   | 1 | 489±37[a] | 0.472±0.073[b] | 1.3469±0.0011[c] |
|   | 1.5 | 461±1[b] | 0.412±0.006[b] | 1.3499±0.0007[b] |
| 10 | 0.5 | 468±9[ab] | 0.440±0.016[b] | 1.3442±0.0001[d] |
|   | 1 | 472±11[ab] | 0.445±0.026[b] | 1.3471±0.0010[c] |
|   | 1.5 | 87±2[c] | 0.678±0.011[a] | 1.3504±0.0003[ab] |
| 11 | 0.5 | 469±8[ab] | 0.437±0.020[b] | 1.3440±0.0002[d] |
|   | 1 | 91±2[c] | 0.673±0.015[a] | 1.3472±0.0006[c] |
|   | 1.5 | 88±5[c] | 0.677±0.006[a] | 1.3513±0.0000[a] |
| 12 | 0.5 | 474±1[ab] | 0.451±0.002[b] | 1.3442±0.0000[d] |
|   | 1 | 87±1[c] | 0.688±0.002[a] | 1.3473±0.0001[c] |
|   | 1.5 | 90±3[c] | 0.666±0.004[a] | 1.3508±0.0001[ab] |
| 12.5 | 0.5 | 474±1[ab] | 0.452±0.000[b] | 1.3440±0.0004[de] |
|   | 1 | 88±2[c] | 0.667±0.016[a] | 1.3474±0.0001[c] |
|   | 1.5 | 93±2[c] | 0.687±0.034[a] | 1.3510±0.0003[a] |
| 13 | 0.5 | 469±8[ab] | 0.438±0.020[b] | 1.3440±0.0001[de] |
|   | 1 | 89±2[c] | 0.672±0.008[a] | 1.3471±0.0001[c] |
|   | 1.5 | 94±5[c] | 0.715±0.095[a] | 1.3509±0.0009[ab] |
| 14 | 0.5 | 472±10[ab] | 0.447±0.026[b] | 1.3436±0.0001[de] |
|   | 1 | 91±0[c] | 0.657±0.002[a] | 1.3472±0.0004[c] |
|   | 1.5 | 94±3[c] | 0.710±0.052[a] | 1.3512±0.0002[a] |
| 15 | 0.5 | 75±6[c] | 0.712±0.072[a] | 1.3429±0.0004[e] |
|   | 1 | 82±14[c] | 0.686±0.017[a] | 1.3474±0.0004[c] |
|   | 1.5 | 72±1[c] | 0.696±0.002[a] | 1.3506±0.0001[ab] |

HLB: hydrophilic lipophilic balance; SOR: surfactant to oil ratio. Means in each column with different superscript letters are significantly different (*p*<0.05).

Irrespective of the SOR, the largest particle sizes were obtained at HLB 9; which means that the final particle size is dependent on the hydrophobicity of emulsifier mixture. In other words, nanodroplets cannot be developed within an O/W emulsion where the hydrophobic characteristics of surfactant molecules exceed a certain level. An increase in the SOR generally decreased the particle size. Tween 80 is derived from polyethoxylated sorbitan and oleic acid. Span 80 is sorbitane monooleate. Both molecules have an oleic acid residue. A decrease in the HLB value led to higher concentrations of Span 80 (as an emulsifier with low affinity toward water) in the mixed emulsifier system. An increase in the surface area to volume ratio and hence higher surfactant concentrations were required to obtain lower particle sizes. At higher HLB

values, the dependence of particle size on the amount of SOR was more than lower HLBs. To obtain lower particle sizes, surfactant molecules should be easily placed at the interface; however, lipophilic molecules of Span 80 were not able to, mainly because of the presence of water as the continuous medium. Therefore, the particle size was independent from low SOR values at low HLBs. Particle size decreased at a SOR value (1.5) higher than a critical SOR (1) likely due to the increased concentration of surfactant molecules in the emulsion. At higher HLB values, the hydrophilic head groups of Tween 80 could be readily positioned at the interface and hence particle size decreased by increasing the surfactant concentration or SOR. In other words, higher concentrations of surfactant molecules could provide adequate interfacial layers for effective coating of newly developed

nanodroplets (i.e., increased specific surface area). An increase in the surfactant concentration can decrease the particle size through various mechanisms (Lamaallam et al., 2005). First, higher amounts of surfactant molecules would result in a higher decrease in the interfacial tension and hence increasing the mobility of the oil− water interfaces, where oil droplets are formed. Second, higher concentration gradient results in more flux of surfactant molecules from the lipid phase into the aqueous phase upon contact and hence enhances the formation of finer droplets at the oil−water boundary. Third, higher surfactant concentrations may lead to favorable rearrangements within the system. Our results are in good agreement with those reported by Li and colleagues (2013). These researchers prepared nanoemulsions of D-limonene using a catastrophic phase inversion method. An increase in the surfactant concentration led to a decrease in the mean particle diameter. Gulotta and colleagues (2014) prepared a mixture of fish and lemon oils nanoemulsions using a spontaneous method and Tween 80 as the emulsifier. The mean particle diameter was large (>1000 nm) at relatively low surfactant levels (SOR<0.75); whereas, small (<200 nm) particle sizes were obtained at higher levels (SOR≥0.75).

As shown in Table 1, a significant (p< 0.05) increase in the Span values was observed by increasing the SOR. The amounts of emulsifiers used to stabilize emulsions are generally larger than the actual amounts required to be loaded at the interfaces. Therefore, a substantial fraction of the emulsifier molecules remains un-adsorbed. This fraction may be partitioned between oil and water phases and hence develops surfactant micelles (Berton-Carabin *et al.*, 2014). The presence of un-adsorbed surfactant molecules may lead to broadening the particle size distribution.

**Effect of SOR value and HLB factor on refractive index**

The effect of surfactant concentration on the refractive index of nanoemulsions is reported in Table 1. The refractive index of nanoemulsions increased significantly ($p<0.05$) by increasing the SOR. The ratio of the refractive index of oil ($n_1$) to that of aqueous phase ($n_2$) has a vital role on the optical properties of the resultant emulsion. As an example, for HLB 15, the refractive index of the oil was 1.477. An upward trend in $n_2$ (from 1.336 to 1.343) was observed by increasing the amount of Tween 80. At higher SORs, when $n_1$:$n_2$ ratio moved toward unity, the emulsions became less opaque. The light detection mechanisms in turbidity and reflectance measurements are different. In turbidity, the propagated light through an emulsion is measured. As the scattering efficiency of the dispersed droplets increases above a certain level, the fraction of the light waves that can transmit through the emulsions falls below a detectable level (i.e., increased turbidity). Reflectance measurement relies on the light that has been reflected from the emulsions. At high droplet concentrations, reflectance occurs more than transmittance (Chantrapornchai *et al.*, 2001). An increase in SOR resulted in increasing the number of smaller droplets and hence the amount of reflected light (i.e., increased refractive index). There is a direct relationship between the particle size and turbidity up to a defined particle size (around 75 nm), above which turbidity increases more rapidly. Kumar Dey et al (2012) reported that the refractive index of fish oil nanoemulsion was slightly higher than that of fish oil emulsion. Rao and McClements (2013) found that incorporating high levels of polar cosolvents into the aqueous phase prior to homogenization led to formation of optically transparent lemon oil nanoemulsions. This observation was due to the fact that cosolvent reduced the refractive index contrast, rather than reducing the particle size.

**Effect of SOR and HLB on surface tension**

Particle size reduction under the effect of increased SOR could be explained by the fact that a larger amount of emulsifier resulted in reducing the surface tension and eventually

creating smaller droplets. There was not any significant difference between the surface tension of nanoemulsions obtained at SOR values of 1 and 1.5 (Table 2). However, a decreasing trend could be detected by increasing the SOR. The surface tension of water and oil were 71.43 and 31.2 mN/m, respectively. During this study we did not measure the effects of a wide range of surfactant concentrations on the reduction of surface tension. It is obvious that the presence of surfactant molecules results in a decrease in the surface tension by disrupting the cohesive forces between water molecules. At higher surfactant concentrations, the adhesive forces between polar head groups of surfactant molecules and continuous medium (water) may compensate the decreased cohesive forces. Therefore, above a certain level, surface tension might be relatively independent from the concentration of surfactant molecules. The ability of emulsifiers to reduce the surface tension was dependent on the total amount as well as the ratio of emulsifiers (HLB). This dependency on HLB was more obvious at SOR of 0.5. An increase in the HLB increased the surface tension. Indeed, the increase in the proportion of the polar head groups of the emulsifiers resulted in

extensive hydrogen bonding with water molecules and surface tension increased for this reason. Previous studies have shown that smaller droplets can be formed when the disperse-to-continuous phases viscosity ratio ($\eta_D/\eta_C$) is close to unity and when the disperse-to-continuous phases interfacial tension is reduced (Wooster et al., 2008; Qian & McClements., 2011). The diffusion of surfactant molecules from the bulk phase onto the droplet surface reduces the interfacial density fluctuations at the thin liquid films. Since the rate of diffusion increases with surfactant concentration, the average droplet diameter is lowered. Joe and colleagues (2012) reported a decrease in the surface tension using a variety of oils by reducing the particle size. Yang and McClements (2013) reported a decrease in the interfacial tension of the aqueous phase and also the particle size by increasing glycerol concentration. Moreover, a decrease in vitamin E concentration resulted in increasing the interfacial tension of the organic phase and hence increasing the particle size. The tensiometer used in this study had a manual movement of the ring. We could not precisely determined the location of the ring at the interface of water and oil and interfacial tension was not measured for this reason.

**Table 2. Effect of SOR and HLB on the surface tension (mN/m), density (Kg/m³) and dynamic viscosity (mPa.s) of nanoemulsions**

| HLB | SOR | Surface tension (mN/m) | Density (kg/m3) | Dynamic viscosity (mPa.s) |
|---|---|---|---|---|
| 9 | 0.5 | 31.05±0.18[bc] | 1008.36±0.10[i] | 1.16±0.001[h] |
| | 1 | 30.68±0.25[bc] | 1009.16±0.10[f] | 1.31±0.001[g] |
| | 1.5 | 30.89±0.19[bc] | 1011.48±0.09[c] | 1.78±0.004[a] |
| 10 | 0.5 | 31.33±0.08[b] | 1007.44±0.11[k] | 1.16±0.001[h] |
| | 1 | 30.49±0.15[c] | 1008.40±0.10[h] | 1.34±0.001[f] |
| | 1.5 | 30.86±0.25[bc] | 1011.20±0.08[d] | 1.61±0.001[b] |
| 11 | 0.5 | 32.67±0.30[a] | 1008.16±0.10[j] | 1.14±0.002[i] |
| | 1 | 30.94±0.29[bc] | 1010.56±0.11[e] | 1.36±0.003[e] |
| | 1.5 | 31.18±0.11[bc] | 1013.24±0.10[a] | 1.53±0.002[d] |
| 12 | 0.5 | 32.29±0.05[a] | 1007.36±0.10[l] | 1.16±0.001[h] |
| | 1 | 31.24±0.17[bc] | 1008.72±0.08[g] | 1.34±0.001[f] |
| | 1.5 | 31.41±0.29[b] | 1012.52±0.11[b] | 1.54±0.003[c] |

HLB: hydrophilic lipophilic balance; SOR: surfactant to oil ratio. Means in each column with different superscript letters are significantly different ($p<0.05$).

**Effect of SOR and HLB on viscosity and density**

An increase in the surfactant concentration increased the amounts of viscosity and density of nanoemulsions (Table. 2). Similar results

have been reported by Ghosh et al (2013) for the viscosity of basil oil nanoemulsion prepared by different amounts of Tween 80.

There are several factors which influence the emulsion viscosity namely, the volume fraction of the dispersed phase, the rheology of component phases, droplet size, colloidal (inter-particle) interactions and droplet charge (McClements, 2005; Pal, 2011). Emulsions of various viscosities can be obtained at different droplet concentrations. Flocculation may appreciably increase the emulsion viscosity. Moreover, incorporation of thickening agents into aqueous phase may have similar effect (McClements, 2002). The viscosity of a nanoemulsion may be significantly greater than that of a macroemulsion at the same lipid concentration, particularly when it contains a thick or charged interfacial layer (Tadros et al., 2004; Weiss & McClements., 2000). Increasing the emulsifier concentration may change the characteristics of the interfacial layers surrounding the oil droplets. After formation of interfacial layers of limited thickness, the non-adsorbed fraction of surfactant molecules might be responsible for increasing the viscosity and density of the continuous phase.

**Storage stability of fish oil nanoemulsions at different temperatures**

α–Tocopherol-free and -loaded fish oil nanoemulsions prepared at SOR 1.5 and HLB 12 were used for the stability tests. During the shelf life of a product, minimum changes in particle size distribution are required. Changes in the droplet size of nanoemulsions during 1-month storage at 4 and 25°C are shown in Table. 3.

**Table 3. Effect of temperature (4 and 25 °C) on particle size, viscosity and surface tension of nanoemulsions as a function of time**

| Day | Sample/ Temperature (°C) | Particle size (nm) | Dynamic viscosity (mPa.s) | Surface tension (mN/m) |
|---|---|---|---|---|
| 1 | TS 4 °C | 102±1.80[h] | 1.68±0.002[a] | 31.97±0.10[a] |
|  | TS 25 °C | 60±1.42[p] | 1.58±0.001[c] | 30.70±0.35[b] |
|  | TSα 4 °C | 88±1.23[k] | 1.55±0.001[b] | 31.92±0.15[a] |
|  | TSα 25 °C | 78±1.28[m] | 1.56±0.001[d] | 31.80±0.40[a] |
| 7 | TS 4 °C | 119±1.73[g] | 1.50±0.001[c] | 31.81±0.29[ab] |
|  | TS 25 °C | 71±1.01[no] | 1.53±0.003[e] | 32.08±0.31[a] |
|  | TSα 4 °C | 81±1.44[l] | 1.45±0.001[e] | 31.88±0.22[a] |
|  | TSα 25 °C | 95±1.02[j] | 1.57±0.002[c] | 31.93±0.20[a] |
| 14 | TS 4 °C | 130±1.43[f] | 1.43±0.004[e] | 31.42±0.19[b] |
|  | TS 25 °C | 70±1.22[o] | 1.60±0.001[b] | 32.53±0.15[a] |
|  | TSα 4 °C | 78±1.56[m] | 1.47±0.002[d] | 32.19±0.41[a] |
|  | TSα 25 °C | 72±1.38[n] | 1.58±0.001[b] | 32.07±0.48[a] |
| 21 | TS 4 °C | 156±1.33[e] | 1.54±0.001[b] | 31.49±0.39[ab] |
|  | TS 25 °C | 467±1.12[b] | 1.55±0.002[d] | 32.22±0.49[a] |
|  | TSα 4 °C | 70±1.45[o] | 1.54±0.002[c] | 32±0.83[a] |
|  | TSα 25 °C | 466±1.87[b] | 1.58±0.003[b] | 32.57±0.48[a] |
| 28 | TS 4 °C | 209±1.28[d] | 1.46±0.002[d] | 31.71±0.26[ab] |
|  | TS 25 °C | 464±1.66[c] | 1.62±0.002[a] | 32.25±0.62[a] |
|  | TSα 4 °C | 98±1.29[i] | 1.57±0.002[a] | 31.89±0.29[a] |
|  | TSα 25 °C | 483±1.57[a] | 1.61±0.003[a] | 32.52±0.46[a] |

Means in each column with different superscript letters are significantly different ($p<0.05$). TS: antioxidant-free samples; TSα: samples incorporated with 100 ppm α-tocopherol.

The increase in the droplet size of α-tocopherol-loaded nanoemulsions kept at 4°C was less than that of α–tocopherol-free ones. At 25°C, the particle size was relatively constant during first 14 days; however, an abrupt increase was observed at longer storage times (>21 days). The increase in the particle size can be explained in terms of droplet collisions within the aqueous phase. Temperature has an enhancing effect on the Brownian motion of nanoparticles. The higher stability of antioxidant-incorporated nanoemulsions was attributed to the presence of antioxidant at interface. Generally, the

viscosity of nanoemulsions increased significantly ($p<0.05$) as a function of time (Table 3). As already mentioned, the viscosity of nanoemulsions is considerably larger than that of conventional emulsions at same oil content. Different destabilization mechanisms may occur during storage of nanoemulsions including flocculation, coalescence and Ostwald ripening. During flocculation, the continuous phase is entrapped within flocculated particles and viscosity increases for this reason. In other words, emulsion viscosity may increase appreciably if the droplets are flocculated because of the continuous phase entrapment (McClements *et al.*, 2010). Therefore, the viscosity of emulsions is a function of both particle size and the amount of flocculated particles. Particle size reduction (via increasing the surface area to volume ratio) and flocculation (through entrapment of continuous phase) have an enhancing effect on the emulsion viscosity. The final viscosity is a consequence of these two parameters. The viscosity of antioxidant-free nanoemulsions stored at 4 °C revealed an opposite trend (i.e., a slight decrease in the viscosity was observed during storage). Surface tension of antioxidant-free nanoemulsions increased as a result of increased particle size; whereas, it remained relatively constant in antioxidant containing system at two studied temperatures (Table 3). The arrangement of surfactant molecules near each other may have an important role in the observed surface tension. Indeed, the interfacial layer is not a homogeneous shell around the oil droplet core and consists of various molecules with specific structures, organizations and interactions (Lam & Nickerson., 2013). Teixeira *et al* (2016) reported that the viscosity of α-tocopherol-loaded nanoemulsions was higher than that of antioxidant-free ones; however, the surface tension of nanoemulsions without antioxidant was higher than those with antioxidant.

**The morphology of droplets**

Nanoemulsions, prepared at HLB 12 and SOR 1.5, were subjected to morphological studies using TEM (Fig. 1). A mean diameter of less than 100 nm could be observed. Based on TEM observation, we can conclude that the dispersed phase of emulsion was in nanometer range confirming the results of laser diffraction technique.
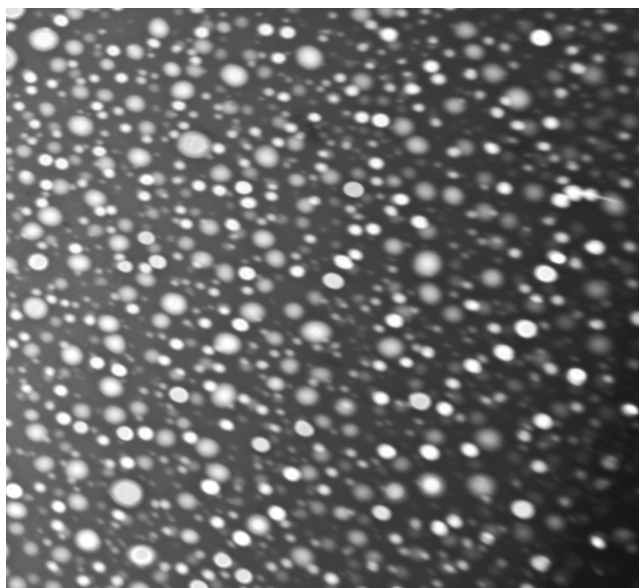


700 nm
**Fig 1. TEM image of nanoemulsions**

## Conclusion

In this work, a high energy approach was used to develop fish oil nanoemulsion. The characteristics of nanoemulsions were studied under the influence of SOR, HLB and storage temperature. A decrease in the particle size and surface tension as well as an increase in the refractive index and viscosity was observed by increasing the SOR and HLB. During storage, incorporating α-tocopherol into nanoemulsions fairly increased the stability of nanoemulsion. The results reported in this study may have implications for the design and utilization of nanoemulsions as delivery systems for food fortification particularly after optimization of formulation using off-flavors masking agents. In future studies, it would be informative to examine the lipid digestion, bioavailability and body intake.

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# تغییرات کشش سطحی و گرانروی نانوامولسیون روغن ماهی تولید شده با روش فراصوت طی انبارمانی

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## چکیده

مصرف کافی از اسیدهای چرب ضروری امگا 3 تاثیری مثبت بر سلامت انسان دارد. برای دستیابی به این هدف از مواد غذایی فراسودمند غنی شده با اسیدهای چرب ضروری می‌توان بهره برد. نانوامولسیون‌ها می‌توانند به‌عنوان یک سیستم تحویل اسیدهای چرب ضروری در موادغـذایی مختلـف و نوشیدنیها مورد استفاده قرار گیرند. در این مطالعه، نانوامولسیون روغن ماهی تولید شده با روش فراصوت در نسبت‌های مختلـف HLB و SOR طـی نگهداری در دو دمای 4 و 25 درجه سانتی‌گراد برای مدت 1 ماه تحت آزمون‌های مختلفی قرار گرفتند. در نیمی از نمونه‌ها از آلفاتوکوفرول بـا غلظـت 100 پی‌پی‌ام استفاده گردید. با افزایش میزان HLB و SOR، اندازه ذره و کشش سطحی کاهش ولی گرانروی و ضریب شکست افزایش یافتند. طـی انبارمانی، اندازه ذرات نانوامولسیون حاوی آنتی‌اکسیدان آلفاتوکوفرول کاهش یافت اما اندازه ذرات نانوامولسیون‌های بدون آلفا توکوفرول (طـی رونـدی وابسته به دمای نگهداری) افزایش یافت. صرف نظر از دمای نگهداری، کشش سطحی نانوامولسیون‌های حاوی آنتی‌اکسیدان آلفا توکوفرول ثابت بـاقی ماند، هرچند که ویسکوزیته آنها افزایش یافت. پایداری شیمیایی نمونه‌های حاوی آنتی‌اکسیدان به دلیل قرار گرفتن آنها در فضای بین سـطحی نسـبتاً افزایش یافت. تصاویر میکروسکوپ الکترونی عبوری وجود ذرات در مقیاس نانومتر را تأیید نمودند. نتایج این تحقیق ممکن است به طراحـی غـذاهای فراسودمند با استفاده از سیستم‌های تحویل بر پایه نانوامولسیون کمک نماید.

**واژه‌های کلیدی:** روغن ماهی، فراصوت با شدت بالا، ویسکوزیته، کشش سطحی، نانوامولسیون

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