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# Nano-encapsulation of fish oil in nano-liposomes and its application in fortification of yogurt

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ABSTRACT

Fish oils have many dietary benefits, but due to their strong odors and rapid deterioration, their application in food formulations is limited. For these reasons, nano-liposome was used to nano-encapsulate fish oil in this study and encapsulated fish oil was utilized in fortifying yogurt. Physicochemical properties of produced yogurt including pH, acidity, syneresis, fatty acid composition, peroxide value as well as sensory tests were investigated during three weeks storage at 4 °C. Nano-liposome encapsulation resulted in a significant reduction in acidity, syneresis and peroxide value. The results of gas chromatography analyses revealed that after 21 days storage, yogurt fortified with nano-encapsulated fish oil had a higher DHA and EPA contents than yogurt containing free fish oil. Overall, the results of this study indicates that adding nano-encapsulated fish oil into yogurt gave closer characteristics to control sample in terms of sensory characteristics than yogurt fortified with free fish oil.



## 1. Introduction

There is evidence that poly unsaturated fatty acids (PUFAs) have beneficial effects on health, including prevention of cardiovascular diseases, decrease the risk of some types of cancer and autoimmune disorders, proper development and function of the brain and retina and prevention and treatment of many diseases. Thus, an adequate intake of omega PUFA is important. Fish and other sea animals are the richest sources of PUFA in human diet. Omega-3 fatty acids are long chain polyunsaturated fats containing methylene-separated double bonds starting from the third carbon atom counted from the methyl-terminus. The quantitatively most important long-chain n-3 PUFA in the diet are *cis*-5,8,11,15,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) (Kolanowski, Ziolkowski, Weißbrodt, Kunz, & Laufenberg, 2006; Ruxton, Reed, Simpson, & Millington, 2004; Siddiqui et al., 2004).

Recent evidence shows that the intake of EPA plus DHA is negatively related to cardiovascular risk in a dose-dependent way up to about 250 mg/d (1–2 servings of oily fish per week) in healthy populations (European Food Safety Authority, 2009). The proposed labeling reference intake value for long chain n-3 PUFA (200 mg) is lower than this value, as are observed average intakes of EPA plus DHA in adults in some European countries, which vary between 80 mg/d and 420 mg/d. The European Food Safety Authority proposes 250 mg/d as the labeling reference intake value for the long-chain n-3 PUFAs EPA plus DHA, which is in agreement with most recent evidence on the relationship between the intake of these fatty acids and cardiovascular health in healthy populations.

Due to low consumption of fish in many societies, supplementation of the diet with fish oil capsules seems to be the easiest way to elevate the level of omega-3 LC PUFA intake (Bender et al., 2014; Kolanowski, 2010). The increased interest of consumers in fortified foods, many containing micronutrients such as omega-3, has been significant (Siro, Kapolna, Kapolna, & Lugasi, 2008). Nevertheless, the challenge in producing fortified foods has been tremendous. The main challenge in producing these foods is related to the stability and undesirable flavors of fish oil. Using highly refined and odorless or microencapsulated fish oil may be an alternative way to mask undesirable sensory characteristics and thus protect the oil during processing (Iafelice et al., 2008).

Encapsulation is a unique way to package materials in the form of micro- and nano-particles and is defined as a process to entrap one substance (active agent) within another (wall material) (Jafari, Assadpoor, He, & Bhandari, 2008; Mahdavi, Jafari, Ghorbani, & Assadpoor, 2014). In the food industry, it involves the incorporation of ingredients such as polyphenols, volatile additives, colors, enzymes and bacteria in small capsules to stabilize, protect and preserve them against processing, nutritional and health losses (Zuidam & Shimoni, 2010). There are a lot of encapsulation techniques among them liposome encapsulation was used in this research.

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Nano-liposome technology is one of the most recent nano-encapsulation techniques (Munin & Edwards-Lévy, 2011). Liposomes (the term refers to artificially constructed capsules of phospholipid bilayers) are spherical particles with sizes in the nanometer to micrometer range. The vesicular particles may consist of one or more bilayer membranes. Liposomes have been widely used in food sectors both in research and industry; it has become feasible to use liposomes to deliver functional components such as nutraceuticals, antimicrobials, and flavors to foods because of having a number of benefits, e.g. possibility of large-scale production using natural ingredients and entrapment and release of water-soluble, lipid-soluble, and amphiphilic materials as well as targetability (Cui, Li, Li, Vittayapadung, & Lin, 2016; Lin et al., 2015; Mozafari et al., 2008). Different procedures have been developed to produce nano-sized liposomes; from thermal methods to non-thermal ones (Mozafari et al., 2008; Mozafari, Reed, Rostron, Kocum, & Piskin, 2002).

Current encapsulation technologies of fish oils are commonly based on spray-drying with the disadvantage that the high temperatures used during the drying process accelerates the oxidation of oils (Jafari, Assadpoor, Bhandari, & He, 2008; Kagami et al., 2003; Kolanowski et al., 2006; Pourashouri et al., 2014a,b). Moreover, there are various types of encapsulated fish oil structures which have been reviewed by Beindorff and Zuidam (2010), but still some challenges of fish oil encapsulation have not been solved; so more recent techniques can be applied to defeat these drawbacks. One of the solutions could be nano-encapsulation. Hence, the objective of this study was to evaluate the incorporation of fish oil nano-encapsulated by nano-liposomes into yogurt and evaluating their effect on the physicochemical and sensory quality of yogurt samples.

## 2. Materials and methods

Soy lecithin was purchased from Merck Company (Germany). Purified fish oil was obtained from Jiangyin Shuji International Trade Co., China, and sunflower oil (Nina, Iran) acquired at a local market. All other chemicals used in this study were of analytical grade and purchased from chemical suppliers.

### 2.1. Preparation of nano-liposomes

Nano-liposomes were prepared according to the modified method of Rasti et al. (Rasti, Jinap, Mozafari, & Yazid, 2012). Briefly, ingredients of the liposomal formulation (lecithin, sunflower oil) were mixed in a heating bath (IKA®HB4, USA) at 30 °C to ensure complete dissolving of lecithin in oil. Then, fish oil (preheated to 30 °C) was added drop wise into the lecithin-oil mixture while stirring at 1000 rpm (Hydolph, Germany) on a hotplate. Finally, this solution was hydrated by adding deionized water and glycerol (final concentration 2%, v/v and preheated to 30 °C) and homogenizing for 10 min by a rotor-stator homogenizer (Basic B50, IKA, Germany). The liposomal dispersions were subjected to sonication (7 min; 1 s on and 1 s off) at 25 °C using a probe sonicator (200 UPS, Dr. Heischler, Germany), and a nominal frequency of 20 kHz at 80% of full power before annealing. Final nano-liposomes were kept at 25 °C (ambient temperature) under nitrogen for at least 1 h after preparation to anneal and stabilize them.

### 2.2. Centrifugal stability measurement

Nano-liposome stability (NS), was determined by centrifugation 5 ml of nano-liposomes at 3500 rpm for 15 min. NS was calculated as:

$$NS = \frac{f_{eV}}{i_{eV}} \times 100 \quad (1)$$

where $f_{ev}$ is the final volume and $i_{ev}$ is the initial volume of liposomal dispersion (Sciarini, Maldonado, Ribotta, Pérez, & León, 2009).

### 2.3. Particle size and size distribution

Average particle size (PS), and size distribution (polydispersity index; PDI) of nano-liposome preparations were measured by dynamic light scattering (Nano ZS90, Malvern Instruments, Worcester, UK) technique at 25 °C, using a He-Ne laser of 633 nm and a detector angle of 173 °C. Three independent measurements were performed for each sample. The Malvern measures the time-dependent fluctuations of light scattered by the liposomes and uses it to calculate the average size and polydispersity of the liposomes. Samples were analyzed 24 h after preparation. Nano-liposomes were appropriately diluted with the aqueous phase of the formulations prior to the measurements. The particle size values given are averages of three measurements and are expressed as mean. An optical microscope (phase contrast) was used to confirm nano-liposome sizes.

### 2.4. Nano-encapsulation efficiency

In order to evaluate the effectiveness of nano-encapsulation, first the nano-liposome dispersions were centrifuged at 4200×*g* for 15 min (Hettich Lab Technology, Germany) to leave only the non-encapsulated active compound (fish oil). After phase separation, 1 ml of supernatant was collected. Then 5 mL chloroform was added and the samples were extracted for 5 min and filtered through 0.45-μm-sized Millipore membrane and left for 24 h after being well mixed to allow enough time for all entrapped active compound to be in the solution (Viriyaroj et al., 2009). The absorbance at 280 nm was measured by a spectrophotometer (PG-instrument-Ltd, UK). Similarly, the fish oil within the bottom layer of liposomal structures was extracted and its content was determined as "encapsulated fish oil". Encapsulation efficiency (%EE) was calculated according to Eq. (2) (Gomes, Moreira, & Castell-Perez, 2011; Hill, Gomes, & Taylor, 2013).

$$\%EE = \frac{(\text{Total fish oil within nanoliposomal disperions}) - (\text{non-encapsulated fish oil})}{\text{Total fish oil content}} \times 100 \quad (2)$$

### 2.5. Yogurt preparation

Yogurt formulations were made by adding 15 mL nano-liposomal emulsions into 100 g yogurt samples separately (8.2% fat, Pegah Dairy Company, Gorgan, Iran) and stored in glass containers in a refrigerator at 4 °C for further analysis. Physicochemical properties and sensory evaluations were studied over 21 days at 4 °C (weekly).

### 2.6. Physicochemical properties of yogurt samples

Titrable acidity and pH were measured according to the AOAC official method 942.15 (AOAC, 2000). Titrable acidity was

expressed as g of lactic acid/100 g product after mixing 10 g of yogurt sample with 10 mL hot distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthalein indicator.

To determine syneresis, 20 g of yogurt was centrifuged at 500 rpm for 5 min. The separated liquid was collected in a graduated cylinder. Syneresis percentage was calculated using the following equation (Achanta, Aryana, & Boeneke, 2007):

$$\text{syneresis} = \frac{\text{Total weight of separated liquid (g)}}{\text{Total weight of yogurt (g)}} \times 100 \quad (3)$$

### 2.7. GC-FID analysis of fatty acids profile

Fat from yogurt samples was extracted using chloroform-methanol (2:1; v/v) and then converted into fatty acids methyl esters (FAME). Fatty acid profile was determined using gas chromatography technique. GC was performed in a CP 3800 Varian Gas Chromatograph fitted with a FID (GC-FID). A highly polar column, BPX 70 (SGE Analytical Science, Australia) was selected for the analysis. The column was 120 m × 0.25 mm × 0.25 lm. The temperature program was as follows: initial temperature, 140 °C (hold for 5 min); temperature rate, 4 °C/min; final temperature, 240 °C for a final holding time of 20 min. Helium was used as the carrier gas at a flow rate of 1.3 ml/min The detector and injector port temperatures were maintained at 260 °C. The individual fatty acid contents were expressed as weight percentages (g/100 g of fat) (AOAC, 996.06).

### 2.8. Peroxide value (PV)

The Peroxide value was determined according to the official methods of AOCS (2007). Briefly, the extracted oil sample (3 g) was dissolved in glacial acetic acid (30 ml) and chloroform (20 ml) (3:2 v/v). Then saturated KI solution (1 ml) was added. The mixture was kept in the dark for 1 min. After adding distilled water (50 ml), the mixture was titrated against sodium thiosulfate (0.01 N). The PV value (mEq of oxygen/kg sample) was calculated using the following equation:

$$\text{PV value} = 1000\,(S \times N)/W \quad (4)$$

where S is the volume of sodium thiosulfate solution (blank corrected) in ml, N is the normality of sodium thiosulfate solution, and W is the weight of oil sample (gram) (AOCS, 2007).

### 2.9. Sensory evaluation

Twenty trained panelists aged between 25 and 35 years old were selected to take part in the sensory panel. Sensory evaluation consisting of color, taste, texture and overall acceptance were based on 5-point hedonic scales (1: dislike extremely; 5: like extremely). Each sample was scored individually, and the samples were presented to the panelists in the individual plastic containers. Yogurts, coded with 3 digits, were randomly presented to the panel group at each session. Water was also presented to rinse their palate between samples (Lawless & Heymann, 2010).

### 2.10. Statistical analysis

Three yogurt samples were produced: (1) Control sample (yogurt without fish oil), (2) Yogurt fortified with unencapsulated (free) fish oil, (3) Yogurt fortified with nano-encapsulated fish oil. All samples were stored for 3 weeks storage time. Each experiment was carried out at least in duplicate and measurements performed at least in triplicate. Statistical analysis of data and analysis of variance was calculated using the SPSS program with a confidence level of 95%, to find any significant differences between treatments.

## 3. Results and discussion

### 3.1. Nano-encapsulation results

#### 3.1.1. Nano-encapsulation efficiency of fish oil within nano-liposomes

By definition, encapsulation efficiency is the amount of core material encapsulated inside the particles. Encapsulation efficiency reflects not only the non-encapsulated oil present on the surface of nano-capsules, but also the proportion of oil extracted from near the surface of the capsules. Nano-encapsulation efficiency of fish oil within nano-liposomes was calculated as 92.22 ± 0.19%. In other words, about 92% of the added fish oil has been encapsulated within nano-liposomes and only less than 8% has been remained unencapsulated.

In the liposomal structure, the aqueous core and bilayer wall are the hydrophilic and hydrophobic parts, respectively; therefore, the phospholipid bilayers act as the reservoir for fish oil. It is generally accepted that the encapsulation efficiency of the active substances within liposomal structure can be affected by the size and/or specific surface areas of the liposomes (Feller, Gawrisch, & MacKerell, 2002; Rasti et al., 2012). Similar results were found in previous studies which reported high yields (83–95%) of nano-liposomes (Carvalho et al., 2015; Colas et al., 2007; Liu & Park, 2009; Lu, Li, & Jiang, 2011; Lu et al., 2014; Toniazzo et al., 2014; Wechtersbach, Poklar Ulrih, & Cigić, 2012; Wu & Xiao, 2005). Our result was in agreement with those reported by Elizondo et al. (2012) who stated that liposomal methods had a significant effect on the physical characteristics and encapsulation efficiency of bioactive compounds. Moreover, Mozafari and Mortazavi (2005) showed increased lipid phase (oil) from 10 to 40 g in the preparation of liposomes resulted in an increase in the stability and efficiency of nanoencapsulated particles so that the half-life increased from 20 min to 3 h.

#### 3.1.2. Stability of nano-liposomes containing fish oil

The results of centrifugal test revealed that the stability of nano-liposomes containing fish oil was 70.03%. Centrifuge stability indicates the numeric value of the aqueous phase separated from the nano-liposome dispersion. So, smaller the number, the less aqueous phase is isolated, and thus, more stable the dispersion. We found that nearly 70% of the nano-liposome samples were stable. High stability of liposomes may be due to the presence of phospholipids in their structure. The stability of liposome vesicles could be influenced by the composition of the external environment, liposome size, the number of layers, the structure of phospholipid and liposome producing techniques (Laridi et al., 2003). According to Taylor, Gaysinsky, Davidson, Bruce, and Weiss (2007), instability of liposomes was attributed to collisions and eventual merging of membranes of two or more liposomes. This process is thermodynamically driven because of the tendency of the system to decrease the energetically unfavorable curvature of the bilayer membrane in spherical liposomes. Collisions may be because of random (Brownian) movement of vesicles in solution or because of superimposed convection (Taylor et al., 2007).

#### 3.1.3. Particle size of nano-liposomes

Size distribution of nano-liposomes containing fish oil has been shown in Fig. 1. DLS results indicated that nano-capsules had a narrow particle-size range (300–500 nm) with a relatively uniform distribution and all particles distributed in less than 1000 nm. The size of fish oil nano-liposomes were within the size range reported by other studies (Liu & Park, 2009; Panya et al., 2010; Wang, Lv, Lu, Jiang, & Lin, 2015; Zou et al., 2014). Mean diameter and polydispersity index (PDI) were 409 ± 1.22 nm and 0.557 ± 0.01, respectively. PDI is a measure of the uniformity of

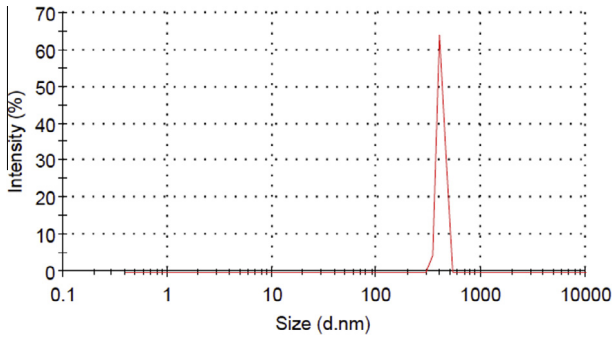



**Fig. 1.** DLS Size distribution of fish oil encapsulated by nano-liposomes.

particle sizes present in the suspension; therefore its value can reflect the homogenous size distribution of vesicles.

### 3.2. Physicochemical characteristics of yogurt fortified with nano-encapsulated fish oil

#### 3.2.1. pH and acidity

We evaluated pH and acidity changes of yogurt samples during 21 days storage at 4 °C. As seen in Fig. 2A, sharpest decline of pH was in control sample and the lowest trend was in the sample containing free (unencapsulated) form of fish oil. The results of this study indicated that the primary pH value in all samples was about 4.43, and in all samples, the pH was reduced over time possibly due to transformation of lactose into lactic acid by starter culture bacteria. The results also indicated that increased acidity in control was higher than other samples so that encapsulated samples were almost more stable during storage because of the effect of encapsulation protection (Fig. 2B). Our results also was in agreement with Bonczar, Wszołek, and Siuta (2002) who reported that increasing fat level in yogurt, increased acidity (Bonczar et al., 2002).

#### 3.2.2. Syneresis of yogurt samples

Syneresis (released whey) is one of the most important physical parameters to measure the quality of yogurt. Changes of syneresis in all samples during 21 days storage at 4 °C has been shown in Fig. 3. Storage time ($P < 0.05$) significantly influenced syneresis. The amount of syneresis during the last week of storage was significantly lower compared with first and second week of storage. This could be explained by the decrease in pH during storage which may have contracting effect on the casein micelle matrix causing more serum to be released. Similar observations of higher syneresis initially followed by decreased syneresis have been observed by other investigators as well (Aryana, Plauche, Rao, McGrew, & Shah, 2007; Estrada, Boeneke, Bechtel, & Sathivel, 2011; Salvador & Fiszman, 2004; Staffolo, Bertola, & Martino, 2004). Syneresis reduction over time in the sample containing nano-encapsulated fish oil was almost similar to the control sample and the one fortified with

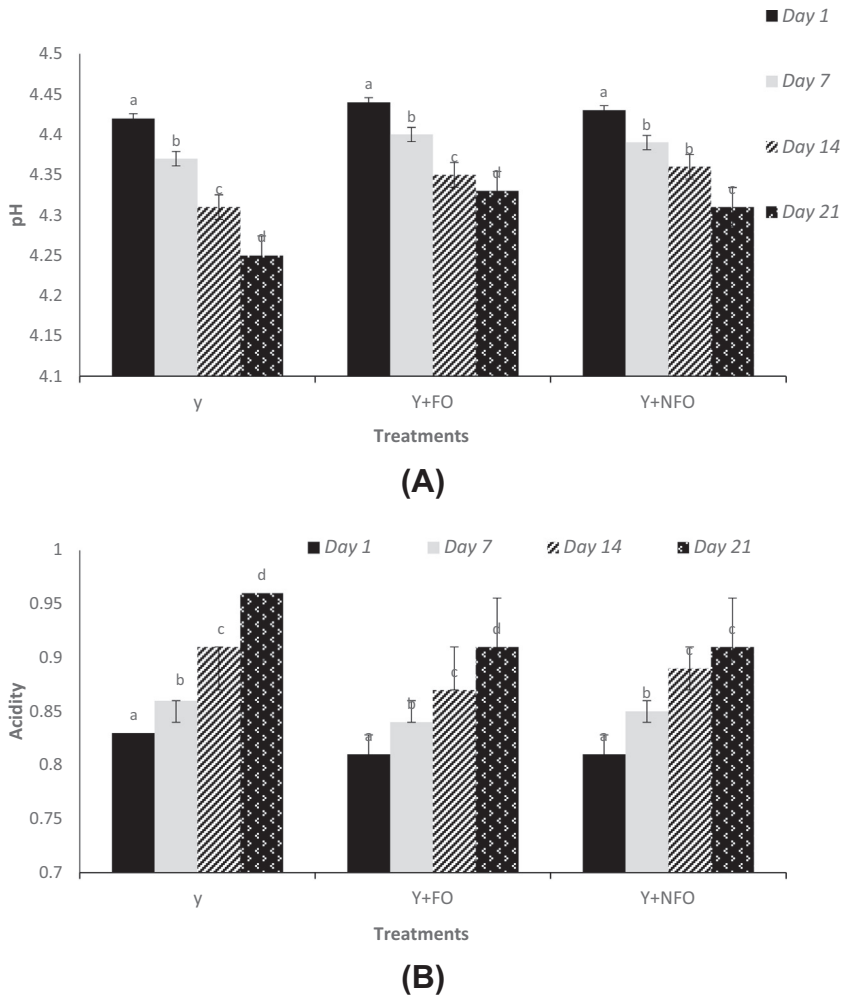



**Fig. 2.** Changes in (A) pH and (B) acidity of different yogurt samples during 21 days storage. Y: control sample (yogurt); Y + FO: yogurt enriched with free form of fish oil; Y + NFO: yogurt enriched with nano-liposomal encapsulated fish oil, Different letters show statistically significant results ($P < 0.05$) of each sample during storage.

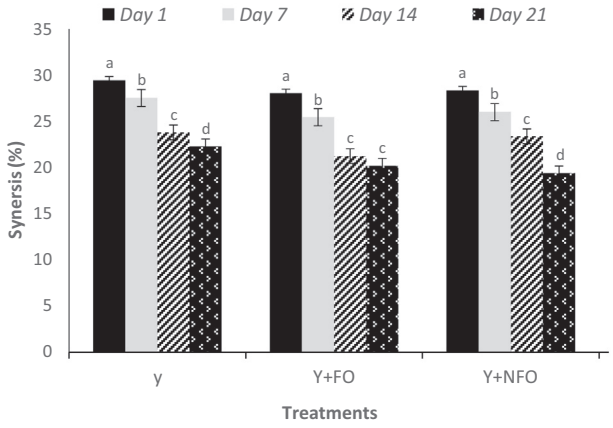
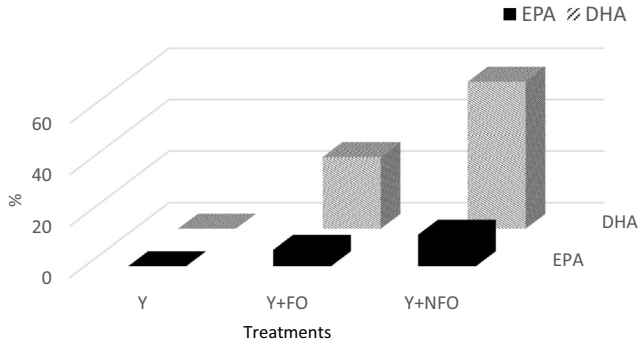



**Fig. 3.** Syneresis changes of different yogurt samples during 21 days storage. Y: control sample (yogurt); Y + FO: yogurt enriched with free form of fish oil; Y + NFO: yogurt enriched with nano-liposomal encapsulated fish oil, Different letters show statistically significant results ($P < 0.05$) of each sample during storage.

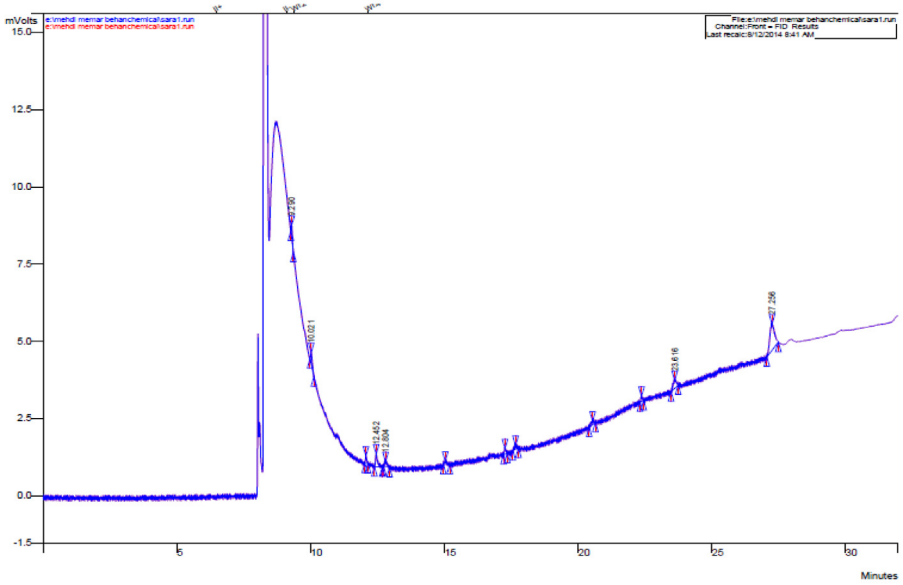
free fish oil. Lecithin as an emulsifier added through liposomes could absorb some water and retard its separation. Also, increased total dry matter, results in higher water holding capacity and increased network stability which then could reduce the syneresis (Achanta et al., 2007). Reduction of free water and increasing the proportion of solids contents, which occur during concentration, are two main factors decreasing the rates of wheying off in the samples with high total solids. Similarly, Shaker, Jumah, and Abu-Jdayil (2000) indicated that the increase in viscosity of yoghurt with highest fat content may be due to increase of total solids of the milk which has a significant effect on the firmness of yoghurt gel and decreasing degree of syneresis.

### 3.2.3. Fatty acid profile of fortified yogurt

Chromatogram profile of fatty acids in fortified yogurt samples was different after 21 days storage at 4 °C, revealing that fortification of yogurt with nano-encapsulated fish oil had a significant effect on the retention of omega-3 fatty acids (DHA + EPA) (Fig. 4A). Our data showed that the maximum retention of DHA and EPA after 21 days storage was in yoghurt samples containing nano-encapsulated fish oil (57% and 12%, respectively) while unencapsulated samples had 27% and 6% DHA and EPA (decreased to about 50% of its initial value), respectively. The reason could be possibly due to the effectiveness of nano-encapsulation process within nano-liposomes and higher protection of omega 3 fatty acids by nano-liposomes against environmental deteriorating




**(A)**




**(B)**

**Fig. 4.** (A) EPA and DHA fatty acids in different yogurt samples after 21 days storage. Y: control sample (yogurt); Y + FO: yogurt enriched with free form of fish oil; Y + NFO: yogurt enriched with nano-liposomal encapsulated fish oil, (B) Fatty acid chromatogram of oil extracted from yogurt with nano-encapsulated fish oil after 21 days storage at 5 °C.

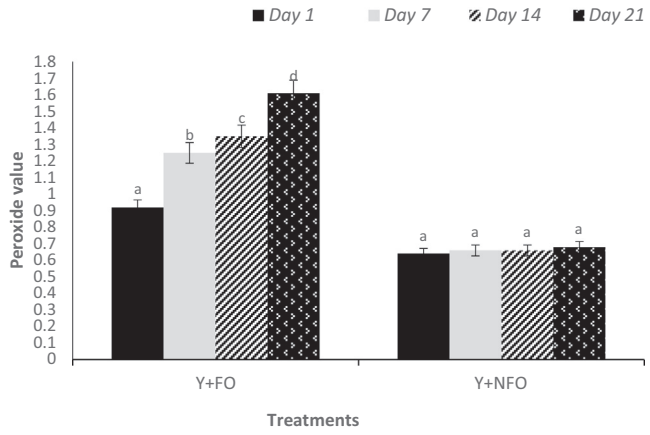



**Fig. 5.** Peroxide value changes of different yogurt samples during 21 days storage. Y + FO: yogurt enriched with free form of fish oil; Y + NFO: yogurt enriched with nano-liposomal encapsulated fish oil, Different letters show statistically significant results ($P < 0.05$) of each sample during storage.

conditions. Our result was in agreement with Borneo, Kocer, Ghai, Tepper, and Karwe (2007) who reported adding fish oil in the form of microencapsulated to food products, is one of the best ways to maintain and increase stability of omega 3 fatty acids in food formulations.

### 3.2.4. Peroxide value

Interestingly, as it can be seen in Fig. 5, peroxide value of oil extracted from samples containing un-encapsulated fish oil was 0.92 (meq/kg) in the first day which increased to 1.61 (meq/kg) in the 21st day while for yogurt samples containing nano-encapsulated fish oil, peroxide value was almost constant (PV = 0.6) during 21 days storage. It is quite obvious that nano-encapsulation protected unsaturated fatty acids well from deteriorating factors such as oxidation. Ye, Cui, Taneja, Zhu, and Singh (2009) reported similar results. They claimed that adding encapsulated fish oil into cheese significantly decreased peroxide and thiobarbituric acid values after 35 days storage (Ye et al., 2009).

### 3.3. Sensory evaluation of yogurt samples

Mean scores of the sensory evaluation parameters were statistically analyzed and the results are given in Table 1. Control and nano-liposomal samples scored the highest value for color and taste. Sensory data showed there was no significant difference among yogurt samples in terms of texture. Concerning the overall acceptability, most of the panelists preferred the control and nano-encapsulated sample and yogurt fortified with free fish oil got the least score. Overall, based on data collected from sensory evaluation in this study, adding nano-encapsulated fish oil into yogurt resulted in close consumer scores to control sample.

**Table 1**
Effect of nano-encapsulation on the sensory characteristics of the yogurt fortified with fish oil.

| Treatments* | Sensory quality score | | | |
|---|---|---|---|---|
| | Taste and aroma | Color | Texture | Overall acceptance |
| Y | $4.03 \pm 0.06^{a}$ | $4.85 \pm 1.02^{a}$ | $4.69 \pm 0.04^{a}$ | $4.72 \pm 0.06^{a}$ |
| Y + FO | $2.84 \pm 0.76^{b}$ | $2.54 \pm 0.45^{c}$ | $4.14 \pm 0.12^{b}$ | $2.65 \pm 0.31^{d}$ |
| Y + NFO | $3.99 \pm 0.56^{a}$ | $4.34 \pm 0.90^{a}$ | $4.55 \pm 0.11^{a}$ | $4.23 \pm 0.11^{a}$ |

Values in the same column bearing different letters are significantly different.
Y + FO: yogurt enriched with free form of fish oil.
Y + NFO: yogurt enriched with nano-liposomal encapsulated fish oil.
* Y: control sample (yogurt).

## 4. Conclusion

This study demonstrated that fish oil could be effectively encapsulated by nano-liposmes. The characterization of the nano-liposomes (efficiency, stability and particle size) suggested liposomal structures as a successful technique for nano-encapsulation. Nano-liposome encapsulation resulted in a significant reduction in acidity, syneresis and proxide value while increasing DHA and EPA stability. Overall, our results revealed that adding nano-encapsulated fish oil into yogurt gave closer characteristics to control sample in terms of sensory parameters than yogurt with free (unencapsulated) fish oil. Further studies should be performed to measure the release of fish oil in simulated gastrointestinal conditions to evaluate this encapsulation technique in detail.

## References


Achanta, K., Aryana, K. J., & Boeneke, C. A. (2007). Fat free plain set yogurts fortified with various minerals. *LWT – Food Science and Technology, 40*(3), 424–429.

AOAC (2000). *The official methods of analysis* (17th ed.). Maryland, USA: Association of Official Analytical Chemists.

AOCS (2007). *Official methods and recommended practices of the American oil chemist's society* (6th ed.). Champaign, IL: AOCS Press.

Aryana, K., Plauche, S., Rao, R., McGrew, P., & Shah, N. (2007). Fat-free plain yogurt manufactured with inulins of various Chain lengths and lactobacillus acidophilus. *Journal of Food Science, 72*(3), M79–M84.

Beindorff, C. M., & Zuidam, N. J. (2010). Microencapsulation of fish oil. In *Encapsulation technologies for active food ingredients and food processing* (pp. 161–185). Springer.

Bender, N., Portmann, M., Heg, Z., Hofmann, K., Zwahlen, M., & Egger, M. (2014). Fish or n3-PUFA intake and body composition: A systematic review and meta-analysis. *Obesity Reviews, 15*(8), 657–665.

Bonczar, G., Wszołek, M., & Siuta, A. (2002). The effects of certain factors on the properties of yoghurt made from ewe's milk. *Food Chemistry, 79*(1), 85–91.

Borneo, R., Kocer, D., Ghai, G., Tepper, B., & Karwe, M. (2007). Stability and consumer acceptance of long-chain omega-3 fatty acids (eicosapentaenoic acid, 20: 5, n-3 and docosahexaenoic acid, 22: 6, n-3) in cream-filled sandwich cookies. *Journal of Food Science, 72*(1), S049–S054.

Carvalho, J. P., Toniazzo, T., Cavalcanti, L., Moraes, I. F., Oliveira, C. P., & Pinho, S. (2015). Physico-chemical stability and structural characterization of thickened multilamellar beta-carotene-loaded liposome dispersions produced using a proliposome method. *Colloid and Polymer Science, 293*(8), 2171–2179.

Colas, J.-C., Shi, W., Rao, V. M., Omri, A., Mozafari, M. R., & Singh, H. (2007). Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. *Micron, 38*(8), 841–847.

Cui, H., Li, W., Li, C., Vittayapadung, S., & Lin, L. (2016). Liposome containing cinnamon oil with antibacterial activity against methicillin-resistant Staphylococcus aureus biofilm. *Biofouling, 32*(2), 215–225.

Elizondo, E., Larsen, J., Hatzakis, N. S., Cabrera, I., Bjørnholm, T., Veciana, J., ... Ventosa, N. (2012). Influence of the preparation route on the supramolecular organization of lipids in a vesicular system. *Journal of the American Chemical Society, 134*(4), 1918–1921.

Estrada, J., Boeneke, C., Bechtel, P., & Sathivel, S. (2011). Developing a strawberry yogurt fortified with marine fish oil. *Journal of Dairy Science, 94*(12), 5760–5769.

Feller, S. E., Gawrisch, K., & MacKerell, A. D. (2002). Polyunsaturated fatty acids in lipid bilayers: Intrinsic and environmental contributions to their unique physical properties. *Journal of the American Chemical Society, 124*(2), 318–326.

Gomes, C., Moreira, R. G., & Castell-Perez, E. (2011). Microencapsulated antimicrobial compounds as a means to enhance electron beam irradiation treatment for inactivation of pathogens on fresh spinach leaves. *Journal of Food Science, 76*(6), E479–E488.

Hill, L. E., Gomes, C., & Taylor, T. M. (2013). Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. *LWT – Food Science and Technology, 51*(1), 86–93.

Iafelice, G., Caboni, M. F., Cubadda, R., Criscio, T. D., Trivisonno, M. C., & Marconi, E. (2008). Development of functional spaghetti enriched with long chain omega-3 fatty acids. *Cereal Chemistry, 85*(2), 146–151.

Jafari, S. M., Assadpoor, E., Bhandari, B., & He, Y. (2008). Nano-particle encapsulation of fish oil by spray drying. *Food Research International, 41*(2), 172–183.

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

Kagami, Y., Sugimura, S., Fujishima, N., Matsuda, K., Kometani, T., & Matsumura, Y. (2003). Oxidative stability, structure, and physical characteristics of microcapsules formed by spray drying of fish oil with protein and dextrin wall materials. *Journal of Food Science, 68*(7), 2248–2255.

Kolanowski, W. (2010). Omega-3 LC PUFA contents and oxidative stability of encapsulated fish oil dietary supplements. *International Journal of Food Properties, 13*(3), 498–511.
Kolanowski, W., Ziolkowski, M., Weißbrodt, J., Kunz, B., & Laufenberg, G. (2006). Microencapsulation of fish oil by spray drying–impact on oxidative stability. Part 1. *European Food Research and Technology, 222*(3–4), 336–342.
Laridi, R., Kheadr, E., Benech, R.-O., Vuillemard, J., Lacroix, C., & Fliss, I. (2003). Liposome encapsulated nisin Z: Optimization, stability and release during milk fermentation. *International Dairy Journal, 13*(4), 325–336.
Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: Principles and practices*. Springer Science & Business Media.
Lin, L., Cui, H., Zhou, H., Zhang, X., Bortolini, C., Chen, M., ... Dong, M. (2015). Nanoliposomes containing Eucalyptus citriodora as antibiotic with specific antimicrobial activity. *Chemical Communications, 51*(13), 2653–2655.
Liu, N., & Park, H.-J. (2009). Chitosan-coated nanoliposome as vitamin E carrier. *Journal of Microencapsulation, 26*(3), 235–242.
Lu, Q., Li, D.-C., & Jiang, J.-G. (2011). Preparation of a tea polyphenol nanoliposome system and its physicochemical properties. *Journal of Agricultural and Food Chemistry, 59*(24), 13004–13011.
Lu, Q., Lu, P.-M., Piao, J.-H., Xu, X.-L., Chen, J., Zhu, L., & Jiang, J.-G. (2014). Preparation and physicochemical characteristics of an allicin nanoliposome and its release behavior. *LWT – Food Science and Technology, 57*(2), 686–695.
Mahdavi, S. A., Jafari, S. M., Ghorbani, M., & Assadpoor, E. (2014). Spray-drying microencapsulation of anthocyanins by natural biopolymers: A review. *Drying Technology, 32*(5), 509–518.
Mozafari, M. R., Khosravi-Darani, K., Borazan, G. G., Cui, J., Pardakhty, A., & Yurdugul, S. (2008). Encapsulation of food ingredients using nanoliposome technology. *International Journal of Food Properties, 11*(4), 833–844.
Mozafari, M. R., & Mortazavi, S. M. (2005). *Nanoliposomes: From fundamentals to recent developments*. Oxford, UK: Trafford Publishing Ltd.
Mozafari, M. R., Reed, C. J., Rostron, C., Kocum, C., & Piskin, E. (2002). Construction of stable anionic liposome-plasmid particles using the heating method: A preliminary investigation. *Cellular and Molecular Biology Letters, 7*(3), 923–928.
Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds; a review. *Pharmaceutics, 3*(4), 793–829.
Panya, A., Laguerre, M., Lecomte, J., Villeneuve, P., Weiss, J., McClements, D. J., & Decker, E. A. (2010). Effects of chitosan and rosmarinate esters on the physical and oxidative stability of liposomes. *Journal of Agricultural and Food Chemistry, 58*(9), 5679–5684.
Pourashouri, P., Shabanpour, B., Razavi, S., Jafari, S., Shabani, A., & Aubourg, S. (2014a). Impact of wall materials on physicochemical properties of microencapsulated fish oil by spray drying. *Food and Bioprocess Technology, 7*(8), 2354–2365.
Pourashouri, P., Shabanpour, B., Razavi, S. H., Jafari, S. M., Shabani, A., & Aubourg, S. P. (2014b). Oxidative stability of spray-dried microencapsulated fish oils with different wall materials. *Journal of Aquatic Food Product Technology, 23*(6), 567–578.
Rasti, B., Jinap, S., Mozafari, M., & Yazid, A. (2012). Comparative study of the oxidative and physical stability of liposomal and nanoliposomal polyunsaturated fatty acids prepared with conventional and Mozafari methods. *Food Chemistry, 135*(4), 2761–2770.
Ruxton, C., Reed, S. C., Simpson, M., & Millington, K. (2004). The health benefits of omega-3 polyunsaturated fatty acids: A review of the evidence. *Journal of Human Nutrition and Dietetics, 17*(5), 449–459.
Salvador, A., & Fiszman, S. (2004). Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage. *Journal of Dairy Science, 87*(12), 4033–4041.
Sciarini, L., Maldonado, F., Ribotta, P., Pérez, G., & León, A. (2009). Chemical composition and functional properties of Gleditsia triacanthos gum. *Food Hydrocolloids, 23*(2), 306–313.
Shaker, R., Jumah, R., & Abu-Jdayil, B. (2000). Rheological properties of plain yogurt during coagulation process: Impact of fat content and preheat treatment of milk. *Journal of Food Engineering, 44*(3), 175–180.
Siddiqui, R. A., Shaikh, S. R., Sech, L. A., Yount, H. R., Stillwell, W., & Zaloga, G. P. (2004). Omega 3-fatty acids: Health benefits and cellular mechanisms of action. *Mini Reviews in Medicinal Chemistry, 4*(8), 859–871.
Siro, I., Kapolna, E., Kapolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. *Appetite, 51*(3), 456–467.
Staffolo, M. D., Bertola, N., & Martino, M. (2004). Influence of dietary fiber addition on sensory and rheological properties of yogurt. *International Dairy Journal, 14*(3), 263–268.
Taylor, T. M., Gaysinsky, S., Davidson, P. M., Bruce, B., & Weiss, J. (2007). Characterization of antimicrobial-bearing liposomes by ζ-potential, vesicle size, and encapsulation efficiency. *Food Biophysics, 2*(1), 1–9.
Toniazzo, T., Berbel, I. F., Cho, S., Fávaro-Trindade, C. S., Moraes, I. C., & Pinho, S. C. (2014). β-carotene-loaded liposome dispersions stabilized with xanthan and guar gums: Physico-chemical stability and feasibility of application in yogurt. *LWT – Food Science and Technology, 59*(2), 1265–1273.
Viriyaroj, A., Ngawhirunpat, T., Sukma, M., Akkaramongkolporn, P., Ruktanonchai, U., & Opanasopit, P. (2009). Physicochemical properties and antioxidant activity of gamma-oryzanol-loaded liposome formulations for topical use. *Pharmaceutical Development and Technology, 14*(6), 665–671.
Wang, Q., Lv, S., Lu, J., Jiang, S., & Lin, L. (2015). Characterization, stability, and in vitro release evaluation of carboxymethyl chitosan coated liposomes containing fish oil. *Journal of Food Science, 80*(7), C1460–C1467.
Wechtersbach, L., Poklar Ulrih, N., & Cigić, B. (2012). Liposomal stabilization of ascorbic acid in model systems and in food matrices. *LWT – Food Science and Technology, 45*(1), 43–49.
Wu, K. G., & Xiao, Q. (2005). Microencapsulation of fish oil by simple coacervation of hydroxypropyl methylcellulose. *Chinese Journal of Chemistry, 23*(11), 1569–1572.
Ye, A., Cui, J., Taneja, A., Zhu, X., & Singh, H. (2009). Evaluation of processed cheese fortified with fish oil emulsion. *Food Research International, 42*(8), 1093–1098.
Zou, L.-Q., Liu, W., Liu, W.-L., Liang, R.-H., Li, T., Liu, C.-M., ... Liu, Z. (2014). Characterization and bioavailability of tea polyphenol nanoliposome prepared by combining an ethanol injection method with dynamic high-pressure microfluidization. *Journal of Agricultural and Food Chemistry, 62*(4), 934–941.
Zuidam, N. J., & Shimoni, E. (2010). Overview of microencapsulates for use in food products or processes and methods to make them. In *Encapsulation technologies for active food ingredients and food processing* (pp. 3–29). Springer.