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## Use of fish oil nanoencapsulated with gum arabic carrier in low fat probiotic fermented milk

**Abstract** Fish oil consists of omega-3 fatty acids which play an important role in human health. Its susceptibility to oxidation causes considerable degradation during the processing and storage of food products. Accordingly, encapsulation of this ingredient through freeze drying was studied with the aim of protecting it against environmental conditions. Gum arabic (GA) was used as the wall material for fish oil nanoencapsulation where tween 80 was applied as the emulsifier. A water- in- oil (W/O) emulsion was prepared by sonication, containing 6% fish oil dispersed in aqueous solutions including 20% and 25% total wall material. The emulsion was sonicated at 24 kHz for 120 s. The emulsion was then freeze-dried and the nanocapsules were incorporated into probiotic fermented milk, with the effects of nanocapsules examined on the milk. The results showed that the nanoparticles encapsulated with 25% gum arabic and 4% emulsifier had the highest encapsulation efficiency (EE) (87.17%) and the lowest surface oil (31.66 mg/100 kg). Using nanoencapsulated fish oil in fermented milk significantly ( $p<0.05$ ) increased the viability of *Lactobacillus plantarum* as well as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents. The fermented milk sample containing fish oil nanoencapsulated with 25% wall material and 4% emulsifier yielded the greatest probiotic bacterial count (8.41 Log CFU/mL) and the lowest peroxide value (0.57 mEq/kg). Moreover, this sample had the highest EPA and DHA contents. Utilizing this nanoencapsulated fish oil did not adversely affect fermented milk overall acceptance. Therefore, it can be used for fortification of low fat probiotic fermented milk.

**Keywords** fermented milk, fish oil, nanoencapsulation, probiotic

## **Introduction**

Scientific publications, especially population-based epidemiologic studies have always emphasized the role of diet in the prevention and control of disease and premature death caused by non-communicable diseases. Nowadays, many people prefer to consume functional food products especially probiotic products. Dairy products such as fermented milks can be good carriers of probiotic bacteria (Delavari et al., 2014). *Lactobacillus plantarum* is one of the probiotic bacteria which regarded as safe. *L. plantarum* has good acidification ability and produces plantaricin bacteriocin in order to increase the safety of food products. So this bacterium can be used as starter for production of fermented milks due to technological and safety properties (Essid et al., 2009).

Omega -3 fatty acids are a group of polyunsaturated fatty acids (PUFA) which are important in keeping the cardiovascular system healthy. Linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most important omega fatty acids whose major resources include seafood and vegetable products such as walnut, rapeseed and linseed (Lavie et al., 2009; Lopez- Huertas, 2010). Omega-3 fatty acids can be used to enrich low-fat foods and to produce functional foods. However, since these compounds are hydrophobic and do not dissolve in water, enrichment of food products and aqueous drinks with them is very difficult. As they possess unsaturated bonds, these components are extremely sensitive to oxidation whereby they undergo off-odor and off-flavor changes because of oxidation, and their health-promoting effects diminish. Application of nanocarriers to transport and protect fat-soluble micronutrients, or in

other words, nanoencapsulation of such substances is a suitable way to overcome these problems (Liu et al., 2008).

Nanoencapsulation is a technique in which carriers, less than 100 nm in size, are produced and utilized to enrich food products and transport nutraceutical and bioactive compounds (essential fatty acids, antioxidants, carotenoids, etc.) to target special sections (Ye et al., 2006). Nanocapsules differ from one another depending on their applications in food matrices. They are divided into two types of biopolymeric and polymeric. Proteins, polysaccharides or their complexes are used in the production of biopolymeric nanocarriers (Zimet and Livney, 2009). In nanoencapsulation, various types of carbohydrates are often used as wall material or carrier (McName et al., 1998). Carbohydrates including gums, different types of starch, maltodextrin and solid corn syrup are abundantly applied for encapsulation, owing to their diversity and low price as well as their properties such as low viscosity at high concentrations and desirable solubility (Mutka et al., 1988).

GA is the most important gum used as wall material in the nanoencapsulation of flavor compounds. Desirable solubility, low viscosity, emulsifying properties and the high holding capacity of volatile compounds and oil droplets have caused this gum to be widely noticed for nanoencapsulation (Shiga et al., 2001).

Some researchers have examined the encapsulation efficiency of fish oil as a core material. The nano-particles were prepared by spray drying and or emulsion preparation via high energy emulsifying techniques such as microfluidization or ultrasonication. Maltodextrin was utilized as wall material in combination with biopolymers (modified starch or WPC) at a ratio of 3:1. The results revealed that microfluidization was an efficient method for emulsification, resulting in a

fish oil powder with the minimum amount of surface oil mostly due to its ability for emulsification within nano range (210-280 nm) (Jafari et al., 2008). Ghorbanzade et al. (2017) encapsulated fish oil in nanoliposomes and used it in yogurt fortification. The results showed that yogurt containing nanoencapsulated fish oil had higher DHA and EPA contents than yogurt containing free fish oil. Moreover, yogurt containing nano-encapsulated fish oil had closer characteristics to control sample in terms of sensory properties than yogurt containing free fish oil.

Given the increased tendency towards application of fish oil in food, pharmaceutical and sanitary products, it should be researched and the findings should be applied to the respective formulations. Accordingly, the aim of the present study was to encapsulate fish oil by preparing an O/W nanoemulsion using GA as wall material and to examine its effects on the properties of probiotic fermented milk.

## **Materials and Methods**

### **Materials**

Fish oil was purchased from Nooshdaru Darya Co. (Iran). GA (density = 1.4 g/cm<sup>3</sup>), sorbitan mono-9-octadecenoate (tween 80), ethanol, n-hexane and 2-propanol were supplied from Merck Co. (Germany). *Lactobacillus plantarum* IBRC-M 10817 was purchased from Iranian Biological Resourco Center. Other high purity chemicals were purchased from Merck Co. (Germany).

### **Nanoemulsion preparation**

In this study, the wall material was considered 20% and 25% (w/w). The solution of GA was prepared in deionized water. This was done using a magnetic stirrer at 500 rpm and 60°C for 1 h until the wall material (GA) completely dissolved in water. Next, the prepared solution was kept in a refrigerator overnight for complete hydration. Then, tween 80 was added to it at 2 and 4% (w/w). Subsequently, fish oil was incorporated into the mixtures dropwise at 6% (w/w). The resulting mixtures were agitated using a magnetic stirrer at 500 rpm for 30 min. Finally, ultrasonic waves were exploited for nanoemulsion preparation at 24 kHz for 120 s (Saber et al., 2014).

#### **Encapsulation and freeze drying of the emulsion**

The prepared emulsions were frozen at -20°C for 24 h. Next, they were placed in a freeze drier at -50°C for 48 h. The dried emulsions were powdered using a pestle and mortar for further experiments (Klinkeson et al., 2005; Kaushik and Ross, 2007).

#### **Particle size of emulsion**

The particle size distribution was determined by dynamic light scattering (DLS; Malvern; UK) to measure the emulsion droplets diameter.

#### **Extraction of surface and total oil of powders**

For this extraction, 8 mL of hexane was added to 1 g of sample powder and mixed for 2 min, then centrifuged at 8000 rpm for 20 min. The supernatant was filtered through filter paper and the solvent was evaporated at 70°C. The extracted oil was dried at 50°C until a constant weight was obtained (Klinkeson et al., 2006). The total oil was extracted as follows: solvent (3:1 isopropanol:hexane) was added to 0.5 g of powder, stirred for 5 min and the clear organic

solution phase was evaporated at 70°C until a constant weight was obtained (Jimenez et al., 2004).

The encapsulation efficiency (EE) was measured as follows (Wang et al., 2006):

$$EE \% = \frac{\text{total oil} - \text{surface oil}}{\text{total oil}} \times 100$$

#### **Determination of DHA and EPA content**

Analysis of the methyl esters of fatty acids was performed using a gas chromatograph (Plus 2010, Germany) equipped with mass spectrometry detector and a sol-gel 1 ms column with a length of 30 m and a diameter of 0.32 mm. Omega-3 fatty acids were extracted from the complex powders with acetone derived by 1 mL of Boron tri-fluoride methanol, 1 mL of NaOH 2 N and 1 mL of n-hexane. It was then injected into GC whereby the fatty acid composition was examined (Ghorbanzade et al., 2017).

#### **Preparation of the probiotic fermented milk containing nanoencapsulated fish oil**

The 24-h MRS broth cultures of *L. plantarum* were separately centrifuged at 5000 g× for 15 min. The supernatant was discarded and the cells were rinsed twice with distilled water. Then, a suspension of the cells was prepared in distilled water at a concentration of 10<sup>9</sup> CFU/mL. The suspension was subsequently diluted to 10<sup>8</sup> CFU/mL. After that, 1% (v/v) of the suspension was inoculated into the pasteurized milk containing 1.5% fat. Next, the inoculated milk samples were incubated at 37°C for 10 h, the nanocapsules were added to them so that 0.3 g of the omega-3 fatty acids existed in each serving size (240 mL) of the low fat probiotic fermented milk. The samples were finally stored at 4°C.

#### **Probiotic bacterial count**



MRS agar was used for enumeration of *L. plantarum*. Incubation was performed at 37°C for 72 h under anaerobic condition (Golestani and Pourahmad, 2017).

### Measurement of the peroxide value

Peroxide value (PV) was determined based on AOCS method (AOCS, 2007). The oil sample (3 g) was dissolved in acetic acid (30 mL) and chloroform (20 mL) at a ratio of 2:3 (v/v). The mixture was kept in the dark for 1 min and then blended with 50 mL distilled water. It was eventually titrated with sodium thiosulfate 0.01 N. PV was calculated by the following equation:

$$PV = 1000(S \cdot N) / W$$

Where S is the volume of sodium thiosulfate (ml), N stands for the normality of sodium thiosulfate and W denotes the oil sample weight (g).

### Sensory evaluation

The sensory attributes (flavor, color, texture and overall acceptance) of the low fat probiotic fermented milk containing fish oil nanocapsules, were assessed by 10 trained panelists through 5-point hedonic scale (Ilyasoglu and Nehir El, 2014).

### Statistical analysis

The experiment was conducted with completely randomized design (CDR). All experiments were performed in triplicate. One-way analysis of variance (ANOVA) and Duncan multiple range tests were employed for statistical evaluation. SPSS 23 software was used.

## Results and Discussion

### Particle size of emulsions

According to Table 1, there was a significant difference ( $p<0.05$ ) between the diameter of emulsion droplet of the samples. The sample with 25% wall material and 4% emulsifier had the lowest diameter of emulsion droplet. The highest diameter of emulsion droplet was related to the sample with 20% wall material and 2% emulsifier. Many factors affect the droplet size. They can include the amount and type of emulsifier, phase type, pH and preparation method of emulsion, the ratio of components, speed of stirrer, sequence and rate of adding ingredients (Izquierdo et al., 2002; Klinkesorn et al., 2005). The wall composition, gum concentration, mixing conditions and emulsifier can influence the nanoparticle size in emulsions containing gum arabic (Ghayempour and Mortazavi, 2015). The results showed that the particle size decreased as the wall material (gum arabic) increased. The highly branched structure of gum arabic acts as an emulsifier and decreases the particle size through covalent bonding of the oil droplets and the branches. As the emulsification capacity of the wall increased, the migration of ingredients to the capsule surface reduced. In a similar study, Najaf-Najafi et al. (2010) investigated the encapsulation of castor oil and reported that the type and concentration of the wall affected the particle size and the modified starch produced larger particles than skim milk.

### **Surface oil of the nanocapsules**

According to Table 2, there was a significant difference ( $p<0.05$ ) between surface oil content of the samples. The highest surface oil content was related to the sample with 20% wall material and 2% emulsifier. The lowest surface oil content was recorded for the sample with 25% wall material and 4% emulsifier. The size of the emulsion can influence the surface oil. Jafari et al. (2008) reported that the surface oil increases significantly with an increase in the size of the emulsion droplets due to destabilization of the emulsion by bigger droplets, which is in agreement with the findings of this study. Davidov-Parado et al. (2008) showed that the particle

size of emulsions influenced the surface oil content. They found that smaller oil droplets were effectively encapsulated in the matrix of the wall component.

### **Encapsulation efficiency (EE) of the nanocapsules**

According to Table 3, sample 4 (the capsule with 25% gum arabic and 2% emulsifier) had the highest EE. The lowest EE was related to samples 1 (the capsule with 20% gum arabic and 2% emulsifier) and 3 (the capsule with 20% gum arabic and 4% emulsifier). EE represents the degree of surface oil on particles and the degree to which the wall can prevent encapsulated oil extraction. It has been observed that larger emulsion droplets allow increased expulsion of the surface oil content. Particle encapsulated with 25% gum arabic and 4% emulsifier had the highest EE value and the lowest surface oil because of the smaller diameter. The difference of the molecular weight of the wall materials may be a reason for difference in oil holding capacity. An increase in molecular weight increased the material holding capacity, effective oil droplet distribution in solid materials and the low percentage of surface oil (Klaypradit and Huang, 2008). An increase in viscosity in the continuous phase reduced the mobility of the droplets and created enough time for adsorption of the emulsifier onto the surface of the water and oil droplets, which reinforced droplets against sedimentation and stabilized the emulsion, creating capsules with higher EE values after drying (Makri and Doxastakis, 2006). In a similar study, Ilyasoglu and Nehir El (2014) encapsulated EPA/DHA with gum arabic and sodium caseinate and recorded an EE for the encapsulated oil of  $78.88 \% \pm 2.98\%$  for the protein-polysaccharose complex. Moreover, Badee et al. (2012) encapsulated peppermint oil with gum arabic, maltodextrin and their combination by spray-drying. They reported that the highest oil retention of 81% and 80%, respectively, was during drying for gum arabic and the gum arabic: maltodextrin 1:1 combination. In another study, Krishnan et al. (2005) encapsulated cardamom oleoresin in maltodextrin-

modified starch-gum arabic. They found that an increase in gum arabic increased encapsulation efficiency.

#### **Fatty acid composition of the fish oil**

Fig. 1 illustrates the fatty acid composition of the fish oil. In the present study, palmitic acid was the major fatty acid accounting for 18.31 mg/g fish oil. Of the unique properties of fish oil is containing large amounts of omega-3 fatty acids, particularly EPA, DHA, alpha linolenic acid (ALA) compared with other oils. The contents of EPA and DHA were determined 15.18 and 11.32 mg/g fish oil, respectively. The content and composition of fish oil fatty acids vary depending on the fish species, fishing season, fish nutrition and many other factors (Ackman et al., 2005). Fish oil, like most animal fats, is so variable in terms of natural antioxidants and has small amounts of tocopherol which is minimized during refining. Moreover, owing to its considerable PUFA content, making up 23.89 mg/g fish oil in the present study, fish oil is prone to oxidation. Therefore, encapsulation could protect this substance from oxidation.

#### **Peroxide value of the fermented milk samples**

According to Table 4, on the first day of storage, the highest PV was related to samples A, B and C. Samples A and B had the highest PV on the 7<sup>th</sup> day. On the 14<sup>th</sup> day, the highest PV was related to sample A. On the first, 7<sup>th</sup> and 14<sup>th</sup> days, sample D and control sample had the lowest PV. Encapsulation efficiency indicates the presence of surface oil on the particle surfaces and the ability of the wall material to prevent the oil from diffusing out of the capsule. In general, the larger the emulsion droplets, the higher the surface oil content (Klaypradit and Huang, 2008). As a result, the capsule in which 25% wall material and 4% emulsifier was used, was smaller in diameter; hence, it had smaller amounts of surface oil and lower PV. The fermented milk sample

226 containing fish oil nanoencapsulated with 4% emulsifier and 25% wall material had the lowest  
227 PV. The reason behind this is that this sample had the smallest particle sizes thus, having the  
228 highest encapsulation efficiency and the lowest surface oil. The lower the surface oil content, the  
229 lesser it is exposed to oxygen and as a result, the smaller the PV. The highest PV was related to  
230 fermented milk sample containing fish oil nanoencapsulated with 20% wall material and 2%  
231 emulsifier which had the lowest encapsulation efficiency and the highest surface oil content. This  
232 led to a larger extent of oxidation and a higher PV. During storage, PV of the fermented milk  
233 samples significantly ( $p<0.05$ ) increased but the sample containing fish oil nanoencapsulated  
234 with 25% wall material and 4% emulsifier had the lowest PV, because this nanocapsule had the  
235 highest encapsulation efficiency and as mentioned earlier, this parameter reflects the presence of  
236 oil on the particle surfaces and the ability of the wall to prevent the oil from being released  
237 (Vahid Moghadam et al., 2018). The higher the encapsulation efficiency, the lower the surface  
238 oil content and hence, the lower the oxidation rate and the smaller the PV so that the PV of the  
239 sample with 25% wall material and 4% emulsifier was equal to that of the control on the first day  
240 of storage. The control sample had the lowest PV because of being free of the fish oil. Since a  
241 decrease in particle size raises the particle surface, this may give rise to PV. Therefore, it is  
242 necessary that the size reduction process to be carried out under controlled atmosphere and the  
243 process conditions reduce the impact of the factors affecting oxidation, otherwise, the process  
244 itself may elevate the oxidation rate. Some researchers found that the PV and anisidine value  
245 (AV) of bread and the milk enriched with omega-3 PUFA significantly raised during storage  
246 (Rasti et al., 2017). Tamjidi et al. (2011) incorporated encapsulated fish oil into yogurt and come  
247 to conclusion that the PV of the controlled sample was equal to zero during the 22 days of  
248 storage. Nonetheless, the samples containing the encapsulated and surface oil increased up to 72

and 260% respectively during storage. Ghorbanzade et al. (2017) reported similar results. They found out that the PV of the fish oil extracted from the yogurt samples containing free fish oil was 0.92 on the first day and increased up to 1.61 after 21 days, while the PV of the yogurt samples containing the nanoencapsulated fish oil was nearly constant during storage. Smet et al. (2008) compared the oxidation of various types of milk with different fatty acid compositions and suggested that the milk with a more unsaturated fatty acid composition was more sensitive to oxidation. Chee et al. (2005) accomplished similar results and reported that the PV of the yogurt enriched with omega-3 PUFA and flavored with strawberry was elevated up to 200% during the 22 days of storage, whereas the PV of the control sample remained equal to zero.

#### **EPA and DHA contents of the fermented milk samples**

According to Table 5, on the first day of storage, the fermented milk sample containing fish oil nanoencapsulated with 4% emulsifier and 25% wall material had the highest EPA content. On the other hand, the fermented milk sample containing fish oil nanoencapsulated with 2% emulsifier and 20% wall material had the lowest EPA content. The same results were observed on the 7<sup>th</sup> and 14<sup>th</sup> days of storage, too. Regarding Table 6, the fermented milk sample containing fish oil nanoencapsulated with 4% emulsifier and 25% wall material had the highest DHA content on the first, 7<sup>th</sup> and 14<sup>th</sup> days. On the first and 7<sup>th</sup> days, the lowest DHA content was related to samples A, B and C. Sample A (the fermented milk sample containing fish oil nanoencapsulated with 2% emulsifier and 20% wall material) had the lowest DHA content on the 14<sup>th</sup> day. Encapsulation efficiency indicates the presence of surface oil on the powder particles and the wall capability for inhibiting the oil from being extracted (Klaypradit and Huang, 2008). Our results also demonstrated that among the test samples, the highest EPA and DHA contents was related to the fermented milk sample

containing fish oil nanoencapsulated with 4% emulsifier and 25% wall material, as this sample had the highest encapsulation efficiency. This, in turn, led to the prevention of oil release thereby yielding the highest EPA and DHA contents. Borneo et al. (2007) reported that the addition of microencapsulated fish oil to food products was one of the best ways of retaining and increasing the stability of omega-3 fatty acids in food formulations. The results of the present study showed that EPA and DHA contents of the samples decreased significantly ( $p<0.05$ ) during storage. In a similar study, Lee et al. (2007) observed that the content of EPA and DHA decreased during the four-week storage of the strawberry yogurt enriched with fish oil. Moreover, Ghorbanzade et al. (2017) also found that the nanoencapsulation of fish oil and yogurt enrichment with it had a significant effect on the residue of omega-3 fatty acids. In the samples containing the encapsulated fish oil, the maximum residues of EPA and DHA were equal to 57 and 12% respectively during 21 days of storage. On the other hand, the sample containing the free oil had EPA and DHA contents of 6 and 27%, respectively. This could be a result of nanoencapsulation which has a protective effect on omega-3 fatty acids against environmental conditions.

#### **Viability of *L. plantarum* in the fermented milk samples**

According to Table 7, on the first day of storage, the highest viability of probiotics was related to the fermented milk sample containing fish oil nanoencapsulated with 25% wall material and 4% emulsifier. In contrast, the lowest viability of probiotics was associated with the control sample. The same results were obtained on the 7<sup>th</sup> and 14<sup>th</sup> days of storage, too. The population of *L. plantarum* significantly ( $p<0.05$ ) decreased during storage. Numerous factors including pH, the presence of other microorganisms, incubation temperature and the presence of oxygen have been identified in fermented milk which can influence the viability of probiotic bacteria. The

294 decrease in the number of lactobacilli in probiotic products is probably due to the damage caused  
295 by acid to the organisms (Damin et al., 2008). Georgieva et al. (2009) performed a study on the  
296 milk fermented with *L. plantarum*. They inoculated  $10^6$  CFU/mL of the bacterium to skim milk.  
297 During fermentation, the number of the bacteria was raised up to 2 logarithmic cycles. During  
298 refrigerated storage until the 28<sup>th</sup> day, the viability of the bacteria was retained to a desirable  
299 extent and reached approximately  $10^7$  CFU/mL. As a result, fermented milk is a suitable carrier  
300 for *L. plantarum* strains, which comprise a large number of viable cells in the final product when  
301 being consumed. The findings of the present study revealed that the addition of the encapsulated  
302 fish oil to the probiotic fermented milk loaded with *L. plantarum* brought about a significant  
303 ( $p<0.05$ ) enhancement in the viability of the probiotic bacteria, compared with the control  
304 sample (the probiotic fermented milk free of the encapsulated fish oil). This could be ascribed to  
305 the unsaturated fatty acids which considerably stimulate the growth of probiotic bacteria.  
306 Because of stimulating the growth and activity of probiotics, the presence of prebiotic  
307 compounds is one of the major reasons behind the viability of most bacteria. Prebiotics could  
308 provide some of the nutrients necessary for the growth of microorganisms (Saad et al., 2013). It  
309 should also be noted that the population of *L. plantarum* significantly ( $P<0.05$ ) decreased in all  
310 the samples at the end of the storage period which could be caused by the aggregation of acid as  
311 a result of the production of lactic acid and other organic acids including acetic acid, formic acid  
312 and acetaldehyde by the yogurt starter culture. This led to a reduction in pH and an increase in  
313 acidity (Joung et al., 2016). In addition, the rise in oxidation-reduction potential ( $E_h$ ) and in the  
314 concentration of hydrogen peroxide resulting from the metabolic activity of bacteria (Dave and  
315 Shah, 1997) are of the factors reducing the population of probiotic bacteria during yogurt  
316 fermentation. However, the final population of *L. plantarum* was higher than the minimum



required for imparting health effects. Sendra et al. (2010) claimed that the incorporation of citrus fiber into the fermented milk enriched with probiotics improved the viability of bacteria. Donkor et al. (2007) reported that the addition of inulin to yogurt also improved the survival of *Lactobacillus casei* during storage.

### **Sensory characteristics of the fermented milk samples**

According to Table 8, the highest flavor score was associated with the control sample on the first, 7<sup>th</sup> and 14<sup>th</sup> days. Samples A, B and C had the lowest score of flavor. During storage, the flavor score of the samples did not change significantly. The results indicated that the control sample had the largest amount of taste and aroma owing to not having the nanoencapsulated fish-oil. As mentioned earlier, EPA and DHA are prone to oxidation and undergo numerous changes during processing because of being unsaturated. When exposed to light, prooxidants and high temperatures, these fatty acids are oxidized rapidly and thus, primary oxidation products are produced. The sensory changes caused by the decomposition of hydroperoxides to secondary oxidation products such as aldehydes, ketones, acids and alcohols, are proportional to the chain length and degree of unsaturation of fatty acids. Some of these compounds have a very low olfactory threshold, thus influencing the sensory quality of a product at low concentrations, leading to off-odor and off-flavor (Bibi et al., 2011). Oxidation of fish, fish oil and the fish oil-enriched emulsions consisting of omega-3 PUFA, indicates the presence of volatile components which are recommended as appropriate factors for detecting the degradation omega-3 fatty acids and play an important role in off-flavor (Rasti et al., 2017). In a similar study, Ghorbanzade et al. (2017) used nanoencapsulated fish oil in yogurt and reported that the control sample attained the highest taste score.

Regarding Table 9, samples A and B had the lowest color score on the first and 7<sup>th</sup> days. On the 14<sup>th</sup> day, there was no significant difference between color score of the samples. During storage, the color score of the samples did not change significantly. Among the test samples, the fermented milk samples containing nanoencapsulated fish oil with 20 and 25% wall material and 2% emulsifier had the lowest score, because of its low encapsulation efficiency and more release of the oil from the nanocapsule wall into the milk during storage which led to color changes in the milk. In a similar study, Ghorbanzade et al. (2017) encapsulated fish oil and used it in yogurt fortification. They reported that the control sample acquired the highest color score.

According to Table 10, there was no significant difference between texture score of the samples. The results demonstrated that adding nanoencapsulated fish oil did not affect the texture of the samples. During storage, the overall acceptance score of the samples did not change significantly.

Based on Table 11, the control sample had the highest overall acceptance score on the first, 7<sup>th</sup> and 14<sup>th</sup> days. The lowest overall acceptance score was related to samples A, B and C. During storage, the overall acceptance score of the samples did not change significantly. The results demonstrated that the control sample was the most acceptable one due to being free of the nanoencapsulated fish oil. Among the test samples, the highest overall acceptance belonged to the fermented milk sample containing fish oil nanoencapsulated with 4% emulsifier and 25% wall material which was caused by the highest encapsulation efficiency and the lowest amount of surface oil, resulting in a lesser extent of oxidation and release of the oil from the capsule walls during the storage of the probiotic low fat fermented milk. Consequently, the off-flavor of the milk was reduced and its overall acceptance increased.

## Conclusion

This study indicated that use of nanoencapsulated fish oil significantly ( $p<0.05$ ) increased the survival of probiotic bacteria in low fat fermented milk. At the end of 14 days of storage, the sample containing fish oil nanoencapsulated with 25% wall material and 4% emulsifier had the highest survival of probiotic bacteria (8.41 Log CFU/mL) and the lowest peroxide value (0.57 mEq/kg). This sample had high EPA and DHA contents. Utilizing fish oil nanoencapsulated with 25% wall material and 4% emulsifier did not adversely affect fermented milk overall acceptability. Therefore, this nanoencapsulated fish oil can be used for fortification of low fat probiotic fermented milk and it is possible to produce functional low fat fermented milk in favor of consumers.

## Conflicts of Interest

The authors declare no potential conflict of interest.

## Author Contributions

Conceptualization: Rezvan Pourahmad, Ali Mortazavi. Data curation: Farideh Vahid Moghadam. Formal analysis: Farideh Vahid Moghadam, Rezvan Pourahmad, Ali Mortazavi. Methodology: Rezvan Pourahmad, Ali Mortazavi, Daryoush Davoodi. Software: Reza Azizinezhad. Validation: Rezvan Pourahmad, Ali Mortazavi. Investigation: Farideh Vahid Moghadam, Rezvan Pourahmad, Ali Mortazavi. Writing-original draft: Farideh Vahid Moghadam. Writing-review & editing: Farideh Vahid Moghadam, Rezvan Pourahmad, Ali Mortazavi, Daryoush Davoodi, Reza Azizinezhad.

## **Ethic Approval**

This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

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**Table 1. Diameter of emulsion droplet (nm) of the samples (mean  $\pm$  SD)**

Sample	Composition (% Emulsifier- Wall material)	Diameter of emulsion droplet
1	2-20	82 $\pm$ 0.58 <sup>a</sup>
2	2-25	68 $\pm$ 0.58 <sup>b</sup>
3	4-20	45 $\pm$ 1.15 <sup>c</sup>
4	4-25	36.67 $\pm$ 1.33 <sup>d</sup>

Values in the same column with same latter have no significant difference (p>0.05)

**Table 2. Surface oil (mg/100 g) of the samples (mean  $\pm$  SD)**

Sample	Composition (% Emulsifier- Wall material)	Surface oil
1	2-20	39 $\pm$ 1.95 <sup>a</sup>
2	2-25	35 $\pm$ 0.58 <sup>b</sup>
3	4-20	37 $\pm$ 0.57 <sup>b</sup>
4	4-25	31.66 $\pm$ 0.54 <sup>d</sup>

Values in the same column with same latter have no significant difference (p>0.05)

**Table 3. Encapsulation efficiency (%) of the samples (mean  $\pm$  SD)**

Sample	Composition (% Emulsifier- Wall material)	Encapsulation efficiency
1	2-20	81.77 $\pm$ 0.68 <sup>c</sup>
2	2-25	85.29 $\pm$ 0.35 <sup>b</sup>
3	4-20	81.79 $\pm$ 0.35 <sup>c</sup>
4	4-25	87.17 $\pm$ 0.28 <sup>a</sup>

Values in the same column with same latter have no significant difference ( $p>0.05$ )

**Table 4. Peroxide values (mEq/kg) of the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition (% Emulsifier- Wall material)	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
Control	0-0	0.5 $\pm$ 0.01 <sup>Bb</sup>	0.53 $\pm$ 0.026 <sup>Dab</sup>	0.54 $\pm$ 0.017 <sup>Da</sup>
A	2-20	0.56 $\pm$ 0.026 <sup>Ab</sup>	0.67 $\pm$ 0.053 <sup>Aa</sup>	0.7 $\pm$ 0.026 <sup>Aa</sup>
B	2-25	0.55 $\pm$ 0.026 <sup>Ab</sup>	0.63 $\pm$ 0.026 <sup>ABab</sup>	0.64 $\pm$ 0.061 <sup>Ba</sup>
C	4-20	0.54 $\pm$ 0.01 <sup>Ab</sup>	0.60 $\pm$ 0.020 <sup>CBa</sup>	0.60 $\pm$ 0.01 <sup>BCa</sup>
D	4-25	0.5 $\pm$ 0.01 <sup>Bb</sup>	0.55 $\pm$ 0.017 <sup>CDa</sup>	0.57 $\pm$ 0.02 <sup>DCa</sup>

Values in the same row with similar lowercase letters are not significantly different ( $p>0.05$ ).

Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).

**Table 5. EPA content (mg/g) of the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition (% Emulsifier-Wall material)	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
A	2-20	10.59 $\pm$ 0.149 <sup>Da</sup>	5.91 $\pm$ 0.077 <sup>Db</sup>	4.76 $\pm$ 0.282 <sup>Dc</sup>
B	2-25	11.66 $\pm$ 0.207 <sup>Ca</sup>	6.92 $\pm$ 0.171 <sup>Cb</sup>	5.76 $\pm$ 0.301 <sup>Cc</sup>
C	4-20	12.02 $\pm$ 0.113 <sup>Ba</sup>	7.20 $\pm$ 0.03 <sup>Bb</sup>	6.30 $\pm$ 0.154 <sup>Bc</sup>
D	4-25	12.52 $\pm$ 0.225 <sup>Aa</sup>	8.78 $\pm$ 0.128 <sup>Ab</sup>	7.42 $\pm$ 0.171 <sup>Ac</sup>

Values in the same row with similar lowercase letters are not significantly different ( $p>0.05$ ).

Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).

**Table 6. DHA content (mg/g) of the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition (% Emulsifier-Wall material)	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
A	2-20	6.86 $\pm$ 0.293 <sup>Ba</sup>	5.35 $\pm$ 0.155 <sup>Bb</sup>	3.96 $\pm$ 0.144 <sup>Dc</sup>
B	2-25	7.04 $\pm$ 0.16 <sup>Ba</sup>	5.38 $\pm$ 0.217 <sup>Bb</sup>	4.66 $\pm$ 0.203 <sup>Cc</sup>
C	4-20	7.19 $\pm$ 0.161 <sup>Ba</sup>	5.42 $\pm$ 0.149 <sup>Bb</sup>	5.05 $\pm$ 0.154 <sup>Bc</sup>
D	4-25	7.64 $\pm$ 0.206 <sup>Aa</sup>	7.14 $\pm$ 0.13 <sup>Ab</sup>	5.96 $\pm$ 0.216 <sup>Ac</sup>

Values in the same row shown with similar lowercase letters are not significantly different ( $p>0.05$ ).

Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).

**Table 7. Population of *Lactobacillus plantarum* (Log CFU/mL) in the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition (% Emulsifier- Wall material)	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
Control	0-0	8.23 $\pm$ 0.02 <sup>Da</sup>	7.63 $\pm$ 0.02 <sup>Eb</sup>	7.44 $\pm$ 0.01 <sup>Dc</sup>
A	2-20	8.56 $\pm$ 0.02 <sup>Ca</sup>	8.22 $\pm$ 0.03 <sup>Db</sup>	8.09 $\pm$ 0.02 <sup>Cc</sup>
B	2-25	8.69 $\pm$ 0.03 <sup>Ba</sup>	8.44 $\pm$ 0.03 <sup>Bb</sup>	8.32 $\pm$ 0.02 <sup>Bc</sup>
C	4-20	8.64 $\pm$ 0.02 <sup>BCa</sup>	8.33 $\pm$ 0.04 <sup>Cb</sup>	8.17 $\pm$ 0.03 <sup>Cc</sup>
D	4-25	8.87 $\pm$ 0.04 <sup>Aa</sup>	8.55 $\pm$ 0.05 <sup>Ab</sup>	8.41 $\pm$ 0.04 <sup>Ac</sup>

Values in the same row with similar lowercase letters are not significantly different ( $p>0.05$ ).

Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).

**Table 8. Flavor scores of the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition (% Emulsifier- Wall material)	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
Control	0-0	4.0 $\pm$ 0.00 <sup>Aa</sup>	4.0 $\pm$ 0.0 <sup>Aa</sup>	4.0 $\pm$ 0.0 <sup>Aa</sup>
A	2-20	3.0 $\pm$ 0.0 <sup>Ca</sup>	3.0 $\pm$ 0.0 <sup>Ba</sup>	2.8 $\pm$ 0.447 <sup>Ca</sup>
B	2-25	3.2 $\pm$ 0.447 <sup>BCa</sup>	3.2 $\pm$ 0.447 <sup>Ba</sup>	3.0 $\pm$ 0.0 <sup>BCa</sup>
C	4-20	3.4 $\pm$ 0.548 <sup>BCa</sup>	3.2 $\pm$ 0.447 <sup>Ba</sup>	3.0 $\pm$ 0.0 <sup>BCa</sup>
D	4-25	3.6 $\pm$ 0.548 <sup>Ba</sup>	3.4 $\pm$ 0.548 <sup>Ba</sup>	3.2 $\pm$ 0.447 <sup>Ba</sup>

Values in the same row shown with similar lowercase letters are not significantly different ( $p>0.05$ ).

Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).

**Table 9. Color scores of the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
	(% Emulsifier- Wall material)			
Control	0-0	4.8 $\pm$ 0.447 <sup>Aa</sup>	4.8 $\pm$ 0.447 <sup>Aa</sup>	4.6 $\pm$ 0.548 <sup>Aa</sup>
A	2-20	4.0 $\pm$ 0.00 <sup>Ba</sup>	4.2 $\pm$ 0.447 <sup>Ba</sup>	4.4 $\pm$ 0.548 <sup>Aa</sup>
B	2-25	4.0 $\pm$ 0.0 <sup>Ba</sup>	4.2 $\pm$ 0.447 <sup>Ba</sup>	4.4 $\pm$ 0.548 <sup>Aa</sup>
C	4-20	4.4 $\pm$ 0.548 <sup>ABa</sup>	4.4 $\pm$ 0.548 <sup>ABa</sup>	4.4 $\pm$ 0.894 <sup>Aa</sup>
D	4-25	4.4 $\pm$ 0.548 <sup>ABa</sup>	4.4 $\pm$ 0.548 <sup>ABa</sup>	4.4 $\pm$ 0.548 <sup>Aa</sup>

Values in the same row shown with similar lowercase letters are not significantly different ( $p>0.05$ ).

Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).

**Table 10. Texture scores of the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
	(% Emulsifier- Wall material)			
Control	0-0	4.6 $\pm$ 0.24 <sup>Aa</sup>	4.4 $\pm$ 0.24 <sup>Aa</sup>	4.4 $\pm$ 0.24 <sup>Aa</sup>
A	2-20	4.6 $\pm$ 0.24 <sup>Ab</sup>	4.2 $\pm$ 0.2 <sup>Aab</sup>	4.6 $\pm$ 0.24 <sup>Aa</sup>
B	2-25	4.8 $\pm$ 0.2 <sup>Aa</sup>	4.2 $\pm$ 0.24 <sup>Aa</sup>	4.8 $\pm$ 0.2 <sup>Aa</sup>
C	4-20	4.6 $\pm$ 0.24 <sup>Ab</sup>	4.4 $\pm$ 0.2 <sup>Aab</sup>	4.0 $\pm$ 0.24 <sup>Aa</sup>
D	4-25	4.8 $\pm$ 0.2 <sup>Aa</sup>	4.8 $\pm$ 0.24 <sup>Aa</sup>	4.8 $\pm$ 0.24 <sup>Aa</sup>

Values in the same row shown with similar lowercase letters are not significantly different ( $p>0.05$ ).

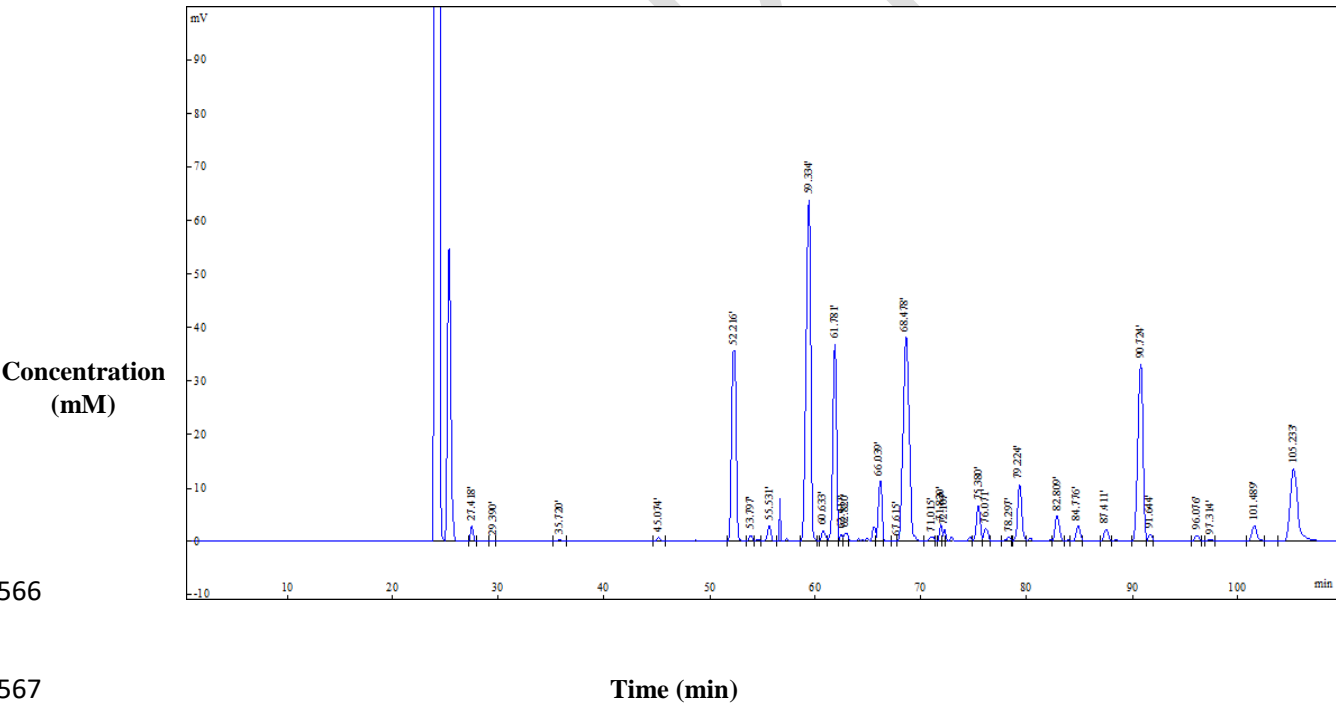
Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).

**Table 11. Overall acceptance scores of the fermented milk samples during storage (mean  $\pm$  standard deviation)**

Sample	Composition (% Emulsifier- Wall material)	First day	7 <sup>th</sup> day	14 <sup>th</sup> day
Control	0-0	4.4 $\pm$ 0.24 <sup>Aa</sup>	4.4 $\pm$ 0.24 <sup>Aa</sup>	4.4 $\pm$ 0.24 <sup>Aa</sup>
A	2-20	3.2 $\pm$ 0.2 <sup>Ca</sup>	3.2 $\pm$ 0.2 <sup>Ca</sup>	3.0 $\pm$ 0.0 <sup>Ca</sup>
B	2-25	3.5 $\pm$ 0.24 <sup>BCa</sup>	3.4 $\pm$ 0.24 <sup>BCa</sup>	3.2 $\pm$ 0.2 <sup>Ca</sup>
C	4-20	3.4 $\pm$ 0.2 <sup>BCa</sup>	3.2 $\pm$ 0.2 <sup>Ca</sup>	3.0 $\pm$ 0.0 <sup>Ca</sup>
D	4-25	4.2 $\pm$ 0.2 <sup>ABa</sup>	3.8 $\pm$ 0.0 <sup>Ba</sup>	3.8 $\pm$ 0.2 <sup>Ba</sup>

Values in the same row shown with similar lowercase letters are not significantly different ( $p>0.05$ ).

Values in the same column shown with similar capital letters are not significantly different ( $p>0.05$ ).



**Fig. 1. Fish oil fatty acid composition**