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Development of EPA-DHA Microcapsules Supplemented Probiotic Fermented Milk

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ABSTRACT

Polyunsaturated fatty acids especially long chain eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), exert strong positive influence on human health. A good way to raise the omega-3 PUFA content in the diet, without radical changes of eating habits, seems to be the enrichment of frequent and common consumed food products. The target of this study was to explore the possibility of selected food products enrichment with omega-3 PUFA, using fish oil preparations in powder-micro-encapsulated (2%) form, without significant taste deterioration of the enriched foods. Different types of microcapsules (one shell and multi shells) were added to the samples prepared by fermented milk starter and probiotic lactobacilli. Sensory evaluation, pH, counting the probiotic bacteria, level of syneresis and viscosity in all samples were evaluated in the first day and 28 days later. There was an insignificant difference between samples after 28 days of storage. It was stored at 4°C for 28 days and all analyses were conducted weekly.

Key Words: PUFA, Probiotic lactobacilli, Fermented milk

Probiyotik Fermente Süt Katkılı EPA-DHA Mikrokapsüllerinin Geliştirilmesi

ÖZET

Çoklu doymamış yağ asitleri, özellikle de uzun zincirli eikosapentaenoik (EPA) ve dokosahekzaenoik asitler (DHA) insan sağlığı üzerine güçlü pozitif etki göstermektedir. Yemek yeme alışkanlıklarında radikal değişiklikler yapmadan diyetteki omega-3 çoklu doymamış yağ asitleri içeriğini arttırmak için sık ve yaygın tüketilen gıdaların zenginleştirilmesi iyi bir yol olarak görünmektedir. Bu çalışmanın amacı toz mikroenkapsüle edilmiş formda (%2) balık yağı kullanarak balık yağı preparasyonları kullanarak ve gıdada tat bozukluklarına yol açmadan omega-3 çoklu doymamış yağ asitleri ile zenginleştirilmiş seçilmiş bazı gıda maddelerinin üretilebilirliğini göstermektedir. Fermente süt starteri ve probiyotik laktobasil ile hazırlanmış örneklere farklı tiplerde mikrokapsüller (tek kapsül ve çoklu kapsüller) ilave edilmiştir. Bütün örneklerdeki duyusal özellikler, pH, probiyotik bakteri sayısı, su salma seviyesi ve vizkozite ilk gün ve 28. gün belirlenmiştir. Depolamanın 28. gününde örnekler arasında önemli bir farklılık görülmemiştir. Örnekler 4°C'de 28 gün süreyle depolanmış ve bütün analizler haftalık olarak gerçekleştirilmiştir.

Anahtar Kelimeler: Çoklu doymamış yağ asitleri (PUFA), Probiyotik laktobasil, Fermente süt

INTRODUCTION

The preparation of fermented foods is a practice with a very long history. Already thousands of years ago, for instance, as reported in the bible, wine and cheese were being made. Foods that help prevent disease are becoming increasingly popular with consumers, with 70% of American shoppers believing that certain foods contain components that reduce the risk of diseases and improve long-term health [1]. Species of Lactobacillus and Streptococcus have traditionally been used in fluid fermented dairy products to promote human health [2, 3]. These probiotic starters may influence the microbial ecology of the host, lactose intolerance, incidence of diarrhea, mucosal immune response, levels of cholesterol, and cancer. The market share of probiotic drinks is expanding rapidly. The most common probiotic strains are lactobacilli [4]. Bifidobacteria have been incorporated into dairy products such as yogurt, [5, 6] fermented milks [7, 8], cheese [9, 10] ice cream, [11, 12] and frozen yogurt [13].

Epidemiological studies have shown that fish consumption within a healthy eating pattern is associated with lower body weight [14, 15]. However, dietary intervention studies which included fish in a weight loss diet are limited [16, 17]. n-3 PUFAs are prone to oxidation while decreasing the rates of lipid synthesis [18, 19]. They have also been shown to decrease plasma lipid concentrations [20] and enhance insulin sensitivity [21]. In addition, they are believed to be preventive in various chronic diseases, including rheumatoid arthritis [22], coronary heart disease [23], stroke [24], and certain types of cancer, including breast, prostate, and colorectal cancers [25, 26]. The $2.9 billion fermented milk industry saw a 10.5% increase in sales from 1999 to 2000 [27]. In 2004, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommended an intake of EPA and DHA of at least 500 mg/d for cardiovascular health [28]. Cardiovascular disease (CVD) is ranked as the number one killer in the United States. In 2010, the total direct and indirect costs of CVD and stroke in the United States were estimated to be $503.2 billion (American Heart Association, 2010).

The main objective of this research project was to develop fish oil microcapsules enriched pre/probiotic fermented milk acceptable by consumers.

MATERIALS and METHODS

Material

Yogurt starter (YC-X11) and probiotic bacteria including *Lactobacillus acidophilus* (LA-5) and *Lactobacillus casei* (LC-01) were bought from Shanghai Pharmaceutical Group Company (Shanghai, China). The packages of starter were prepared based on producer's instructions. The contents of packages were poured in to 1000 mL reconstitued milk (prepared with 10% skimmed milk powder) and were stirred till the whole starters granules solved completely. Commercial fresh milk (Shanghai Milk Company, Shanghai, China) and inulin (Sinopharm Chemical Group, Shanghai, China) were used as carrier agents. Sodium benzoate and all the solvents were bought from Sinopharm Chemical Group.

Methods

Preparation of Microencapsulated Fish Oil (MFO)

Microencapsulated fish oil was produced as described by Yan [29]. A stable emulsion was prepared with homogenizer (FJ 200-S, Shanghai Specimen Model factory, China) using different ratio of components: fish oil (FO), gum Arabic, sodium carboxymethyl cellulose (NaCMC), Sodium polyphosphate and water so that to obtain single-shell, double-shell microcapsules; The emulsion was spray-dried in a centrifugal wheel atomizer (QZ-5, Wuxi Linzhou Drying Equipment Co. Ltd., China) at an inlet temperature of 180°C and outlet of 90°C to produce MFO with moisture content of 4.6%.

Microencapsulation Efficiency (MEE) and Particle Yield (PY)

Microencapsulation Efficiency (MEE)

The microencapsulation efficiency is defined as the ratio of core material in the final dried microcapsules to that in the original emulsion [30] and was calculated according to Eq. (1), as provided elsewhere [31-34].

$$MEE\ (\%) = \frac{(Total\ \ oil - Surface\ \ oil)}{Total\ \ oil} \times 100 \qquad 1$$

Particle yield (PY)

The particle yield for each experimental assay was calculated using Eq. (2) as reported by Zhong [35]:

$$PY = \frac{Mass\ \ of\ \ \ collected\ \ \ product}{Non-solvent\ \ mass\ \ \ in\ \ \ the\ \ \ feed} \times 100 \qquad 2$$

Peroxide Value (PV), Anisidine Value (AV), Total Oxidation (TOTOX), Free Fatty Acids (FFA), Color and Moisture of Microcapsules and Crude Fish Oil

PV, AV, TOTOX, FFA

The peroxide value (PV), anisidine value (AV), and

free fatty acids (FFA) were determined following American Oil Chemists Society (AOCS) Official Methods (1997). The acetic acid-chloroform method [36] was used to determine the PV. The PV of oils was calculated as indicated in Eq. (3) in which V_s is the volume (mL) of $Na_2S_2O_3$ used to titrate the sample, V_b is the volume (mL) of $Na_2S_2O_3$ used to titrate the blank, M is the molar concentration of the $Na_2S_2O_3$ solution (N), and W is the weight of the sample in grams.

$$PV = \frac{(Vs - Vb) \times M}{W} \times 1000 \quad (3)$$

$$AV = \frac{25 \times (1.2As - Ab)}{m} \quad (4)$$

TOTOX values were calculated as described by Wai [37].

$$TOTOX = 2PV + AV \quad (5)$$

Free fatty acids were measured in PFO, and MFO as described by AOCS method Ca 5a-40 [38] with slight modifications.

$$FFA\ (\%) = \frac{NaOH\ (mL) \times N \times 28.2}{Mass\ (g)} \quad (6)$$

N stands for the normality of the NaOH and mass (g) refers to the mass of sample used.

Moisture Content

The moisture content of each sample (500 mg) was determined by loss of weight in an oven (DHG – 9076A) at 105 °C, and figured out by the formula as follows [39]. Each analysis was repeated three times.

$$C\ (\%) = \frac{W1 - W2}{W2} \times 100 \quad (7)$$

Water Activity

The water activity of each sample (500 mg) was measured by an FA-ST Lab System (GBX Water Activity Meter, Romans, France). Each analysis was repeated three times.

Preparation of Yogurt Starter Organisms and Probiotics

The bacterial suspension was prepared following the procedures described by Fritzen-Freire [40]. Pure freeze-dried cells of Bifidobacterium BB-12 were rehydrated at 2.5% (w/v) using a 12% (w/v) of sterile solution of reconstituted skim milk (RSM) and were stirred till the whole starters granules solved completely then frozen as stock solution at 18±1°C in sterile glass bottles.

Processing of Functional Fermented Milk

Functional fermented milk was prepared in stainless steel containers (sanitized before use with a 200 ppm sodium hypochlorite solution) from partially skimmed fluid milk. Mixes for plain yogurt with MFO1 (FMFO1) and MFO2 (FMFO2) were produced and PF (plain functional fermented milk) was used as controls.

Set fermented milk was prepared using milk with 2.5% fat that was standardized to 8.6% solids not fat. Milk was preheated to 45 °C, at which stage the skim milk and inulin of added. The level of inulin addition was 1%. Preheated mixes were homogenized milk samples were heated at 85±1°C for 30 min, then cooled down to 43±2°C for inoculation. The samples were inoculated commercial yogurt starter (1.5%) and mixture of probiotic culture (1%). Incubation was carried in an incubator until a pH around 4.50 was reached. The inoculated samples were mixed thoroughly and dispensed in 500 mL polystyrene cups with lids then incubated at 43±2°C until the pH dropped to 4.5. The fermentation was stopped by transferring the cups immediately to cool down at 4°C.

Determination of Fatty Acid Profile

Extraction of lipids from raw ingredients yogurt, fish oil and microencapsulated fish oil powder) was carried out according to the modified procedure of Folch [41] using chloroform/methanol/water (8:4:3,v/v/v) as the extraction solvent. FAME was expressed as percentage of total fatty acid.

Thiobarbituric Acid Values of Yogurts

The analysis was conducted following the procedure described by Hekmat [42].

Determination of Alpha Tocopherol Content

The α-tocopherol concentration of lipids extracted from PF, FMFO1 and FMFO2 was determined in duplicate by Waters 600 HPLC according to the Official AOCS Method Ce 8-89 [43] and Ubaldi *et al.* [44].

pH, Water Holding Capacity and Color

pH was determined by means of a pH meter at about 6±1°C. The pH meter was calibrated using commercial pH 4.00 and 7.00 buffer solutions. The water holding capacity of yogurt, expressed as syneresis, was determined using the method described by Cueva [45]. The spectrophotometer was standardized using white and black tiles and results were reported in CIE L*, a*, and b* values. Total color differences (ΔE*) were

calculated as follows (Eq. 8):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad 8$$

ΔL^*, Δa^*, and Δb^* are the differences of the L*, a* and b* values between the storage samples and day 1 samples.

Microbial Counts

For propagation of bifidobacteria, sterile MRS broth was supplemented with 0.05% L-cysteine to provide anaerobic condition and stimulate their growth [45]; Culture media and supplements used were: bacteriological peptone, MRS broth and MRS agar, M-17 and MRS fermentation broth; D-sorbitol; L-cysteine, NNLP (nalidixic acid, neomycine sulfate, lithium chloride, paromycine sulfate) and vancomycine.

Rheological Measurements

The rheological measurements of the samples were carried out during 1 month determined weekly using an AR 2000 ex Rheometer and Universal Analysis (TA Instrument) software with fitted plate geometry using plates of 40 mm in diameter.

Sensory Evaluation

Sensory analysis was run on 2 and 28 days stored fermented milks. All sensory work was carried out according to the International Standards [46].

Statistical Analysis

SPSS 19.0 (IBM, USA) for Windows was used.

RESULTS and DISCUSSION

Physicochemical Properties

Physicochemical characteristics of microencapsulated fish oil one shell (MFO1) and microencapsulated fish oil multi shells (MFO2). The EE for the encapsulation process of MFO1 and MFO2 were 75.2±0.73% and 82.81±0.618%, respectively (Table 1). It reflects not only the non-encapsulated oil present on the surface of microcapsules but also the proportion of oil extracted from near the surface of the capsules [47]. According to Gracey [48], oil with a PV below 5 meq/ kg can be considered fresh oil or one in which hydroperoxides have degraded into secondary oxidation products. The secondary oxidation products measured as AV were determined to be 3.92±0.04 and 2.67±0.13 for MFO1 and MFO2 respectively.

Table 1. Physicochemical properties of fish oil microcapsules

Property, Unit	Fish oil	
	MFO1	MFO2
PV, mEq/kg of oil	2.98 ± 0.12	2.09 ± 0.05
AV	3.92 ± 0.04	2.67 ± 0.13
TOTOX	9.88 ± 0.06	6.87 ± 0.13
FFA, %	0.84 ± 0.03	0.78 ± 0.02
Moisture, %	4.80 ± 1.60	6.00 ± 1.90
Water activity	0.13 ± 0.01	0.14 ± 0.02
Hygroscopicity, %	30.00 ± 0.60	28.90 ± 0.50
Carr's Index, %	1.44 ± 0.20	1.42 ± 0.11
Hausner Ratio	30.02 ± 1.60	29.04 ±5.97
Total oil, %	10.86 ± 0.33	11.92 ± 0.25
Surface oil, %	2.69 ± 0.89	1.99 ± 0.67
Microencapsulation efficiency, %	75.20 ± 0.73	82.81± 0.61
Encapsulation yield, %	72.68 ± 0.51	76.86 ± 0.46
Color L*	73.07 ± 0.02	72.01 ± 0.01
Color a*	6.35 ± 0.02	4.84 ± 0.02
Color b*	8.76 ± 0.01	12.8 ± 0.01

Fatty Acid Methyl Ester Profile

The fatty acid compositions of lipids extracted from microencapsulated fish oil one shell (FMFO1) and multi shells (FMFO2) are given in Table 2. Polyunsaturated fatty acids in MFO accounted for around 19% of the fatty acids detected, and EPA and DHA were the predominant fatty acids, accounting for 86% of the total PUFA content (Table 2). The percentage reduction of total n-3 content after 28 days of refrigerated storage was 1.6 and 1.73% for FMFO1 and FMFO2, respectively. That's mean that consuming our fermented milk can theoretically supplement your daily need of EPA and EDH as described by many others authors [49-51].

Probiotic Counts

Number of probiotic bacteria in the samples one day and 28 days after production is presented in Table 3. Counts of probiotic bacteria of the samples were not different significantly ($p>0.05$). Probiotics counts were above 7 log cfu/mL. Counts were always higher (but not significantly) in fish oil-enriched fermented milks although pH was below 4.5 and in control yogurt pH was over 4.7 [52]. Counts in fermented milks were much higher than those in MRS broth after 28 days; stated that *S. thermophilus* has antagonistic effect(s) on the growth of Bifidobacteria. Shankar et al., [53] reported that *B. bifidum* grows better in the presence of *L. bulgaricus* due to their symbiotic relation. The free amino acids resulting from the proteolytic activity of *L. bulgaricus* may promote the growth of Bifidobacteria [54]. The highest number was on first week in our samples. After that the counts declined with time but this decrease was not significant. These results are similar to findings of Akalin *et al.* [55] who reported higher counts of Bifidobacteria in yogurts containing fructooligosaccharide compared to yogurts without this prebiotic. They reported declining counts of bifidobacteria after 28 d storage at 4°C. Akalin et al., found that there is a steady decrease in counts of yogurt bacteria and *B. longum* and *B. animalis* in yogurts with and without fructooligosaccharide over 28 days of storage at 4°C. Shin *et al.* [56] reported that there is a decrease in generation time and increase in viability of Bifidobacterium spp. in skim milk with increasing concentration of inulin and oligosaccharides up to a maximum of 50 g/L implying that incorporation of inulin and oligosaccharides has a limit of 50 g/L beyond which they have a reducing effect on the survival of the probiotics. Also some authors have studied the interactions among Bifidobacteria and commercial fibers in probiotic fermented milks: Varga *et al.* [57] reported that the presence of inulin at 1–5% (w/v) did not influence significantly ($P>0.05$) the survival rates of either *S. thermophilus* or *L. acidophilus*. However, the addition of inulin at 5% (w/v) had a significant beneficial effect ($P<0.05$) on the viability of Bifidobacteria after 28 days of refrigerated storage.

Table 2. FAME profile (% of total fatty acids) of lipids extracted from MFO on two different storage times

Fatty Acid	FMFO1		FMFO2	
	Day-1	Day-28	Day-1	Day-28
C16:0 (palmitic)	3.28[a]	3.29[a]	3.30[a]	3.29[a]
C18:0 (stearic)	6.58[a]	6.57[b]	9.61[b]	8.60[a]
C18:1n-9 (oleic)	7.04[a]	7.17[b]	9.74[a]	9.05[b]
C18:1n-7 (vaccenic)	3.16[a]	3.06[b]	3.28[b]	3.24[a]
C18:2n-6 (linoleic)	1.95[a]	1.93[a]	2.04[a]	2.02[a]
C18:3n-3 (α-linolenic)	0.87[a]	0.83[b]	0.92[a]	0.91[b]
C18:4n-3 (octadecatetraenoic)	2.67[a]	2.50[a]	3.02[a]	3.01[a]
C20:1n-9 (eicosenoic)	1.68[a]	1.49[b]	2.01[a]	1.05[b]
C20:4n-6 (arachidonic)	0.63[a]	0.52[b]	0.84[a]	0.79[a]
C20:5n-3 (eicosapentaenoic)	13.09[a]	12.7[b]	12.9[a]	11.6[b]
C22:1n-11 (cetoleic)	0.93[a]	0.91[a]	1.03[a]	1.01[a]
C22:5n-3 (docosapentaenoic)	3.79[a]	3.74[b]	3.82[a]	3.81[a]
C22:6n-3 (docosahexaenoic)	7.50[a]	6.9[b]	7.06[a]	7.03[a]
∑Omega-3 PUFA	16.92[a]	14.67[b]	17.72[a]	16.36[a]
∑Omega -6 PUFA	2.58[a]	2.45[a]	2.88[a]	2.81[a]
∑SFA	9.88[a]	9.86[b]	12.90[a]	11.890[b]
∑PUFA	19.50[a]	17.12[b]	20.60[a]	19.16[a]
∑MUFA	12.81[a]	12.63[b]	16.06[a]	14.34[b]

Table 3. Bacterial counts in different types of yoghurts on two different storage times.

Storage time (days)	Type of yogurt	*S.thermophilus*		*L.bulgaricus*		*B.bifidum*	
		Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
Day -1	Control	6.67E+08	4.23E+05	6.65E+08	2.35E+05	8.71E+08	5.03E+04
	MFO1	7.94E+08	6.12E+05	8.78E+08	3.92E+05	6.95E+08	4.56E+04
	MFO2	6.28E+08	4.33E+05	7.68E+08	3.34E+04	7.85E+08	4.70E+04
Day-28	Control	4.49E+07	1.63E+03	4.87E+07	5.10E+04	5.35E+07	8.69E+03
	MFO1	4.43E+07	1.03E+04	5.90E+07	6.50E+05	4.97E+07	2.01E+04
	MFO2	3.93E+07	3.84E+03	3.90E+07	7.03E+05	5.02E+07	7.50E+03

Color

Color is one of the most important visual attributes in food and changes in physical, chemical or microbiological parameters of yogurt affect storage, shelf life and may cause color deterioration [58]. According to Table 4, a decrease in a* values was observed during 28 days of storage, from 0.22±0.07 to -1.35±0.05 in control sample and from 2.8±0.04 to -0.44±0.89 in FMFO2.

Table 4. Color changes of different yoghurt samples on two different storage times.

Storage time	Color	Control		MFO1		MFO2	
(days)	Value	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
Day -1	L*	86.2[a]	1.64	86.03[a]	0.03	79.25[a]	0.05
	a*	0.22[a]	0.07	0.24[a]	0.07	2.80[a]	0.04
	b*	9.41[a]	0.03	10.12[a]	0.01	9.29[a]	0.02
Day-28	L*	73.5[b]	0.37	75.63[b]	0.78	75.90[b]	0.05
	a*	-1.35[b]	0.05	-1.34[b]	0.03	-0.44[b]	0.89
	b*	5.67[b]	0.10	6.02[b]	0.07	6.92[b]	0.04

The a* values were negative implying that they were in the green color space and the instrument detected greenness was increasing with an increase in fish oil microcapsules. The same results have been obtained by Olga Cueva [45]. The control sample (initial b* = 9.41±0.03) was less yellow than FMFO1 (initial b* = 10.12±0.01). The significantly ($P < 0.05$) greater yellowness of FMFO1 can be attributed to the addition of fish oil microcapsules MFO, which has a yellowish color imparted by the nonencapsulated fish oil. These results are similar with those obtained by Estrada et al. [59].

pH, Syneresis and TBA

pH of sample decreased significantly ($p<0.05$) during cold storage. Similary, Estrada et al. manufactured fermented milk with fish oil microcapsules and reported a final pH value of 4.4 after 4 weeks. There was a decline in pH with storage time. The pH on 14th day was lower than the other days [59].

The syneresis (released serum) values of the best sample are presented in Table 5. Syneresis decreased over the 28 days. The 28th day was significantly different ($p<0.05$) from other days. Reduced syneresis of probiotic yogurt containing *L.casei* in the presence of lactolose-inulin has been confirmed in another study [60].

The evaluation of the oxidative stability of microencapsulated [61] and non-encapsulated [62] oils has been conducted using thiobarbituric acid reactive substances analysis. It is well documented that fish oils and milk fat are prone to oxidation upon heating [63], nevertheless, Boran et al. [64] reported that fish oils can also deteriorate to an unacceptable level during refrigerated storage (4°C) due to lipid oxidation. Lee et al. [65] reported a slow TBA absorbance increase from 0.08 to 0.10 over the initial 6 days of storage of evening primrose oil (EPO)-enriched yogurt, followed by a dramatic increase up to 0.17 after 15 days. Lee reported a slow TBA absorbance increase from 0.08 to 0.10 over the initial 6 days of storage of evening primrose oil (EPO)-enriched yogurt, followed by a dramatic increase up to 0.17 after 15 days.

Determination of Vitamine E by HPLC

The α-tocopherol content during 28 days of storage of yogurt has been successful determined. At Day-1, control, FMFO1 and FMFO2 α-tocopherol contents were 54.7±1.23, 63.47±1.23, and 69.16±1.12 µg/ g yogurt, respectively. After 28 days of storage, α-tocopherol contents were reduced by 32.8%, 26.0% and 20.4%, respectively; however FMFO1 and FMFO2 α-tocopherol contents were still greater than the control samples. A number of factors such as oxygen, light, heat, alkali, trace minerals, and hydroperoxides can cause decomposition of vitamin E [66]. The decrease in α-tocopherol content during refrigerated storage may be primarily caused by oxygen dissolved in the fermented milk matrix, and reaction with hydroperoxides produced by initial lipid oxidation reactions. α-Tocopherol is expected to have chain-breaking antioxidant activity in fermented milk as well as in human body tissues. Burton [67, 68] showed that α-tocopherol donates its phenolic hydrogen atom to peroxyl radicals arising and in the process becomes an α- tocopheroxyl radical. The remarkable degree of oxidative protection afforded by small amounts of α-tocopherol may be partly explained by the fact that peroxyl radicals react with α-tocopherol about 10,000 times faster than they react with PUFAs, making it less likely that an oxidative chain reaction will be propagated than that it will be quenched [69]. Furthermore, α-tocopherol is stable at high temperatures if no oxygen is present [70].

Rheology Properties

The flow behavior properties of control sample,

FMFO1, and FMFO2 were measured in day 1, and during 28 days of refrigerated storage (Table 5). The flow behavior index (η) values were all less than 1.0 regardless of storage time, indicating that all yogurts were pseudoplastic fluids with shear thinning behavior. The addition of fish oil microcapsules did not significantly affect consistency index (K) values when compared to control sample. The slightly higher K values may be related to the high hygroscopicity and water holding capacity of fish oil microcapsules. K values change (P>0.05) during storage. A higher K value indicates a more viscous consistency [71].

Table 5. Viscosity behavior of three different yoghurt samples on two different storage times

Storage time (days)	Item	Control		MFO1		MFO2	
		Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.
Day-1	n	0.033^{a}	0.005	0.04^{a}	0.003	0.058^{a}	0.007
	K (Pa.s^{n})	3.42^{a}	0.07	3.788^{a}	0.05	3.324^{a}	0.06
	pH	4.52^{a}	0.003	4.53^{a}	0.003	4.52^{a}	0.003
	Syneresis	30.76^{a}	0.406	28.13^{a}	0.2906	27.86^{a}	0.14
	TBA	0.059^{a}	0.0018	0.133^{a}	0.0088	0.130^{a}	0.005
Day-28	n	0.0504^{b}	0.003	0.014^{b}	0.007	0.0175^{b}	0.008
	K (Pa.s^{n})	2.2029^{b}	0.008	2.1643^{b}	0.003	2.877^{b}	0.009
	pH	4.37^{b}	0.07	4.35^{b}	0.08	4.36^{b}	0.09
	Syneresis	34.48^{b}	0.23	33.6^{b}	0.3	33.12^{b}	0.02
	TBA	0.09^{b}	0.004	0.23^{b}	0.001	0.21^{b}	0.06

^{a,b}Different superscripts indicate a statistical difference (P < 0.05) between initial and d-28 means of Control, MFO1 or MFO2, separately.

Sensory Analysis

All types of fermented milks received average scores of at least 5.65 on a seven-point scale. Control sample enriched fermented milks got the best results for overall acceptability followed by FMFO1 and FMFO2 (Table 6). It has to be pointed out that acceptability increased with storage time and that is probably due to the increased perception of creaminess. The fermentation process which includes pH decrease and formation of exopolysaccharides may be responsible for the increased creaminess of fermented milks stored for 28 days. [72]

Table 6. Sensory evaluation profile

Product Code	Aroma/flavor		Off-flavor		Texture		Creaminess		Acceptability	
	Day-1	Day-28	Day-1	Day-28	Day-1	Day-28	Day-1	Day-28	Day-1	Day-28
Control	4.12±0.16	4.53±0.23	0.12±0.17	0.15±0.24	5.74±0.14	6.05±0.13	4.65±0.18	4.83±0.21	6.01±0.23	6.21±0.21
FMFO1	3.42±0.18	3.50±0.14	1.25±0.23	1.89±0.20	5.80±0.19	5.89±0.18	4.62±0.12	4.77±0.19	5.88±0.17	5.65±0.12
FMFO2	3.67±0.13	3.94±0.15	0.94±0.21	1.37±0.14	5.82±0.22	5.91±0.21	4.64±0.13	4.85±0.20	6.03±0.21	6.32±0.23

CONCLUSION

Fish oil microcapsules supplemented probiotic fermented milk using a bifidobacteria strain was successfully developed and delivered an excellent source of micronutrients and probiotic bacteria. Fermented milk is a relatively low risk food product for pathogenic contamination which increases its appeal in a resource-poor context where refrigeration and other quality control measures are difficult to access and not always reliable. Use of inulin (1%) in fermented milk containing *L.acidophillus* and *L.casei* increased viability of probiotic bacteria and improved organoleptic quality. During cold storage, pH of synbiotic yogurt decreased whereas viscosity increased. Syneresis decreased and counts of probiotic bacteria increased until 7th day and then declined with time but this decrease was not significant. The number of probiotic bacteria in synbiotic yogurt was above 7 log cfu/mL.

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