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Preparation of Functional Yoghurt Fortified with Fish Oil-In-Water Nanoemulsion

S.F. Hamed[1], T. N. Soliman*[2], L.K. Hassan[2], Ghada.A. Abo-Elwafa[1]
Fats and Oils Department,Food Industries and Nutrition Division, National Research Centre, El-Buhouthst, Dokki, Cairo, Egypt.
[2]Dairy Department, Food Industries and Nutrition Division, National Research Centre, El-Buhouthst, Dokki, Cairo, Egypt.

ALTHOUGH being one of the richest sources of long chain omega-3 fatty acids, its stringent odor, high susceptibility to oxidation and hydrophobicity make fish oil application in food formulae very restricted. The main objective of this study was to fortify yoghurt, with fish oil nanoemulsion to increase its nutritional benefits. Olive and orange oils as well as α- tocopherol were also used in the preparation of the nanoemulsion to enhance the oxidative stability and palatability of yoghurt. Whey protein isolate (WPI) was utilized in different concentrations as a more safer emulsifier. Different characteristics of the prepared nanoemulsion like droplet size, polydispersity index (PDI), zeta potential, turbidity, and physicalparameters were assessed. The prepared nanoemulsion was then used to prepare yoghurt and its physicochemical and sensory attributes were examined. The oxidative stability of the incorporated fish oil was greatly improved as indicated by retention of polyunsaturated fatty acids (PUFA) content which was accounted to be 90.40 and 85.17 % for nanoemulsion fish oil (NFO) andplain fish oil (FFO) yoghurt samples respectively. Generally, Results indicated that yoghurt fortified with nanoemulsified fish-olive oils (NFO) gave closer characteristics to normal yoghurt and had better acceptability and sensory attributes than that fortified with plain fish-olive oils which let the door opened for more application of nanoemulsified fish oil in food formulations.

Keywords: Functional yoghurt, fish oil, whey protein isolate, nano-emulsion, ultra-high pressure homogenizer.

Introduction

Fish oil is known to be one of the richest sources of long-chain polyunsaturated fatty acids (LCPUFA) like eicosapentanoic acid (EPA) and eicosahexanoic acid (DHA) in human diet, that its inadequate intake can have a great influence on health[1,2]. Omega-3 PUFA are known to be protective agents against cardiovascular diseases, decrease the risk of some types of cancer and autoimmune disorders and they are essential for a proper development of the brain and retina functions. Due to the strong odors, distinctive aroma and rapid deterioration of fish oil, as PUFA are very sensitive toward oxidation, their application in food formulations is limited which is considered to be a great challenge for food industry and researchers. In addition, they are added in small quantities and are difficult to distribute in the food matrix [3]. Olive oil and especially virgin type is known to be one of the healthiest oils among vegetable oils as it is rich in polyphenolic compounds and other antioxidants [4]. It also has a superior balance between monounsaturated and polyunsaturated fatty acids, where the beneficial oleic acid is the major fatty acidconstituent. This structure makes olive oil

*Corresponding author e-mail: tariknour.nrc@gmail.com; Mobil: 01155945511
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stable against oxidative deterioration and has the ability to enhance the oxidative stability of other oils [5]. It also has an interesting content of other components such as carotenoids and chlorophyll, which plays a stimulating part in many organs and accelerates the healing process, vitamin A and magnesium, both with a possible anticancer action [5]. All of these beneficial constituents make olive oil a superior nutrient that has antioxidant, anti-inflammatory and anticancer effects. Also many authors reported that olive oil may have a role in the control of chronic diseases such as arteriosclerosis and heart diseases [6], Parkinson's disease [7], the regulation of cholesterol plasma level, obesity, and hypertension control [8]. Hashtjin and Abbasi[9] reported that orange peel essential oil (OPEO) is one of the most popular and important essential oils used in food, cosmetics, and pharmaceutical industries due to its pleasant aromatic scent and therapeutic properties.

Recently, there is a growing demand for the use of edible nanoemulsions as delivery systems for lipophilic active substances, such as oils, flavors, and nutraceuticals, because of their unique physicochemical properties. Theoretically, the small droplet size of nanoemulsionleads was to an improvement in gravitational stability, separation, and aggregation. Furthermore, small droplet size with the high droplets surface area led to high reactivity with biological cells and macromolecules [10].

Whey protein isolates (WPI) are widely used in food emulsions as emulsifiers/ stabilizers, where itplays an important role in stabilizing the droplets against flocculation and coalescence during long-term storage [11]. It is also, known to inhibit lipid oxidation by preventing pro-oxidants from accessing the droplets, and masking odor [12]. Whey proteins exhibit a highly stable pharmacokinetic profile, as they do not coagulate under acidic environments and they can also resist the destructive action of enzyme Chymosin in the stomach. The denatured whey proteins unfold and expose reactive groups for protein aggregation [13]. Additionally, whey proteins can improve nutritional values because of their compatibility, high nutritional value, and excellent functional properties [14].

Development of functional foods based mainly on the fortification of natural foods with one or a mixture of health-promoting bioactive compounds [15].Yoghurt has gained wide acceptance among consumers as it is perceived as a healthy product rich in nutrients such as calcium and high-quality proteins [16]. The use of nanomaterials in food fortification has experienced significant growth in recent years and is very promising [17]. The nanoemulsion used for the fortification of the yoghurt has been previously designed, prepared and evaluated by our research group.

So, the main objective of this studywas to:(1) formulationnanoemulsion consisted of fish, olive and orange oils emulsified with whey protein isolates;(2)evaluate the effect of incorporating the formulated nanoemulsiononyoghurt physico chemical properties and; (3) monitor the stability of fish oil during cold storage of yoghurt samples.

Materials and Methods

Materials

- Fish oil gelatin capsules (Cod-liver oil) were purchased from a pharmacy in Poland (producer: Olimp Laboratories Sp.zo.o.) and stored at – 20˚C till use.
- Olive and orange oils were obtained as pure (crude) oils from the oil extraction unit in National Research Centre (NRC), Dokki, Cairo, Egypt.
- BiPro®, a commercial whey protein isolate (WPI) was obtained from Davisco Foods International Inc. (Le Sueur, MN, USA) as a gift. Fish oil from local market of Lublin, Poland.
- α-Tocopherol was purchased from Sigma – Aldrich International Company.
- Skim milk powder was purchased from DAIRYAMERICA, Inc. California, USA. The chemical composition of SMP of 34% Protein, 51% lactose, 1.2% Fat, 8.2% Minerals, 4% Moisture as declared by the producer.
- Starter culture (Chr. Hansen Pty. Ltd., Bayswater, Australia) in the freeze-dried direct-to-vat set form containing *Lactobacillus bulgaricus* and *Streptococcus thermophiles.*
- All solvents and reagents were of analytical grades needed for each application.

Methods

Fatty acid composition

For the determination of fatty acid composition of the tested oils, fatty acid methyl esters were prepared according toAOAC [18]. Determination of fatty acids composition was carried out according toHamed et al.,[19] using a Hewlett Packard HP 6890 gas chromatograph, operated under the following conditions: Detector, flame ionization (FID); column, capillary, 30.0 m X

530 µm, 1.0 µm thickness, polyethylene glycol phase(INNO Wax); N2 with flow rate, 15 ml/min with average velocity 89 cm/s (8.2 psi); H2 flow rate, 30 ml/min;air flow rate, 300 ml/min; split ratio, 8:1, split flow, 120 ml/min; gas saver, 20 ml/min. Detector temperature,280 °C; column temperature, 240 °C; injection temperature, 280 °C. Programmed temperature starting from 100°C to reach a maximum of 240 °C was used for eluting the fatty acid methyl esters. The identification of thepeaks was made as compared with chromatograms of standard fatty acids methyl esters (Sigma, USA).

Preparation of nanoemulsion

Oil-in-water emulsions were prepared by homogenizing 5 wt% lipid phase (fish oil: olive oil, 1:1 wt/wt 1000 ppm orange oil + 40mg tocopherol) with 95 wt% aqueous phasecontaining1, 5 or 10 wt% whey protein as an emulsifier and completed with distilled water. A coarse emulsion premix was formulated by blending the lipid and aqueous phases together using a high-speed homogenizer (Ingenieubüro CAT, M. ZippererGmbh, Germany) at 22.000 rpm for 2 min at room temperature. Nano-emulsions were formed by passing the coarse emulsions three times through a high-pressure homogenizer (HPH, IKA-LABOR- PILOT, 2000/4, Germany) at a homogenization pressure of 1000 bar.

Droplet Size Measurement

Characterization of the nanoemulsion was described by the mean globule size diameter and globule size distribution (reported as polydispersity index, or PDI), together with the globule electrical charge (ζ-potential). These properties were all determined using a Zetasizer NICOMP 380 ZLS dynamic light scattering (DLS) instrument (PSS, Santa Barbara, CA, USA), using the 632 nm line of a He Ne laser as the incident light with angel 90^{o} and Zeta potential with external angel 18.9^{o}.

Turbidity and physical stability of nanoemulsions

The physical stability of the nanoemulsions was determined as described previously by Li et al., [20]. The nanoemulsions were diluted 500-fold in deionized water and then centrifuged at 3000 g for 15 min. A sample of the supernatant was withdrawn from the bottom of the tube into a pipette with slow and steady motion. Then, the sample was vortex-mixed for 5 s before the absorbance measurement. The absorbance was determined spectrophotometrically at a wavelength of 500 nm. The centrifugal stability (Ke) was calculated according to the following formula:

$$Ke\% = \frac{A0 - A}{A0} \times 100 \quad (1)$$

Where A0 and A are the absorbance values of the diluted nanoemulsion before and after centrifugation, respectively.

The turbidity was calculated using Eq. (2) [21]:

$$T = 2.303 \times \frac{A.V}{L} \quad (2)$$

Where T is the turbidity of the nanoemulsions in m1, A is the observed absorbance, V is the dilution factor, and L is the path length of the cuvette, which is 0.01 m.

Yoghurt preparation

Powdered skim milk was reconstituted to 12% in waterand divided into three equal portions. Fat was added to each portion to constitute 3% of total weight. In the first treatment fresh cream was used as the fat source, in the second treatment the fat was plain fish-olive oil mixture (FFO), whereas in the third treatmentfish-olive oil nanoemulsion(NFO) (prepared as in section 2.2.2) was the fat source. Each treatment was stirred well, heated to 85-90º C for 5 min, cooled to 42ºC, then inoculated with 3% of starter cultures, dispensed into a plastic container (100ml)and incubated at 42ºC until uniform coagulum was formed. Chemical composition, physicochemical properties, and sensory evaluations were studied over 21 days at 5º C (weekly).

Physicochemical properties of yoghurt samples

Acidity, pH, total solids, total protein, fat contentwere determined according to the methods described by AOAC [18].

To determine syneresis, 20 g of yoghurt 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 [22]:

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

Sensory evaluation

Organoleptic evaluation of yoghurt samples was judged for flavor (60 points), body and texture (30 points), and color and appearance (10 points) by 15 panelists from the staff members of

the Dairy Science Department, National Research Center, Dokki, Cairo, Egypt. The scorecard was designed as described by Nelson and Trout [23].

Oxidative Stability Study

To follow up the oxidative stability of the optimized nanoemulsion formulation, fatty acid composition was determined for the oil recovered by n-hexane from the zero time and 21 days stored yoghurt samples. Where 10 gm of yoghurt sample were soaked in 200 ml n-hexane and the hexane layer containing the extracted oil was separated using a separating funnel. The previous step was repeated three times and hexane layers were collected and sodium sulfate anhydrous was added to remove moisture. n-Hexane was then evaporated using rotary evaporator and the extracted oil was then transesterified to obtain fatty acids methyl esters in order to determine fatty acid composition using GC as previously mentioned in section (2.2.1).

Statistical analysis

Statistical analysis was performed according to SAS [24] using General Linear Model (GLM) with the main effect of treatments. Duncan's multiple ranges were used to separate among three replicates at $P \leq 0.05$.All measurements were performed on two or three freshly prepared samples and are reported as means and standard deviations.

Results and Discussion

Fatty acid composition

Table 1 presents the fatty acid composition of investigated oils and oil blend. Results revealed that the plain fish oil (F) was characterized by EPA, DHA, and oleic acids as major fatty acids constituting 48.6, 33.31 and 8.13 % respectively. While the major fatty acids in plain olive oil (O) fatty acids were oleic, palmitic and linoleic (LA) acids 67.12 %, 19.26 %, and 8.92 % respectively. After blending the two oils (1:1 wt/wt), significant changes in fatty acid levels were observed. Compared to the pure fish oil, FO mixture resulted in significant increases ($p < 0.05$) in its content of oleic and palmitic acids, while significant decreases ($p < 0.05$) were observed in EPA, DHA and linoleic acid contents (Table 1). Oleic acid and palmitic acid have increased by more than 4, and 8 folds, respectively. On the other hand, the long chain polyunsaturated fatty acids EPA and DHA contents decreased significantly by more than 50% (Table 1). As shown in Table 1, although total unsaturated fatty acids decreased only by about 10 % but the total polyunsaturated fatty acids (PUFA, sum of LA, EPA , and DHA) decreased by more than 50% (Table 1). Such noticeable decrease in PUFA content would share significantly in enhancing the stability of the oil against oxidation [25].

TABLE 1. Fatty acid composition of investigated oils and their admixture.

Fatty acid (%)	Fish Oil (F)	Olive Oil (O)	FO Mix	Change %
Myristic	0	0	0	
Palmitic	1.06	19.26	10.19	861
Palmitoleic	1.27	1.5	1.29	
Stearic	2.27	2.41	2.25	
Oleic	8.13	67.12	44.39	446
Linoleic	5.35	8.92	4.73	-12
Linolenic	0	0.79	0	
EPA	48.6	0	22.43	-54
DHA	33.31	0	14.73	-56
Others	0	0	0	
TSF	3.33	21.67	12.44	274
TUFA	96.66	78.33	87.57	-9
PUFA	87.26	9.71	41.89	-52

Impact of emulsifier concentration and number of homogenization cycles on droplet size of nanoemulsion

Whey proteins are considered important emulsifiers in foods for their amphiphilic structure (they have both hydrophilic and hydrophobic moieties in the same molecule) [26, 27, 28]. The whey protein hydrophobic part break through the oil phase and therefore bind the protein molecules to the oil droplet surfaces, whereas its hydrophilic part protrudes into the continuous aqueous phase decreasing droplet agglomeration or aggregation through a combined action of steric and electrostatic repulsion [29].

Droplets size, polydispersity index (PDI), and zeta potential (ζ) were followed up as markers of the effect of whey protein concentrations and number of homogenization cycles (i.e. number of passing through homogenizer) on the stability of the formed nanoemulsions [30].

As shown in Fig.1 and Table 2 droplets diameter of the formed nanoemulsions decreased significantly ($p<0.05$) as we increased emulsifier concentration from 1 % up to 10 % as well as with increasing homogenization cycles. The lowest size (257.6 nm) was registered for 10 % emulsifier concentration and 3 homogenization cycles. Such decreases in droplet diameters contribute well with nanoemulsion stability [31]. These findings were in agreement with those of Håkansson et al., [32] who stated that asthe length of time the emulsion droplets spend in the disruption chamber of homogenizer increases, the fragmentation of oil droplets in the emulsion also increases. Regarding the emulsifier concentration the droplet size is expected to be decreased with increasing surfactant concentration due to increasing the capacity of the emulsifier to cover all oil droplets produced during homogenization.

Total particle size distribution (polydispersity index) rather than just the particle diameter is very crucial for the practical application of nanoemulsions [33]. Fig. 2 and Table 2 show the influence of emulsifier concentrations and number of homogenization cycles on the PDI of the formed nanoemulsions. Results revealed that PDI ranged from 0.162 to 0.412 (Table 2) that demonstrated monomodal and uniform particle size distribution at all studied emulsifier concentrations as well as homogenization cycles. As shown in Fig. 2 at emulsifier concentrations 1 and 5 %, the PDI numerical valuechanged up and down with increasing homogenization cycles, whereas at 10% concentration the PDI decreased continuously with increasing homogenization cycles.

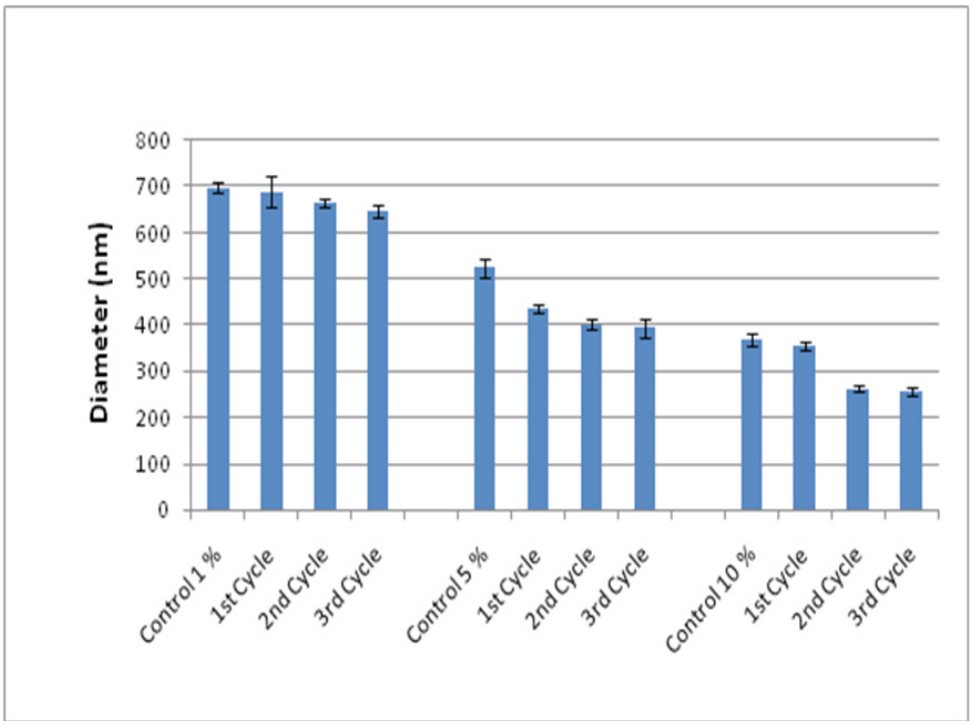

Fig. 1. Effect of emulsifier concentrations and number of homogenization cycles on particle size.

TABLE 2. Effect of emulsifier concentration and number of homogenization cycles on hydrodynamic diameter size, polydispersity index (PDI), and zeta potential of nanoemulsion droplets.

Samples	Diameter (nm)	PDI	Z-Potential (mV)
Control 1 %	697.8 ± 12.15^{ad}	0.342 ± 0.06^{ac}	-1.78 ± 0.48^{ad}
1st Cycle	688.4 ± 33.50^{a}	0.375 ± 0.06^{b}	-13.4 ± 0.79^{b}
2nd Cycle	665.1 ± 9.99^{b}	0.339 ± 0.09^{a}	-14.99 ± 2.22^{b}
3rd Cycle	647.8 ± 12.25^{c}	0.383 ± 0.08^{b}	-39.28 ± 2.35^{c}
Control 5 %	524.1 ± 21.68^{ef}	0.370 ± 0.02^{dc}	-2.14 ± 0.56^{ed}
1st Cycle	435.9 ± 9.71^{g}	0.321 ± 0.03^{d}	-11.23 ± 0.57^{f}
2nd Cycle	401.6 ± 11.45^{h}	0.370 ± 0.01^{d}	-25.21 ± 1.79^{g}
3rd Cycle	393.7 ± 20.77^{i}	0.412 ± 0.10^{e}	-36.11 ± 0.69^{h}
Control 10 %	370.4 ± 14.05^{jk}	0.411 ± 0.02^{gk}	-5.69 ± 1.92^{im}
1st Cycle	356.4 ± 8.50^{j}	0.292 ± 0.04^{h}	-11.64 ± 1.05^{j}
2nd Cycle	262.1 ± 7.53^{l}	0.170 ± 0.02^{i}	-17.45 ± 1.82^{k}
3rd Cycle	257.6 ± 9.00^{m}	0.162 ± 0.01^{i}	-42.44 ± 1.27^{l}

Data in the same column with different superscript letters are significantly different ($P < 0.05$).
Each value represents mean ± SD (n = 3).

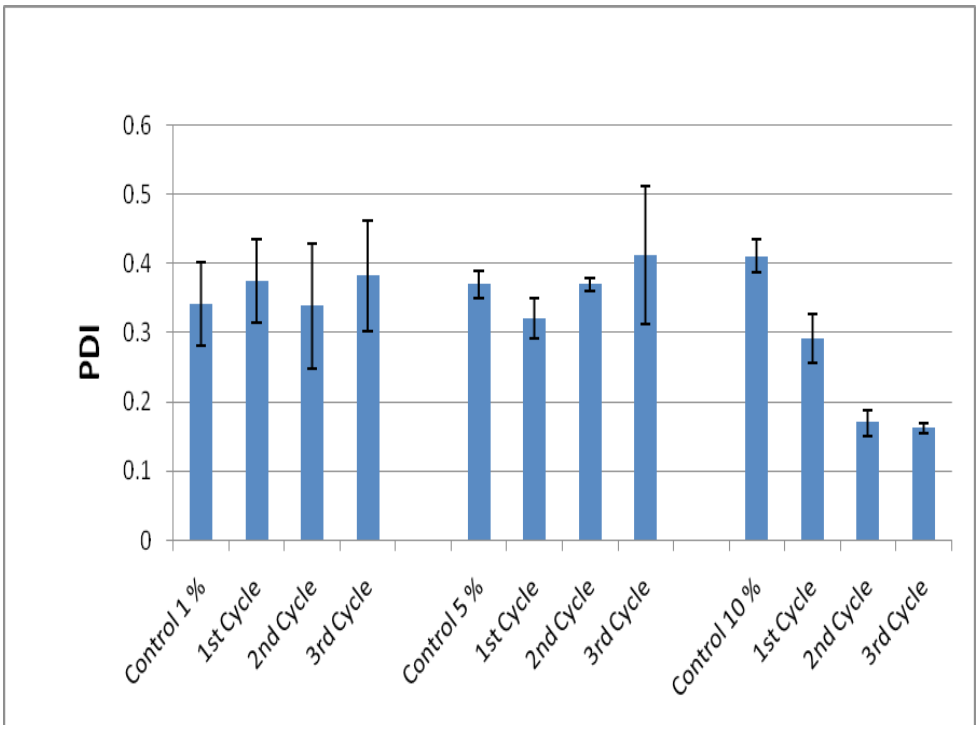

Fig. 2. Effect of emulsifier concentrations and number of homogenization cycles on particle size distribution (PDI).

Regarding zeta potential, it is a term describes the electro-kinetic potential in colloidal systems [34]. In these colloidal systems, this potential is a measure of the difference between the dispersed phase and the stationary layer of the continuous phase attached to the dispersed particle [35]. According to the repulsion degree between adjacent and similarly charged particles (negative or positive), zeta potential value can be related to the stability of colloidal dispersions. Table 2 indicated the impact of the emulsifier concentration and homogenization cycles on the of the zeta potential of the fabricated nanoemulsions in the present work. Table 2 demonstrated that 3 homogenization cycles induced quantitatively high zeta potential (more than 35 mV) for all concentrations of the used emulsifier. According to Preetz [36], a value of 30 mV (either positive or negative) could be taken as postulated value separates low-charged surfaces from highly charged surfaces. Also, ASTM [37] concluded that zeta potentials higher than ±30 mV indicate stability. From an economic point of view, usage of 5 % whey protein isolate concentration was sufficient to prepare stabilized the nanoemulsion.

So, from results of particle size, polydispersity index, and zeta potential 5% WPI concentration and 3 homogenization cycles at homogenization pressure of 1000 bar where the proper conditions to fabricate oil-in-water nanoemulsion in this study and would be fixed in the experiments.

Effect of Stabilizer WPI Concentration on the physical stability of the nanoemulsion

The physical stability was followed up by measuring the centrifugal stability (Ke%) and turbidity of the formulated nanoemulsions (Figure 3, A & B). Figure 3 (A & B) depicts the effect of increasing WPI concentration on the centrifugal stability (Ke %) and turbidity. As shown in Fig. 3 (A & B) there was sharpdecreasingin either centrifugal stability or turbidity accompanying the increase in WPI up to 5% which reflected enhanced physical stability of nanoemulsions. However, any increase in WPI concentration beyond 5% did not have marked improves in the physical stability of the nanoemulsion as shown by the negligible decrease in Ke % orturbidity. Such improvement in centrifugal stability and turbidity of the formulated nanoemulsion could be explained in the light of decreasing droplet size with increasing surfactant concentration due to increasing the capacity of the emulsifier to cover all oil droplets produced during homogenization and inhibiting the aggregation and the collisions of oil droplets [20]. On the other hand the negligible changes occurred after increasing WPI more than 5% may be understood bypostulating that the surfaces of the droplets became saturated with WPI at concentrations 5%. In agreement with our results [38] reported that stability of nanoemulsion enhanced with increasing protein concentration due to increasing electrostatic repulsive forces between colloiding droplets.

Physicochemical characteristics of yoghurt fortified with fish oil nanoemulsion

Chemical composition

Table (3) showed that non-significant changes in the chemical composition of the control yoghurt, as well as the yoghurt fortified with plain fish oil. While there were significant differences intotal protein and total solid contents of yoghurt fortified with nanoemulsified fish oil compared to the other two yoghurt samples which could be related to the presence of WPI in nanoemulsifiedyoghurt sample (Table 3).

The pH and acidity are considered two important parameters determining the taste of yoghurt. The pH and acidity of yoghurt for all fresh samples ranged 4.57–4.60 and 0.77-0.82 (as lactic acid), respectively (Figure 4). The pH has been decreased and acidity increased in all samples during storage time possibly because of the metabolism of lactose into lactic acid by the starter bacteria. As shown in Figure 4 the positive control sample (yoghurt fortified with free fish oil) suffered the sharpest decline of pH accompanied by the highest increase in acidity whereas thehighest pH and lowest acidity were that of yoghurt sample fortified with the fish oil nanoemulsion. These results reflected the superior stability of the yoghurt fortified with NFO during storage as a direct effect of nanoencapsulation protection. This protection may be interpreted by postulating that the oil droplets in the nanoemulsion are covered by a layer of the surfactants which can reduce chemical reactions between the oil and air or oxygen. In agreement to our results [39] reported that plain yoghurt sample showed higher acidity and lower pH than yoghurt fortified with free fish oil, while yoghurt fortified with fish oil/γ-oryzanolnanoemulsion displayed better values of pH and acidity over 21 days of storage than the plain yoghurt and yoghurt containing free fish oil.

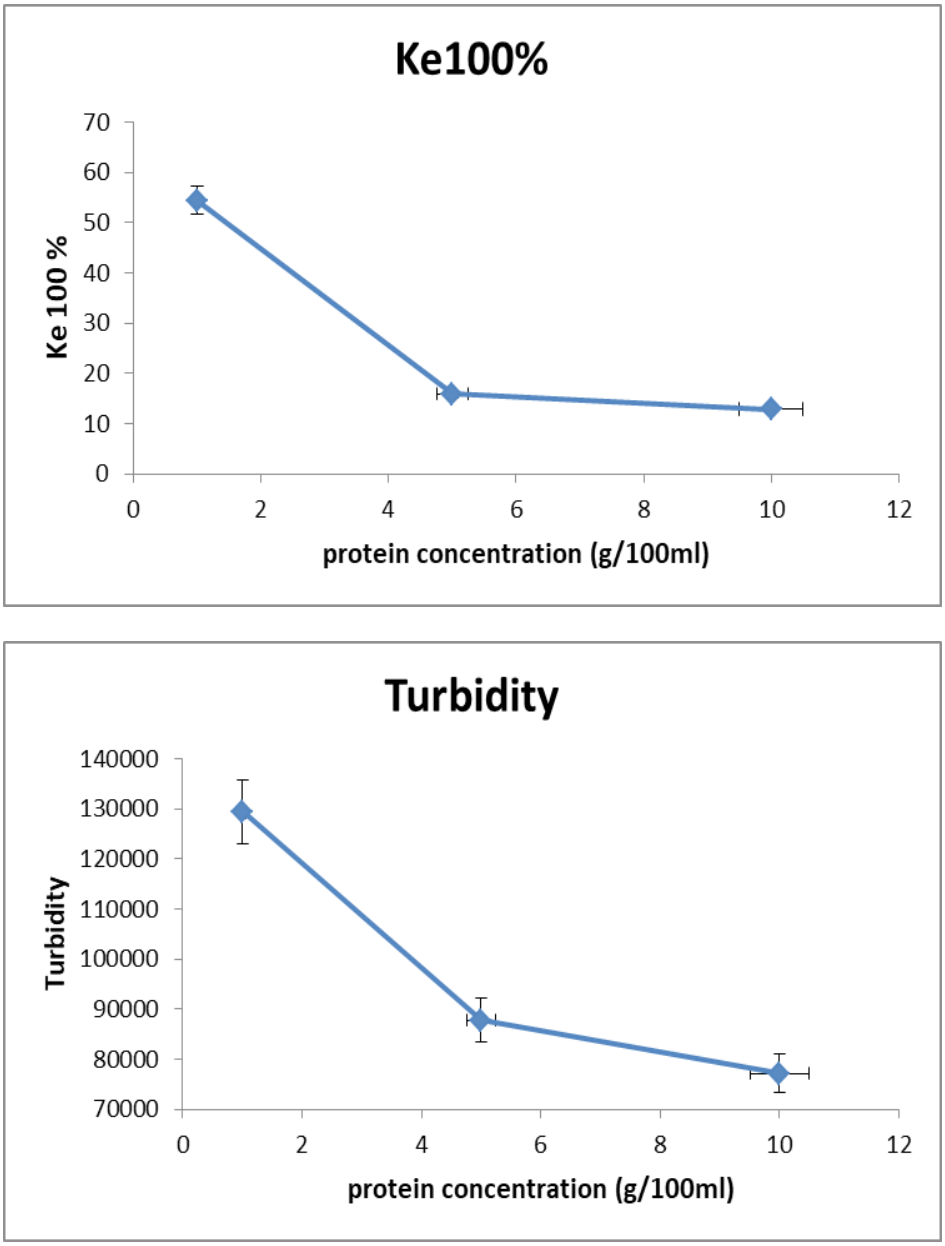

Fig. 3. Effect of WPI concentration on the: centrifugal stability (A) and turbidity (B), of nanoemulsions with a 10 % v/v oil phase and a 1000 bar homogenization pressure for 3 cycles.

TABLE 3. Chemical composition of fresh (Control) and fortified yoghurt samples.

Sample	Total Solids %	Total Protein %	Fat %	Lactose
Control	14.99 ± 0.17^{c}	4.01 ± 0.06^{b}	3.07 ± 0.50^{b}	7.23 ± 0.23^{a}
FFO	15.01 ± 0.15^{b}	4.00 ± 0.05^{b}	3.03 ± 0.55^{b}	7.24 ± 0.15^{a}
NFO	15.46 ± 0.25^{a}	5.20 ± 0.05^{a}	3.11 ± 0.50^{a}	7.15 ± 0.20^{a}

Data in the same column with different superscript letters are significantly different ($P < 0.05$).
Each value represents mean ± SD (n = 3).

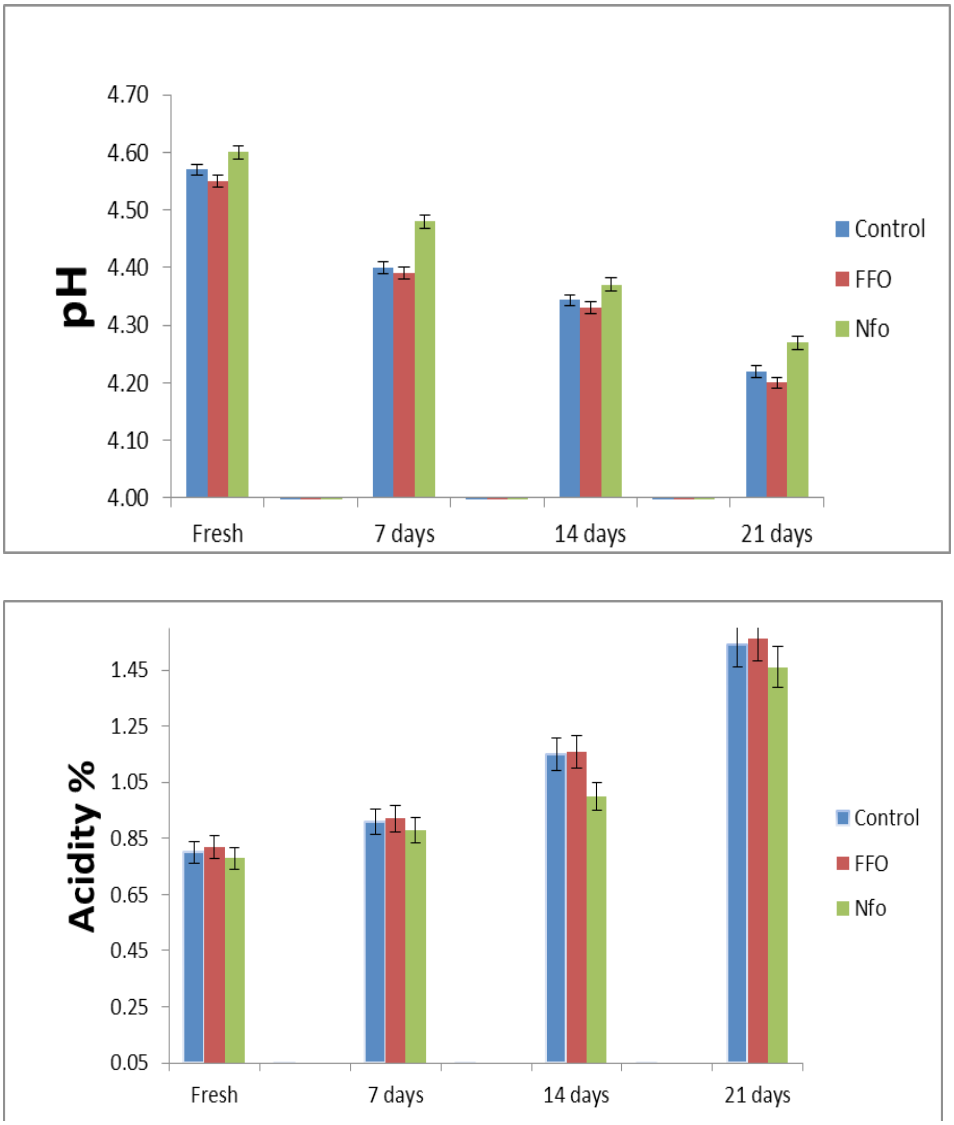

Fig. 4. The change in the pH and acidity (%) of fresh yoghurt (Control) and fortified samples during cold storage.

Syneresis is one of the important physical parameters to measure yoghurt quality. Figure (5) showed changes of syneresis in all samples during cold storage of 21 days. As shown in Figure 5, syneresiswas affected significantly ($P < 0.05$) by storage time in all samples. Results indicated that the highest percentage of synersis of all samples were recorded at the beginning of storage time. Zhong et al.,[39] returned such increase in synersisat the beginning to the pH of yoghurt which was about 4.5 very close to the isoelectric point of casein present in yoghurt samples that lowers the water holding capacityof all samples. In addition, gel backbone of all yoghurt samples could be brocken before storage due to the mixing process which couldalso contribute to more synersis at the beginning times of storage [39]. Although there were no significant differences in synersisbetween all samples, but yoghurt fortified with fish oil nanoemulsion had the least amount of synersis among all samples, which may be due to the presence of WPI in the yoghurt sample fortified with the nanoemulsified fish oil that can absorb some water and retard synersis [3].

Omega-3 rich oils and specially fish oils are highly prone to oxidation due to their very reactive polyunsaturated fatty acids (PUFA) content [3, 40, 41]. Oxidative stability of edible oils is conventionally followed up by determining primary oxidation (e.g. peroxide value) and

secondary oxidation products (e.g. thiobarbituric acid reactive spaecies, TBA and anisidine value). There are many literatures that used either calculated oxidizability index of edible oils (COX value) or the peroxidability index (PI) instead of measuring primary or secondary oxidation products. In all these literatures [42,43,44,45,46], change in fatty acid composition and specifically polyunsaturated fatty acids content was considered as a reliable parameter of oxidative stability during storage period. So, in our present work we followed up the oxidative stability of the prepared yoghurt, stored at 4 °C for 21 days, through investigating the total changes in polyunsaturated fatty acids content.

Figure 6 and Table 4 showed changes in total saturated, unsaturated and polyunsaturated fatty acid contents and the percentage changes of these fatty acids of yoghurt samples fortified with plain fish-olive oil mixture (FFO) and nanoemulsified fish-olive oil mixture (NFO). Although decrease in total unsaturated fatty acids content seemed not to be greatly different between FFO (-1.4%) and NFO (-2.55%), but FFO showed more decrease in polyunsaturates (-14.93 %) while NFO showed less decrease in polyunsaturaes (-9.60 %). This large difference in polyunsaturated fatty acid content revealed more stability of the fish oil incorporated in NFO yoghurt compared to the FFO yoghurt. Retention of polyunsaturated fatty acids (PUFA) was accounted to be 90.40 and 85.17 % for NFO and FFO yoghurt samples respectively. This finding could be interpreted by the protection afforded by the emulsifier (whey protein) around the polyunsaturated fatty acid moieties which act as a barrier decreasing its susceptibility to oxidations. In agreement with our results [41, 3] reported that addition of liposomal nanoemulsified fish oil enhance the oxidation stability of fortified yoghurt 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. Also, whey protein isolate could enhance the oxidative stability of the nanoemulsifiedyoghurt samples through chelation of pro-oxidative transition metals, inactivation of reactive oxygen species (ROS), scavenging of free radicals, and reduction in hydroperoxides [47, 48].

Table 5 shows the scores of sensory evaluation of all yoghurt samples after one and twenty one days of storage.As shown in Table 5 the control yoghurt sample (without fish oil) recordedthe highest (best) scores in terms of all characteristics for fresh or stored among the three yoghurt samples. On the other hand, NFO yoghurt sample revealed closer sensory attributes to control yoghurt sample compared to that of FFO yoghurt. In addition, NFO yoghurt sample showed better scores compared with FFO Yoghurt sample regarding all sensory attributes of the fresh and stored Yoghurts which may be returned to the attenuation of the fishy odor offered byincorporating the nanoemulsified fish oil sample (NFO) rather than incorporating plain fish oil (FFO).

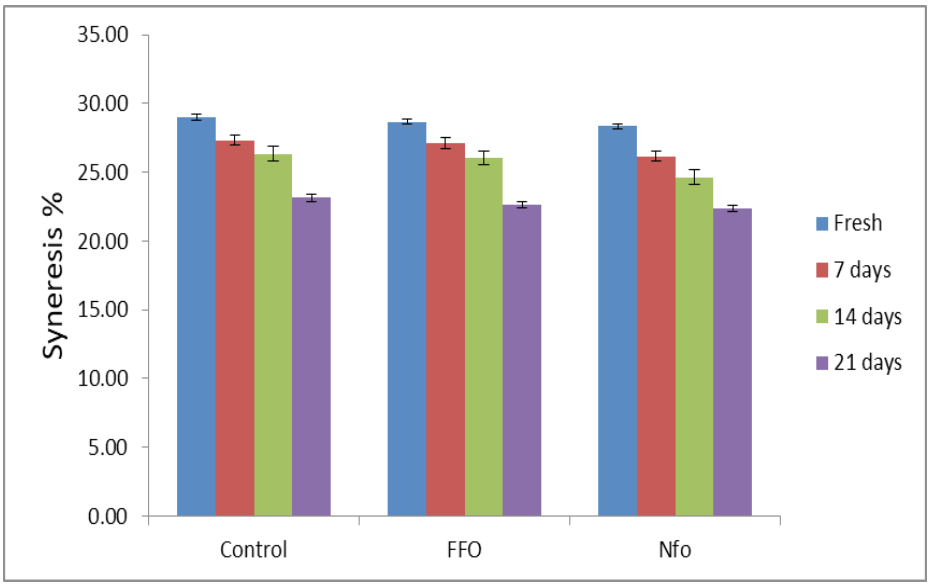

Fig. 5. Syneresis changes of fresh yoghurt (Control) and fortified samples during storage.

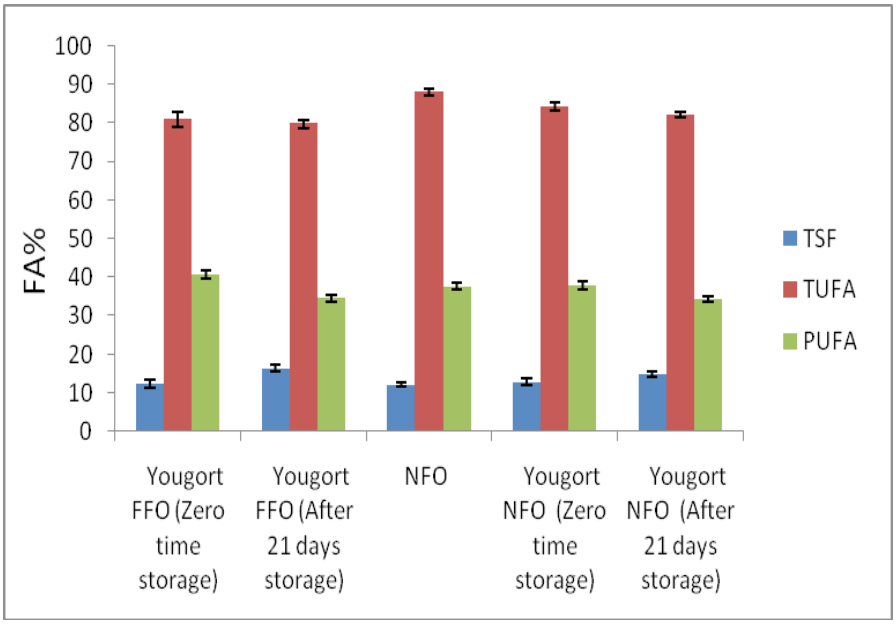

Fig. 6. Effect of storage period (21 days at 4 ºC) on total saturated (TSF), total unsaturated (TUFA) and polyunsaturated fatty acid (PUFA) contents of yogurt fortified with fish : olive oil mixture (FFO) and nanoemulsified fish : olive oil mixture (NFO).

TABLE 4. Effect of storage period (21 days at 4 ºC) on changes in total saturated (TSF), total unsaturated (TUFA) and polyunsaturated fatty acid (PUFA) contents of yogurt fortified with fish : olive oil mixture (FFO) and nanoemulsified fish : olive oil mixture (NFO).

	Change %	
	FFO	NFO
TSF	32.79	16.22
TUFA	-1.40	-2.55
PUFA	-14.93	-9.60

TABLE 5. Sensory properties of plain yoghurt and fortification yoghurt at 5±2°C.

Sample	Flavor (60)		Body & Texture (30)		Color & Appearance (10)	
	Fresh	21 days	Fresh	21 days	Fresh	21 days
Control	53.00 ± 3.29^{a}	49.12 ± 4.15^{a}	25.75 ± 1.61^{a}	23.85 ± 1.40^{a}	9.65 ± 0.45^{a}	8.50 ± 0.50^{a}
FFO	42.57 ± 4.27^{c}	37.24 ± 3.45^{c}	22.50 ± 2.19^{c}	19.75 ± 0.95^{b}	5.80 ± 0.44^{b}	5.00 ± 0.45^{b}
NFO	50.00 ±2.34^{b}	47.15 ± 1.75^{b}	24.5 ± 0.58^{b}	23.75 ± 1.25^{a}	9.40 ± 0.44^{a}	8.60 ± 0.35^{a}

Data in the same column with different superscript letters are significantly different ($P < 0.05$).
Each value represents mean ± SD (n = 3).

Conclusion

The present work was an attempt to produce functionalyoghurtfortified with fish oil based nanoemulsion. The omega-3 richoil-in-water nanoemulsions were prepared by high pressure homogenization of 5 wt% lipid phase (fish oil: olive oil, 1:1 wt/wt + 1000 ppm orange oil + 40 mg tocopherol) with 95 wt % aqueous phase containing 5 wt % whey protein as an emulsifier at 1000 bar and three homogenization cycles. Such emulsification procedure demonstrated good nanoemulsion characteristics in terms of the acceptable small droplet size, unimodal polydispersity index and good zeta potential. The prepared yoghurt fortified with the fish oil-based nanoemulsionshowed relatively good physicochemical characteristics andacceptable sensory attributes and high oxidative stability.

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