Development of stable flaxseed oil emulsions as a potential delivery system of ω -3 fatty acids

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Revised: 23 February 2014 / Accepted: 10 April 2014 / Published online: 2 September 2014
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Abstract The objective of the present study was to develop a stable flaxseed oil emulsion for the delivery of omega-3 (ω -3) fatty acids through food fortification. Oil-in-water emulsions containing 12.5 % flaxseed oil, 10 % lactose and whey protein concentrate (WPC)-80 ranging from 5 to 12.5 % were prepared at 1,500, 3,000 and 4,500 psi homogenization pressure. Flaxseed oil emulsions were studied for its physical stability, oxidative stability (peroxide value), particle size distribution, zeta (ζ)-potential and rheological properties. Emulsions homogenized at 1,500 and 4,500 psi pressure showed oil separation and curdling of WPC, respectively, during preparation or storage. All the combinations of emulsions (homogenized at 3,000 psi) were physically stable for 28 days at 4–7°C temperature and did not show separation of phases. Emulsion with 7.5 % WPC showed the narrowest particle size distribution (190 to 615 nm) and maximum zeta (ζ)-potential (–33.5 mV). There was a slight increase in peroxide value (~20.98 %) of all the emulsions (except 5 % WPC emulsion),

as compared to that of free flaxseed oil (~44.26 %) after 4 weeks of storage. Emulsions showed flow behavior index (n) in the range of 0.206 to 0.591, indicating higher shear thinning behavior, which is a characteristic of food emulsions. Results indicated that the most stable emulsion of flaxseed oil (12.5 %) can be formulated with 7.5 % WPC-80 and 10 % lactose (filler), homogenized at 3,000 psi pressure. The formulated emulsion can be used as potential omega-3 (ω -3) fatty acids delivery system in developing functional foods such as pastry, ice-creams, curd, milk, yogurt, cakes, etc.

Keywords Flaxseed oil · Whey protein concentrate · Emulsion · Oxidative stability · Rheology

Introduction

Flaxseed (*Linum usitatissimum*) also known as linseed, a member of the genus *Linum* in the family *Linaceae*, is an economically important oilseed crop (Oomah 2001; Lei et al. 2003) containing about 40 % oil in the seed. Flaxseed oil is the richest source of ω -3 fatty acid (α -linolenic acid: ALA, 50–60 %), an essential fatty acid, which gets converted into long chain polyunsaturated fatty acids (PUFAs): eicosapentaenoic acid (EPA) & docosahexaenoic acid (DHA) (Sharma et al. 2012). These PUFAs are further converted into three- and five- series prostaglandins and prostacyclins in human body, which play a vital role in promoting eye health (Chiu et al. 2009), development of brain and nervous system in infants, reducing the risk of hypertension, hypercholesterol and cancer including colon, breast & prostate, improving intelligence and memory, inhibition of aging, reduction of inflammatory bowel diseases & coronary heart diseases, lessening neurodegenerative disorders and controlling diabetes; which have been comprehensively reviewed by several authors (Goyal et al. 2014; Rodriguez-Leyva et al. 2010; Carraro et al. 2012; Singh et al.

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2011a). In spite of imparting aforementioned health benefits, it is surprising that one in three adults has raised blood pressure, which is the third leading cause of death after the heart attacks and strokes; and one in ten adults has diabetes worldwide (WHO 2012). According to a report on global status of non-communicable diseases, cardiovascular diseases (CVDs) were responsible for over 17.3 million deaths in 2008, representing 30 % of all global deaths and were the leading cause of death in the world (WHO 2011). It is obvious that these monstrous situations depend upon various factors, but the diet remains the most influencing one. Due to lower consumption of ω -3 and higher intakes of ω -6 fatty acids (in the form of refined vegetable oil such as soybean, sunflower, safflower, cottonseed and groundnut oil) in regular diet, the frequency of such life-threatening diseases has increased drastically in last two decades.

Although fish is the greatest contributor to food sources of ω -3 fatty acids (EPA and DHA) but the Indian and Western diet does not include enough oily fish to meet dietary recommendations of ω -3 fatty acids. Moreover, addition of fish oil preparations (in the form of emulsions or spray dried powder) in food is literally impossible for vegetarians due to religious beliefs and practices. In such a situation, it is very difficult to meet recommended intakes of ω -3 for vegetarians. It has been reported that ω -6: ω -3 ratio in current Indian urban and Western diet is 38–50:1 and 16:1, respectively (Singh et al. 2011a; Singh et al. 2011b), which appears to be very high as compared to the recommended ratio, i.e. 5:1 (FAO/WHO 2008). The International Society for the Study of Fatty acids and Lipids (ISSFAL 2004) recommended 2.2 g/day of ALA per day. Flaxseed oil, being the richest source of ω -3 fatty acids, can be exploited to fill this ω -3 gap in vegetarian diet. Although there are some other sources of ω -3 such as walnut and algal oil; but walnuts are neither consumed on regular basis nor by all age groups; while the algal oil is not economically feasible. Flaxseed oil is inexpensive and easily available; but due to its highly polyunsaturated nature (>75 %), it is extremely susceptible to oxidation in presence of oxygen, metal ions and at high temperatures, which leads to the production of toxic hydroperoxides and off-flavor compounds during processing conditions. It is reported that α -linolenic acid (ALA, C18:3, ω -3) is 20 times more susceptible to oxidation as compared to oleic acid (C18:1) (Decker et al. 2012). All these factors limit its applications as cooking/frying oil as well as its fortification in foods. So, there is an intense need to produce such form of flaxseed oil, which can be supplemented in food products for the delivery of ω -3 fatty acids, without affecting its taste and flavor adversely. This can be achieved through preparation of stable oil-in-water (O/W) emulsions containing flaxseed oil. Generally, an emulsion consists of at least two immiscible liquids (usually oil and water), with one of the liquids being dispersed as small spherical droplets in the other. An emulsion that consists of oil droplets dispersed in an aqueous phase is called an oil-in-

water (O/W) emulsion (McClements et al. 2007). Oil-in-water emulsions may be easier to disperse into water-based foods such as beverages, dairy products, salad dressings, bakery products and muscle foods than bulk oil that could physically separate from the aqueous phase during storage. Emulsions are thermodynamically unfavorable systems that tend to break down over time due to a variety of physico-chemical mechanisms, including gravitational separation, flocculation, coalescence and Ostwald ripening (McClements 2005). It is possible to form emulsions that are kinetically stable for a reasonable period of time by using encapsulating agents and/or emulsifiers. Emulsifiers are surface-active molecules that adsorb to the surface of freshly formed oil droplets during homogenization, forming a protective layer that prevents the droplets from aggregation. Proteins are ingredients widely used in food emulsions as emulsifiers/stabilizers due to their amphiphilic character. The ability of proteins to generate repulsive interactions (steric and electrostatic) between oil droplets, and at the same time form an interfacial membrane that is resistant to rupture, plays an important role in stabilizing the droplets against flocculation and coalescence during long-term storage (McClements 2004). Owing to its hydrophobic and hydrophilic regions, whey proteins are most commonly used in food emulsions.

A number of studies have been reported in literature on the preparation of O/W emulsions using soybean, corn, rapeseed, walnut, sunflower and fish oil (Khalloufi et al. 2008; Sun and Gunasekaran 2009; Djordjevic et al. 2004; Dybowska 2011; Nikovska 2010; Bellalta et al. 2012; Taherian et al. 2011; Ye et al. 2009), but the preparation and storage study of flaxseed oil micro-emulsions for the delivery of ω -3 fatty acids is hardly described (Kuhn and Cunha 2012, Ma et al. 2012). Aforementioned vegetable oils are not highly polyunsaturated unlike flaxseed oil and thus not susceptible to oxidation in presence of oxygen and at high temperatures. However, flaxseed oil, being highly polyunsaturated, needs more attention towards controlling its oxidation during processing and storage. Kuhn and Cunha (2012) studied the effects of homogenization pressure and number of homogenization cycles on the physico-chemical characteristics of flaxseed oil-whey protein isolate emulsions. Kentish et al. (2008) studied only particle size distribution of flaxseed oil emulsions homogenized at very high pressure (100 MPa) in presence of tween-40 surfactant. Although some of the researchers have also worked on the microencapsulation of flaxseed oil to obtain flaxseed oil powder (Gallardo et al. 2013; Carneiro et al. 2012; Tonon et al. 2011), which includes the step of emulsion preparation; but their focus was the preparation of powder just after the homogenization and not the optimization & study of the emulsions for a long period.

The objective of the present study was to develop stable flaxseed oil emulsion by optimizing the level of whey protein concentrate-80, which could serve as potential delivery

system of ω -3 fatty acids. Lactose was used as filler at a fixed concentration, which is reported to provide stability to the emulsions. To accomplish this goal firstly flaxseed oil : whey protein concentrate-80 emulsions were developed and optimized in terms of homogenization pressure, physical as well as chemical stability, particle size distribution, ζ -potential and rheological properties. The results will have important implications for the design of WPC-stabilized flaxseed oil emulsions that could be used to incorporate ω -3 fatty acids, which are otherwise sensitive to oxidation.

Materials and methods

Materials

Flaxseed oil was purchased from local market, Karnal, India. Whey protein concentrate (WPC)-80 (Davisco, USA) having 82.5 % (on dry basis) protein, 6.4 % fat, 0.2 % moisture, 7.5 % lactose, 2.4 % ash was obtained from Mahaan Foods Ltd, New Delhi, India. Lactose was purchased from Fischer Scientific; and other chemicals were of analytical grade purchased from Sigma and Himedia, India.

Preparation of emulsions

Oil-in-water emulsions were essentially prepared by the method given by Kuhn and Cunha (2012). WPC-80 and lactose were mixed in distilled water (50 ± 1 °C) in ratios as shown in Table 1. A coarse emulsion was prepared by mixing flaxseed oil into the solution of WPC-80 and lactose by using high speed hand blender (Havells, India) followed by the homogenization (Goma Engineering Pvt. Ltd., India) at 1,500, 3,000 and 4,500 psi (10, 20 and 30 MPa) pressures. Prepared microemulsions were filled in amber colored plastic bottles and stored at low temperature (4–7 °C) for 28 days.

Physico-chemical characterization of emulsions

Physical stability

Physical stability was studied in terms of creaming index using the method given by Kuhn and Cunha (2012). Immediately after preparation, 25 mL of each emulsion were poured into a cylindrical glass tube (internal diameter = 10 mm, height = 95 mm), sealed with a plastic cap and stored at low temperature (7 ± 1 °C) for a period of 28 days. The emulsion stability was measured by the change in height of the bottom serum phase (H) with storage time, using the following equation:

$$\% \text{Separation} = 100 \times (H/H_0) \quad (1)$$

Table 1 Concentration of different ingredients used in preparation of emulsions

Ratio			
Flaxseed oil (%)	WPC-80 (%)	Lactose (%)	Water (%)
12.5	5.0	10.0	72.5
12.5	7.5	10.0	70.0
12.5	10.0	10.0	68.5
12.5	12.5	10.0	65.0

Where H_0 represents the initial height of the emulsion and H represents the height of the emulsion after phase separation.

Oxidative stability (Peroxide value)

To determine the peroxide value, oil was extracted from emulsion by the method of Folch et al. (1957) with slight modifications. Twenty grams of sample was mixed in 200 mL cold mixture of chloroform: methanol (2:1) in a separating funnel. After shaking gently for 3 min, mixture was allowed to stand for 10 min. A lower chloroform layer was removed separately. Upper layer was washed with 100 mL of chloroform: methanol (2:1) mixture and again lower chloroform layer was removed and mixed with previous one followed by mixing with 40 mL distilled water. After phase separation, lower chloroform layer was collected, passed through anhydrous sodium sulphate and dried using flash evaporator (Metrex Scientific Instruments, India) under vacuum at 40 °C. Peroxide value of extracted oil was evaluated at every week during storage of 28 days by the standard iodometric method of AOAC (2005).

Particle size distribution and zeta (ζ) - potential

A Zetasizer nano series *Ver 6.30* (Malvern Instruments Ltd., UK) was used to determine the particle size of the emulsions. About 1 mL of the emulsion was added to 99 mL of distilled water at 25 °C to measure the particle size. The emulsions were analyzed 1 day after their preparation. Emulsion particle size is expressed as Z-average diameter (nm) and ζ -potential in mV. The particle size distribution curves are expressed as percent intensity vs diameter (nm).

Rheological measurements

Steady shear measurements were performed using a dynamic rheometer (Anton Paar Rheometer, MCR-52, Austria, Europe). The 75 mm dia, 1° cone angle cone-and-plate geometry (CP 75/1°) was used for viscosity measurements. Emulsion viscosity was measured at 25 ± 0.1 °C, over a range of shear rate 5–150 s. Viscosity was measured every 7th day till 28 days of storage at low temperature (4–7 °C).

Modeling of flow behavior of the O/W emulsions

The rheological behavior of emulsions is important to food scientists for a number of reasons. Many of the sensory attributes of emulsions are directly related to their rheological properties (e.g., texture, creaminess, thickness, smoothness, spreadability, pourability, flowability, brittleness and hardness). These properties can be described in mathematical terms by different models like Bingham model, Power-law model and Newtonian model. In present study, as the Power law model was the best fitted (on the basis of R^2 value), the experimental flow curves of the flaxseed oil emulsions were described by Power Law model (Ostwald de Waele) over the range of shear rates ($5\text{--}150\text{ s}^{-1}$) as follows:

$$\tau = k \cdot \gamma^n$$

where τ is the shear stress (Pa), k is the consistency index ($\text{Pa} \cdot \text{s}^n$), γ is the shear rate (s^{-1}) and n is the flow behavior index (dimensionless). For a Newtonian emulsion $n=1$, for an emulsion which exhibits shear-thinning behavior $n<1$, and for an emulsion which exhibits shear thickening $n>1$.

Statistical analysis

All the data was analyzed and expressed as means of three replicates. The data was subjected to analysis of variance (ANOVA) technique and analyzed according to two factorial completely randomized designs (CRD). The critical difference value at 5 % level was used for making comparison among different samples during storage.

Results and discussion

Physical stability

Amount of encapsulating agent (or emulsifier) plays a crucial role in stabilizing the emulsion. During homogenization when oil is disrupted into very small droplets, encapsulating agent covers oil droplets and protects them against coalescence. Lack of the encapsulating material (i.e., insufficient concentration) causes sharing the active material between adjacent droplets and leads to irreversible bridging flocculation (Dickinson 2001). However, excess of the encapsulant/emulsifier, above that required to complete oil droplet covering, may increase its surface load and negatively influence emulsion properties (McClements 2004).

In order to find the optimum whey protein concentration to stabilize the fixed amount of flaxseed oil (means the most stable emulsion), and to exclude lack or excess of the protein

material in the system, series of whey protein based emulsions were tested. The stability study revealed that all the emulsions were kinetically stable when homogenized at 20 MPa (3,000 psi) pressure and stored at $7\text{--}8^\circ\text{C}$ for 28 days. There was no separation observed of phases in emulsions produced with different concentration of WPC-80. Although a small non-significant amount of oil droplets was observed on the surface of emulsion prepared by 5 % WPC-80 on 21st day of storage, but no sedimentation or cream layer was observed till the end of storage period. Similarly, Pedro et al. (2011) observed no separation in flaxseed oil emulsions (10–30 % oil) stabilized by gum arabic till 24 h at room temperature. However, Carneiro et al. (2012) reported a small separation (16.8 %) and a foam phase, 24 h after its homogenization in flaxseed oil emulsions encapsulated by maltodextrin: WPC-80 (25:75).

In case of emulsion prepared by 12.5 % WPC-80, gelling was observed (visually) just after the homogenization at 4,500 psi. It could be explained by the fact that at such a high pressure, temperature of emulsion increased rapidly and caused droplet coalescence and the formation of high molecular weight protein aggregates due to shear and increase in temperature. Similarly, Kuhn and Cunha (2012) studied the effect of homogenization pressure (20 to 80 MPa) on the stability of flaxseed oil-whey proteins isolate emulsions and reported that none of the emulsions showed creaming till 9 days of storage; but the emulsions homogenized at 80 MPa showed high molecular weight aggregates, thus concluded that 20 MPa (3,000 psi) was the optimum homogenization pressure. In present study, emulsion stability data showed that all the emulsions were stable to 28 days when homogenized at 3,000 psi pressure except one that was prepared using 5 % of WPC-80. Study also showed that WPC-80 is a good encapsulating agent for preparing a stable O/W flaxseed oil emulsion.

Oxidative stability

Encapsulated oil in the form of emulsion is more oxidative stable as compared to bulk oil. The progress of lipid oxidation was monitored by measuring the formation of primary oxidation products (lipid hydroperoxides) in O/W emulsions. Peroxide values of different flaxseed oil emulsions stored at low temperature for 4 weeks are shown in Fig. 1. The initial PV of flaxseed oil (control) was 12.20, which increased to 17.60 meq peroxide/kg oil during storage. There was no significant difference between PV of samples and control on zero day (just after the preparation of emulsions), suggesting that homogenization did not affect PV of flaxseed oil significantly ($p < 0.05$). It was observed that there was a gradual increase in PV of all of the emulsions in first 2 weeks (from 12.20 to 14.63 meq peroxides/kg oil), which did not further increase significantly (except 5 % WPC emulsion) till the end of storage. However, a significant increase (from 14.63 to

Fig. 1 Peroxide value of emulsions during storage period of 4 weeks at low temperature (4–7 °C). different lowercase letters on bars indicate significant difference within a week of storage period ($p < 0.05$). Control: free flaxseed oil. Values are the mean of three replicates ($n=3$)

16.90 meq peroxides/kg oil) was observed in PV of 5 % WPC emulsion from second to fourth week of storage. Highest PV or lower oxidative stability of 5 % WPC emulsion could be attributed to the thinner layer or lower amount of encapsulating agent around the oil droplets, leading to higher susceptibility to the oxidation. Data showed that there was only ~20.98 % increase in PV of all the emulsions (except prepared with 5 % WPC), as compared to free flaxseed oil (~44.26 %) after 4 weeks of storage. Similarly, Kuhn and Cunha (2012) reported about 41.70 % increase in PV of free flaxseed oil, which increased from 0.420 to 0.714 meq peroxides/kg oil during 30 days of storage. The present results are in agreement with Karaca et al. (2013), Partanen et al. (2008) and Grattard et al. (2002), who reported improved oxidative stability of flaxseed oil (in powder form) encapsulated by different proteins. Peroxide value of flaxseed oil in emulsions (prepared with 7.5–12.5 % WPC) remained well within the limit of up to 15 meq peroxide/kg oil under the Codex Alimentarius Commission (1999) standard for cold-pressed and virgin oils (Choo et al. 2007). The high stability of emulsions containing higher WPC concentrations (7.5, 10 and 12.5 %) may also be attributed to their antioxidative properties and their ability to bind some pro-oxidant impurities (such as transient metals) due to presence of histidine, glutamic acid, aspartic acid, and phosphorylated serine and threonine residues (McClements and Decker 2000; Tong et al. 2000); thus protecting oil against oxidation. Ma et al. (2012) reported improved oxidative stability of flaxseed oil emulsions encapsulated by sodium caseinate cross-linked by transglutaminase during 30 days of storage. Kuhn and Cunha (2012) reported a significant increase in PV from 0 to 1.777 meq peroxides/kg oil of flaxseed oil emulsion homogenized at 80 MPa. Carneiro et al. (2012) reported that flaxseed oil encapsulated with Maltodextrin:Hi Cap (modified starch) and Maltodextrin: Gum arabic presented peroxide values of 22.6 and 24.8 meq peroxides/kg oil, respectively after 1 week of storage. Jimenez et al. (2006)

encapsulated conjugated linoleic acid using WPC, WPC with maltodextrin and gum Arabic (GA), and concluded that WPC was more effective in providing protection against lipid oxidation than GA.

Particle size distribution

Many of the most important properties of emulsion-based food products (e.g., shelf life, appearance, texture, and flavor) are determined by the size of the droplets they contain. The particle size distribution (PSD) of an emulsion represents the fraction of particles in different size classes (McClements 2005). PSD can be changed by varying amount of encapsulating agent, stages and pressure of homogenization and composition of the emulsion. Figure 2 shows the particle size distribution of flaxseed oil emulsions homogenized with various concentration of WPC-80. Average particle size (Z-average) ranged from 255.0 ± 0.72 to 332.9 ± 1.80 nm. Emulsion prepared with 5 % WPC showed the highest Z-average, while the lowest size was observed for 10 & 12.5 % WPC based emulsions (Table 2). Highest Z-average in 5 % WPC emulsion suggested that there was insufficient amount of whey proteins to cover the oil droplets, which led to the droplets flocculation and thus increase in Z-average, similar to the observations reported by Wang et al. (2010). Ma et al. (2012) prepared flaxseed oil emulsion using cross-linked sodium caseinate and reported mean droplet diameter of 0.16 μ m (160 nm) at zero day. Similarly, Kentish et al. (2008) reported mean droplet size in the range of 135 ± 5 nm when flaxseed oil emulsion was homogenized at very high pressure (100 MPa) in presence of tween 40 surfactant. According to Fig. 2, all of the curves showed a monomodal distribution with a single peak representing predominant size in a particular range, except 5 % WPC emulsion, which showed bimodal distribution with two peaks, larger one ranged from 141 to 712 nm and smaller one ranged from

Fig. 2 Droplet size distributions of emulsions prepared with different concentration of WPC-80 (at 0th day)

1,281 to 5,560 nm suggesting the polydisperse nature of the particles. Bimodal distribution in 5 % WPC also indicated that there was a droplet-droplet flocculation, and thus, emulsion was comparatively less stable than others prepared using higher concentration of WPC. Similarly, Wang et al. (2010) reported bimodal distribution when lower amount of encapsulant (0.5–1 %) was used for soybean oil emulsion. No significant difference was observed between the Z-average of 10 and 12.5 % WPC emulsions ($p < 0.05$). However, 7.5 % WPC emulsion showed the narrowest particle size distribution, indicating more homogeneity, least aggregation and stability of the droplets. It was observed that as the concentration of WPC increased from 5 to 10 or 12.5 %, Z-average decreased and the distribution curve turned to monomodal, suggesting that there were more whey proteins to cover oil droplets surfaces, thus decreased the flocculation (Wang et al.

2010). Smaller sized particles can be produced by increasing the homogenization pressure and amount of emulsifier used (Walstra 1993). The higher size class of particles (in second peak of bimodal distribution) could be due either to coalescence of fat globules or to formation of covalently bound aggregates between proteins adsorbed on to different fat droplets (Sourd et al. 2003). Dybowska (2011) reported bimodal distribution of particles of rapeseed oil: WPC emulsions with particle size ranging from 122.4 to 342 nm and 458–2,669 nm. Emulsion showing particle size distribution with a single peak and in a narrow range would be the most homogenous and physically stable as in case of emulsion prepared with 7.5 % WPC (particle size range from 190.10 to 615.10 nm).

Polydispersity index (PDI) is a dimensionless scale between zero and one, which indicates the polydispersity of the particles. In fact, PDI is more appropriate in explaining

Table 2 Particle size distribution and zeta (ζ)-potential of emulsion droplets

Type of Emulsion* (% WPC)	Average Particle size (z-average) (nm)	Particle size range of Peak		ζ -potential (mV)	Polydispersity index (PDI)
		1 (nm)	2 (nm)		
5.0	332.9 $\pm$ 1.80 ^a	141.80–712.4	1,281–5,560	–30.6 ^a	0.593 ^a
7.5	320.1 $\pm$ 1.12 ^b	190.10–615.1	–	–33.0 ^b	0.294 ^b
10.0	255.0 $\pm$ 0.72 ^c	91.28–4801.0	–	–33.5 ^b	0.396 ^c
12.5	255.6 $\pm$ 1.21 ^c	91.28–955.4	–	–28.6 ^c	0.402 ^c

*Type of emulsion indicates the amount of WPC-80 in respective emulsion containing 12.5 % flaxseed oil and 10 % lactose

Values with different small superscript letters represent significant difference within column ($n=3$)

bimodal distribution profiles than z-average of particles. A lower PDI indicates narrower particle size distribution and vice versa. Data showed that PDI varied from 0.294 to 0.593 for all the emulsions (Table 2), suggesting all the emulsions having good dispersity. Emulsion with 7.5 % WPC showed the lowest PDI (0.294), supporting the narrowest particle size distribution and more homogenous as compared to other emulsions. In general, $PDI > 0.7$ indicates more heterogeneous nature and wide distribution of the particles (Nidhin et al. 2008), which increases the chances of aggregation of the emulsion droplets. Our data was consistent with the results reported by Wang et al. (2010), who observed PDI of 0.29 to 0.42 of soybean oil emulsions prepared with flaxseed proteins.

Zeta (ζ)-potential

The charge on droplet can influence the rheological properties of an emulsion. Emulsions with high zeta-potential (negative or positive) are electrically stabilized while emulsions with low zeta-potential tend to coagulate or flocculate. The ζ -potential represents the charge of the droplets with adsorbed protein and/or biopolymer, plus the charge associated with any ions that move along with the droplet in the electric field (Surh et al. 2006). Table 2 summarizes the ζ -potential of the emulsion droplets as a function of concentration of the encapsulating agent (WPC-80). Whey proteins concentrate being negatively charged at neutral pH, showed negative ζ -potential on emulsion droplets, and ranged from -28.6 to -33.5 mV. Similar findings have been reported by authors that studied the different oil-in-water emulsions and observed negative zeta potential on emulsion droplets stabilized by whey proteins (Nikovska 2012; Chanamai and McClements 2002; Saglam et al. 2013). There was no significant difference between the ζ -potential of 7.5 and 10 % WPC emulsions, which was comparatively higher than that of other emulsions studied. However, on increasing the WPC concentration from 5 to 7.5 or 10 %, a significant increase was observed ($p < 0.05$). Higher ζ -potential in 7.5 and 10 % WPC emulsions could be explained by the maximum utilization of whey proteins for the coverage of oil droplets, leaving very non-significant amount of unadsorbed protein or uncovered oil droplets. Similar results were reported by Wang et al. (2010) for the soybean oil-flaxseed protein emulsions. They reported that ζ -potential of the emulsions varied from -30.7 to -49.5 mV, with significant increase with flaxseed protein concentration. Our data suggested that 7.5 and 10 % WPC emulsions were the most stable systems among all emulsions in terms of ζ -potential. Khalloufi et al. (2008) reported around -50 mV ζ -potential on soybean oil based emulsions droplets stabilized by WPI. The ζ -potential results lead to the hypothesis that electrostatic repulsion occurs between the oil droplets covered by negatively charged whey proteins. The relatively higher negative

ζ -potential of whey protein concentrate coated droplets may account for greater intensity of the electrostatic repulsion force and superior stability of emulsion (Taherian et al. 2011). It can be concluded from the results of particle size distribution and ζ -potential that flaxseed oil emulsion produced by using 7.5 % WPC-80 was the most stable showing narrowest particle size distribution and highest zeta potential.

Rheological characteristics

Depending upon the composition, particle size & its charge and viscosity, emulsions show different rheological properties like Newtonian (ideal fluid) and Non-Newtonian (shear thinning, shear thickening, bingham plastics, etc.) behavior. Rheological properties of an emulsion play a significant role in determining the optimized conditions during the processing conditions (like pumping, mixing, flowing in pipes) or in designing a delivery system for a particular food application. Certain food systems like juices and beverages, which have very low viscosity, should not be changed during mixing with other ingredients, or during flowing & filling operations. Other food systems are highly viscous or gel like (for example, dressings, desserts) and in these cases the delivery system should not decrease the viscosity or disrupt the gel network (McClements et al. 2007).

Figure 3 represents the apparent viscosity (cP) under the shear rate (5 – 150 s^{-1}) for emulsions having different concentration of WPC-80 during storage of 28 days. All the emulsions showed non-Newtonian, shear thinning (Pseudoplastic) behavior as viscosity decreased with increase in shear rate. Figure shows that at lower shear rate (10 – 20 s^{-1}), high viscosity was observed irrespective of the emulsion composition. Similarly, high viscosities at low-shear rates have been reported for emulsions stabilized by whey protein isolate (Laplanche et al. 2005; Zinoviadou et al. 2012). Shear-thinning may occur for a variety of reasons in food emulsions (e.g., the spatial distribution of the particles may be altered by the shear field, or flocs may be deformed and disrupted) (Hunter 1993). Shear thinning behavior was also observed by Sun and Gunasekaran (2009), who worked on whey protein isolate stabilized oil-in-water emulsions. However, Saglam et al. (2013) reported shear thickening behavior of emulsions stabilized by whey protein isolates. Pseudoplastic behavior is the most common type of non-ideal behavior exhibited by food emulsions. In present study, viscosity increased with increase in protein concentration, highest and lowest for emulsions containing 12.5 and 5 % WPC, respectively. Normally, viscosity of an emulsion increases with increasing droplet or total solids concentration. Viscosity also increased during the storage period ranging from 7.85 to 23.7 cP at 150 s^{-1} shear rate.

For most non-Newtonian liquids, the viscosity decreases with an increase in shear rate, giving rise to what is known as pseudoplasticity or shear thinning behavior (Rao 1977).

Fig. 3 Apparent viscosity as a function of shear rate, obtained from flaxseed oil emulsions stabilized by different WPC-80 concentrations

Dybowska (2011) and Wang et al. (2010, 2011) also reported shear thinning behavior at lower shear rate ($<100 \text{ s}^{-1}$) for rapeseed oil and soybean oil emulsions, respectively. Similarly, shear thinning behavior was observed by Taherian et al. (2011) and Dybowska (2011) in O/W fish oil and

rapeseed oil emulsion, respectively. However, Lizarraga et al. (2008) found that corn oil-in-water emulsions (50 g oil/100 g) stabilized by WPC presented a Newtonian behavior.

Shear thinning behavior can be observed due to irreversible deformation and breakdown of flocs under the shear stress

Table 3 Rheological parameters obtained from the power law model for the WPC based o/w emulsions containing flaxseed oil

Type of Emulsion* (% WPC)	Storage period (weeks)														
	(Pa.s ⁿ)														
	0			1			2			3			4		
	<i>n</i>	<i>k</i>	<i>R</i> ²	<i>n</i>	<i>k</i>	<i>R</i> ²	<i>n</i>	<i>k</i>	<i>R</i> ²	<i>n</i>	<i>k</i>	<i>R</i> ²	<i>n</i>	<i>k</i>	<i>R</i> ²
5.0	0.330	0.160	0.950	0.206	0.150	0.959	0.390	0.168	0.975	0.286	0.163	0.988	0.289	0.178	0.988
7.5	0.360	0.170	0.960	0.393	0.176	0.968	0.350	0.197	0.940	0.377	0.189	0.934	0.396	0.199	0.935
10.0	0.500	0.200	0.980	0.470	0.220	0.971	0.533	0.211	0.981	0.493	0.236	0.975	0.443	0.274	0.984
12.5	0.570	0.200	0.970	0.499	0.259	0.979	0.566	0.473	0.980	0.577	0.494	0.990	0.591	0.503	0.990

*Type of emulsion indicates the amount of WPC-80 in respective emulsion containing 12.5 % flaxseed oil and 10 % lactose

n flow behavior index, *k* consistency index, *R*² Regression coefficient. (*n*=3)

(McClements 2005). The flow curves data for all the emulsions fitted well to the power law model equation. Initially, the rheology of all the emulsions was studied by applying 3 models: Bingham model, Newtonian model and Power law model. But the values of regression coefficient (R^2) obtained by fitting Bingham and Newtonian model were comparatively low in the range of 0.900–0.930 and 0.000–0.735, respectively, than the values obtained in Power law model (0.934–0.990). On the basis of R^2 , only the best fitted model, i.e. Power law model was selected. The values for various coefficients at a shear rate of $5\text{--}150\text{ s}^{-1}$ are shown in Table 3. These parameters were evaluated under the said shear range as it is typical of food processes, such as flow through a pipe, stirring or mastication. It is clear from the table that all the emulsions showed very high pseudoplasticity, since the flow behavior index (n) of all the emulsions was in the range from 0.206 to 0.591. However, Kuhn and Cunha (2012) studied the flaxseed oil emulsions stabilized by whey protein isolates (total solids 33 %) and reported that all O/W emulsions showed very low pseudoplasticity with flow behavior index in the range of 0.78–0.95. It can be observed that consistency index (k) (Pa.s^n) increased from 0.154 to 0.511 Pa.s^n with increase in concentration of whey proteins, suggesting the increase in viscosity and droplets concentration. Similar increase in consistency index (from 0.012 to 0.472 Pa.s^n) was reported by Wang et al. (2011) in soybean oil emulsions stabilized by flax proteins and soy protein isolates.

Conclusions

It can be concluded for the study that flaxseed oil emulsions stabilized by whey protein concentrate-80 at a level of 7.5–12.5 %, showed good physical stability with no sign of phase separation, when homogenized at 3,000 psi and stored at low temperature ($4\text{--}7^\circ\text{C}$) for 28 days. Higher homogenization pressure (4,500 psi) with higher concentration of whey proteins led to gelation of the emulsion spontaneously, while lower pressure resulted in free/surface fats during storage. Results indicated that emulsion containing 7.5 % WPC showed the best results among all the emulsions due to its narrowest particle size distribution, lowest PDI and highest ζ -potential. In emulsions, narrow sized particles contribute smoothness and improve texture & mouthfeel. Rheological data revealed that all the emulsions showed shear thinning behavior, which is a characteristic of food emulsions. Pseudoplastic behavior suggested the stability and suitability of emulsions during processing conditions, such as flowing in pipes, shearing or stirring, etc. A significant difference in PV of free oil and the oil encapsulated by WPC (at 7.5–12.5 %) indicated better protection of flaxseed oil provided by WPC, which was not only due to the formation of interfacial film around the oil droplet, but also due to their antioxidative and

metal ions binding properties. However, no significant difference was observed in PV of the emulsions prepared with 7.5–12.5 % WPC even after 28 days of storage. After 4 weeks of storage, lower increase in PV ($\sim 20.98\%$) of flaxseed oil emulsions (made up of 7.5–12.5 % WPC), as compared to the free oil ($\sim 44.26\%$), suggested that highly polyunsaturated flaxseed oil can be stabilized successfully by preparation of stable O/W emulsions using WPC-80; which can be used as a delivery system of ω -3 fatty acid through food fortification.

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