

Whey protein concentrate emulsion biology essay

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Abstract

The objective of the present study was to develop a delivery system for ω -3 fatty acids in the form of emulsion, which is stable physically, chemically as well as during processing conditions. Oil-in-water emulsions containing 12.5% flaxseed oil and whey protein concentrate (WPC)-80 ranging from 5 to 12.5% were prepared at 3000 psi homogenization pressure. The results revealed that all the emulsions were physically stable for 28 days at 4-7°C temperature of storage and did not show separation of phases. Emulsion with 7.5% WPC showed the narrower particle size distribution (190 to 615 nm) and maximum ζ -potential (-33.5 mV). All emulsions showed an increase in peroxide value from 17.62 to 29.35 meq/kg oil, indicating good oxidation stability. All emulsions showed shear thinning behavior (pseudoplasticity) with flow behavior index (n) in the range of 0.206 to 0.591. These results have important implications for the design of WPC-stabilized emulsions that could be used to stabilize omega-3 fatty acids containing flaxseed oil that is otherwise sensitive to oxidation.

Practical applications

It is well known that flaxseed oil is the richest source of omega-3 fatty acids (i. e. α -linolenic acid: ALA). There are some other sources like canola oil, soybean oil, walnuts, etc. but their ALA content is too low to meet daily requirements. Therefore, it is very difficult to meet RDA for the vegetarians, who do not consume fish & marine products, which are otherwise rich source of other omega-3 fatty acids (EPA & DHA). Due to its highly unsaturated nature, ALA rich flaxseed oil is highly oxidative unstable and becomes rancid

easily. Emulsions (o/w) are more oxidative stable and can be easily incorporated into various food products than bulk oil. Therefore emulsion technology provides a method to improve stability of flaxseed oil; and it could be a better approach in delivery of omega-3 fatty acids. Another approach of studying rheological properties will be useful in formulation of emulsion having desired flowing properties and viscosity, which finally affects the texture and mouthfeel of the product.

Introduction

Flaxseed (*Linum usitatissimum*) commonly 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 is regaining popularity from its traditional usage as a raw material in oil production to food oil because of its higher ω -3 fatty acid (ALA) content (> 50%) (Daun et al. 2003). ALA desaturates and elongates in the human body to other ω -3 fatty acids, i. e. eicosapentanoic acid (EPA) and docosahexanoic acid (DHA); and by itself may have beneficial effects in health and in control of chronic diseases (Mantzioris et al. 1994; Wiesenfeld et al. 2003). Epidemiological studies, animal experiments and human clinical trials suggest a role for flaxseed oil in preventing and treating chronic diseases such as cardiovascular disease (Kristensen et al. 2011, 2012; Hassan et al. 2012; Park and Velasque, 2012; Khalesi et al. 2011; Mani et al. 2011; Cardozo et al. 2010; Barakat and Mehmoud, 2011; Leyvaa et al. 2011), diabetes (Kapoor et al. 2011; Mani et al. 2011), cancer (Truan et al. 2012; Sturgeon et al. 2011) and osteoporosis. The ω -3 PUFAs in flaxseed oil have anti-inflammatory properties that are mediated by the production of anti-

inflammatory eicosanoids (Cohen et al. 2005). However, fish is by far the greatest contributor to food sources of ω -3 fatty acids (EPA and DHA) but the Western and Indian diets do not include enough oily fish to meet dietary recommendations. Moreover, flaxseed oil is preferred over fish oil by vegetarian population; but high content of ALA makes the flaxseed oil highly susceptible to oxidation, due to its highly unsaturated nature. During processing, distribution and handling, flaxseed oil can easily oxidize and produce unpleasant tastes and odors, and consequently lead to a reduction in product shelf life (Tonon et al. 2011). The best way to counter the problem of instability is to prepare a stable emulsion. Therefore, in the present study, an emulsion has been prepared using whey protein concentrate; and its stability has been established.

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 ($50\pm 1^\circ\text{C}$) 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). After preparation of pre-emulsion, it was warmed to 50°C in water bath followed by homogenization (Goma Engineering Pvt. Ltd., India) at 20 MPa (3000 psi). Prepared microemulsions were filled in amber colored plastic bottles and stored at low temperature (4-7°C) for 28 days.

Emulsion characterization

Emulsion stability

Emulsion stability was monitored by 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 ($7\pm 1^\circ\text{C}$) 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, according to Eq. (1).% separation = $(H/H_0) \times 100$ (1) 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)

The peroxide value (PV) of the WPC-stabilized emulsions was determined during the 28 days of storage after every week. To determine the peroxide value, fat was extracted from emulsion by the method of Folch et al. (1957) with slight modifications. Twenty g 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 and mixed 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. PV of extracted fat was evaluated by the method of AOAC (2005). About 5±0.5 g extracted fat was mixed with 30 mL of acetic acid : chloroform (3: 2) solution. 0.5 ml of saturated KI solution was added with occasional shaking for 1 minute on boiling water bath followed by the addition of 30 mL water. The solution was titrated with 0.01 N Sodium thiosulphate solutions with vigorous shaking until yellow color is almost gone. 0.5 ml of starch solution (1%) was then added and titration was continued to release all I₂ from chloroform layer until blue color just disappears. Blank determination was also conducted.

Peroxide Value (milli equivalent peroxide / kg oil) = $S \times N \times 1000 / \text{Weight of sample (g)}$ Where S = mL of Na₂S₂O₃ (blank corrected) used N = Normality of Na₂S₂O₃ solution

Particle size distribution and 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 % 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 $25 \pm 0.1^\circ\text{C}$, over a range of shear rate 5-150 per second. 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 (Sherman 1970, Rao et al. 1995). 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. As Power law model is the best suited to several food-grade emulsions, the experimental flow curves of the flaxseed oil emulsions were described by Power Law model (Ostwald de Waele) over the range of shear rates (5-150 s⁻¹) as follows: $\tau = k \cdot \dot{\gamma}^n$ where τ is the shear stress (Pa), k is the consistency index (Pa · sⁿ), $\dot{\gamma}$ 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 were analyzed and expressed as means. The data was subjected to analysis of variance (ANOVA) technique and analyzed according to two factorial completely randomized designs (CRD). Three replicates of each sample were used for analysis. The critical difference value at 5% level was used for making comparison among different samples during storage.

Results and discussion

Creaming index

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, 1997). 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 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 (3000 psi) pressure and stored at 7-8°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 were observed on the surface of emulsion

prepared using 5% of 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 wall material. 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 using 12.5% WPC-80, at higher homogenization pressure (4500 psi), gelling was observed (visually) just after the homogenization. It could be explained by the fact that at such 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. Kuhn and Cunha, (2012) studied that increase in homogenization pressure from 20 to 80 MPa led to the formation of high molecular weight aggregates (> 200 kDa). Kuhn and Cunha, (2012) studied the effect of homogenization pressure on the emulsion stability and concluded that 20 MPa (3000 psi) was the best homogenization pressure in preparation of flaxseed oil: whey protein isolate emulsions without any phase separation till 9 days of storage. In the present study, emulsion stability data showed that all the emulsions were stable to 28 days when homogenized at 3000 psi pressure. This study also showed that WPC-80 is a good encapsulating agent for preparing a stable o/w flaxseed oil emulsion.

Lipid oxidation [Peroxide value (PV)]

The progress of lipid oxidation was monitored by measuring the formation of primary oxidation products (lipid hydroperoxides) in O/W emulsions. Peroxide

values of flaxseed oil coated with different levels of WPC-80 are shown in Figure 2. On zero day, all samples showed a low level of peroxide value ranging from 17.62 to 18.11 meq/kg oil, which was close to peroxide value of pure flaxseed oil (16.50 meq/kg) used in the preparation of emulsions. During the storage period of 4 weeks, peroxide value of all samples increased gradually from 17.62 to 29.35 meq/kg oil. Overall, emulsion containing 5% WPC showed higher peroxide value (29.35 meq/kg oil) as compared to other emulsions during the storage. It could be due to lesser amount of encapsulating agent around the oil droplets in emulsion containing 5% WPC, leading to higher susceptibility of the emulsion to oxidation. There was no significant difference in peroxide value on 28th day of storage for different samples except for the sample prepared with 5% WPC. The high stability of emulsions containing WPC concentrations (7.5, 10 and 12.5%) may also be due to the protein which prevents the binding of some pro-oxidant impurities (such as transient metals); thus protecting oil against oxidation (Fomuso et al. 2002; McClements and Decker, 2000). The findings of the present study are in accordance with the reports given by Carneiro et al. (2012). They reported that flaxseed oil encapsulated with Maltodextrin: Hi Cap (modified starch) and Maltodextrin: Gum arabic presented higher peroxide values of 22.6 and 24.8 meq peroxides/kg oil, respectively after one 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 the 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 (Dickinson 1992). The particle size distribution (PSD) of an emulsion represents the fraction of particles in different size classes (McClements, 2005). The PSD of an emulsion can usually be controlled by varying homogenization conditions or system composition (e. g., the type and concentration of encapsulant/emulsifier used). Figure 1 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 maximum z-average, while minimum size was observed for 10 and 12. 5% WPC based emulsions. Z-average and size range for different emulsions is presented in Table 2. It was observed that as the concentration of WPC increased, average particle size decreased. Smaller droplets can usually be produced by increasing the intensity or duration of homogenization, or by increasing the concentration of emulsifier used (Walstra, 1993; Schubert and Engel, 2004). According to Figure 1, most of the curves of particle size distribution presented a monomodal distribution with a single peak representing a predominant size, with exception of the emulsion prepared with 5% WPC concentration, which showed a bimodal distribution. Although there was no significant difference between the Z-average of 10 and 12. 5% WPC emulsions, but narrower distribution was observed in 7. 5% WPC emulsion as compared to other distribution curves. It shows more homogeneity of particles in 7. 5% WPC emulsion. Flaxseed oil

emulsion homogenized with 5% WPC showed two peaks, larger one ranged from 141 to 712 nm and smaller one ranged from 1281 to 5560 nm suggesting the polydispersive nature (bimodal distribution) of the emulsion. The higher size class of particles could be due either to coalescence of fat globules or to formation of covalently bound aggregates between proteins adsorbed onto different fat droplets (Sourd et al. 2003). Dybowska, (2011) reported bimodal particle size distribution of rapeseed oil: WPC emulsions with particle size range from 122.4 to 342 nm and 458-2669 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).

Zeta (ζ)-potential

The charge on an emulsion droplet can influence the rheological properties of an emulsion. The charge determines whether the droplets are aggregated or unaggregated; and thus affects the viscosity. 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. 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. It suggested that 7.5 and 10% WPC emulsions were the most stable systems in terms of ζ -potential. Khalloufi et al. (2008)

reported around -50mV ζ -potential on the droplets of soybean oil based emulsions 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 narrow droplets size distribution and highest zeta potential.

Rheological characteristics

Emulsions exhibit a wide variety of different rheological behaviors depending on their composition, structure, and droplet interactions: Newtonian and Non-Newtonian (shear thinning, shear thickening, bingham plastics, etc.). The impact of overall rheology of an emulsion may be an important consideration during the processing conditions (like pumping, flowing in pipes) or when designing a delivery system for a particular food application. Some food systems have a relatively low viscosity (such as beverages) and therefore the delivery system itself should not significantly increase the viscosity. 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. Figure 3 represents the apparent viscosity (cP) under the shear rate ($5\text{-}150\text{ s}^{-1}$) for emulsions having different concentration of WPC-80 during 28 days of storage. All the emulsions showed non-newtonian, shear thinning (Pseudoplastic) behavior

as viscosity decreased with increase in shear rate. Pseudoplastic behavior is the most common type of non-ideal behavior exhibited by food emulsions. 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, Mewis and Macosko 1994). Viscosity increased with increase in protein concentration, maximum and minimum for emulsions containing 12. 5 and 5% WPC, respectively. Normally, the viscosity of an emulsion increases with increasing droplet or total solids concentration. Viscosity also increased during the storage period; ranged from 7. 85 to 23. 7 cP at shear rate of 150 s⁻¹. For most non-Newtonian liquids, the viscosity decreases with an increase in shear rate, giving rise to what is known as shear thinning behavior or pseudoplasticity (Rao, 1977). Debowska, (2011) reported that rapeseed oil (30%) : WPC emulsions showed a non-Newtonian, shear thinning behavior. Shear thinning behavior was observed due to irreversible deformation and breakdown of flocs under the shear stress (McClements, 2005). The flow curves data for all emulsions fitted well to the power law model equation. The values for various coefficients at a shear rate of 5-150 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 low pseudoplasticity, since the flow behavior index (n) of all the emulsions was in the range from 0. 206 to 0. 591. Similarly, 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 low pseudoplasticity with flow behavior index

in the range of 0.78-0.95. It can be observed that consistency index (k) (Pa·sn) increased (from 0.154 to 0.511 Pa·sn) with increase in concentration of whey proteins, suggesting the increase in viscosity. Shear thinning behavior was also observed by Taherian et al. (2011) in o/w emulsion containing fish oil (10%): whey protein isolate (1%) emulsion. However, Lizarraga et al. (2008) found that corn oil-in-water emulsions (50g oil/100g) stabilized by WPC presented a Newtonian behavior.

Conclusions

The results of this study revealed that emulsions stabilized by whey protein concentrate showed good physical stability with no sign of phase separation, when homogenized at 3000 psi and stored at low temperature (4-7°C) for 28 days. Higher pressure (4500 psi) with higher concentration of whey proteins led to gelation emulsion spontaneously. Results showed that emulsion containing 7.5% WPC was the most stable in terms of particle size distribution, Z-average and ζ -potential. Rheological data revealed that all the emulsions showed shear thinning behavior, which is a characteristic of food emulsions. Pseudoplastic behavior suggested that emulsions were suitable during processing conditions, such as flowing in pipes, shearing or stirring, etc. From the data it could be suggested that flaxseed oil-in-water emulsions stabilized by WPC may be used to produce oxidatively and physically stable ω -3 fatty acid delivery systems for incorporation of nutritionally significant amounts of these important bioactive lipids into foods.