Characterization of flaxseed oil emulsions

Pei-En Lee · Wee-Sim Choo

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Abstract The emulsifying capacity of surfactants (polysorbate 20, polysorbate 80 and soy lecithin) and proteins (soy protein isolate and whey protein isolate) in flaxseed oil was measured based on 1 % (w/w) of emulsifier. Surfactants showed significantly higher emulsifying capacity compared to the proteins (soy protein isolate and whey protein isolate) in flaxseed oil. The emulsion stability of the flaxseed oil emulsions with whey protein isolate (10 % w/w) prepared using a mixer was ranked in the following order: 1,000 rpm (58 min) $\approx$ 1,000 rpm (29 min) $\approx$ 2,000 rpm (35 min) $>$ 2,000 rpm (17.5 min). The emulsion stability of the flaxseed oil emulsions with whey protein isolate (10 % w/w) prepared using a homogenizer (Ultra Turrax) was independent of the speed and mixing time. The mean particle size of the flaxseed oil emulsions prepared using the two mixing devices ranged from $23.99 \pm 1.34 \mu\text{m}$ to $47.22 \pm 1.99 \mu\text{m}$ where else the particle size distribution and microstructure of the flaxseed oil emulsions demonstrated using microscopic imaging were quite similar. The flaxseed oil emulsions had a similar apparent viscosity and exhibited shear thinning (pseudoplastic) behavior. The flaxseed oil emulsions had L^* value above 70 and was in the red-yellow color region (positive a^* and b^* values).

Keywords Flaxseed oil · Emulsion · Emulsifier · Surfactant · Whey protein isolate

Introduction

Flaxseed oil is a major plant source of omega-3 fatty acids which is rich in α -linolenic acid (ALA). ALA is recognized as

the essential fatty acid precursor of the omega-3 fatty acid family which can be converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by desaturase enzyme (DeFilippis and Sperling 2006). The high content of ALA ($>50\%$) in flaxseed oil, however, renders the oil susceptible to oxidation (Choo et al. 2007a). Flaxseed oil is not recommended to be used with heat treatment such as stir frying or pan heating (Choo et al. 2007b).

In the food industry, there is a wide variety of natural and manufactured food product in an emulsified form or emulsified state during production (McClements 2005). Emulsions are dispersion of a liquid phase in the form of fine droplets in another immiscible liquid phase. The immiscible phases are referring to oil and water and emulsions are generally regarded as oil-in-water or water-in oil emulsion depending on the dispersed phase composition (Fustier et al. 2010). Various products like ice cream, desserts, butter, salad dressing, margarine and beverage are sold in an emulsified state. The formulation of the food emulsions such as addition of food additives (emulsifiers, thickeners, flavouring, preservatives) and formation of small droplet size during emulsification process contributed to their stability, sensory quality and ability to act as delivery systems for bioactive compounds (Fustier et al. 2010).

There are several advantages of emulsions. Firstly, the unpleasant smell of the material can be minimized by processing into an emulsion. For example, unfavorable smell of castor and cod liver oils can be made almost palatable and foul smelling oily substances are made almost odorless by means of emulsification. This is due to the oil globules being completely surrounded by an aqueous medium which markedly suppressed the odor of the oil (Durgin and Hanan 2004). Furthermore, emulsion has better organoleptic properties (taste, smell, texture and mouthfeel) than oily solution or pill form that is administered orally (McKenna and Kilcast 2003). Emulsion also offers better gastro-intestinal absorption of the

P.-E. Lee · W.-S. Choo (✉)
School of Science, Monash University Malaysia, Jalan Lagoon
Selatan, 46150 Bandar Sunway, Selangor, Malaysia
e-mail: choo.wee.sim@monash.edu

essential fatty acids due to the smaller particles size of the emulsion that ranged from micro to nano particles (Remington and Beringer 2005). Couedelo et al. (2011) found that ALA bioavailability was improved with the flaxseed oil ingested in an emulsified state. The objectives of this study were to determine the emulsifying capacity of surfactants and proteins in flaxseed oil and the effect of using two mixing devices on the characteristics of flaxseed oil emulsions produced.

Materials and methods

Materials

Polysorbate 20 (Tween-20) and polysorbate 80 (Tween-80) were obtained from Kolb Distribution Ltd. (Hedingen, Switzerland). Soy lecithin was purchased from American Lecithin Company (Connecticut, U.S.A). Whey protein isolate was obtained from Muscle Feast, LLC (Newark, U.S.A). Citric acid anhydrous was purchased from Weifang Ensign Industry Company Ltd (Shandong, China). Xanthan gum was obtained from Deosen Biochemical Ltd. (Shandong, China). Gum arabic was obtained from Nantong Kaixin Pharma Chemical Company Ltd. (Jiangsu, China). Soy protein isolate was obtained from Nantong Sun Green Bio-Tech Company Ltd. (Jiangsu, China). Potassium sorbate was obtained from Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). Deionized water with resistivity of $18.2 \Omega\text{cm}^{-1}$ was used for preparation of all emulsions. Extra virgin flaxseed oil without the addition of antioxidants (approximately 58 C18:3, 17 C18:2, 16 C18:1, 5.5 C16:0 and 3.5 % C18:0) from U.S.A was purchased from a local distributor (Biogreen, Malaysia).

Emulsifying capacity of emulsifiers

The emulsifying capacity of emulsifiers was determined according to the method of Gurov et al. (1983) with some modifications. Emulsifying capacity of an emulsifier was determined by adding 1 % (w/w) of emulsifier, 0.1 % (w/w) potassium sorbate and 0.4 % (w/w) citric acid into a beaker. Flaxseed oil (10 g) was initially added and mixed using a mixer (RW20 digital, IKA-Werke GmbH & Co. KG, Germany) to produce a crude emulsion for 2 min at 1,000 rpm. Then, the oil was continuously added to the emulsion using a burette until the reading of a conductivity meter (model HI 98312, Hanna Instruments, Inc., U.S.A.) shows $<0.1 \text{ mScm}^{-1}$.

Preparation of flaxseed oil emulsions using a mixer

Emulsion was prepared according to the method of Mirhosseini et al. (2008) with some modifications. A blend of emulsifiers (gum arabic and whey protein isolate) was used.

Xanthan gum was added as a stabilizer. The aqueous phase of an emulsion was prepared by dispersing gum arabic (10 % w/w), whey protein isolate (10 % w/w), xanthan gum (0.3 % w/w), potassium sorbate (0.1 % w/w) and citric acid (0.4 % w/w) in deionized water. The mixture was then mixed using a glass rod for 3 min and kept overnight to facilitate hydration at room temperature. The aqueous phase (60 %) and oil phase (40 %) was pre-mixed at 2,000 rpm for 5 min using a mixer (RW20 digital, IKA-Werke GmbH & Co. KG, Germany) to produce a crude emulsion. The emulsion was continued to mix at two different speeds (2,000 rpm or 1,000 rpm). The mixing time to form an emulsion at each speed was recorded. Then, the mixing time was doubled to study the relationship of time-speed dependence.

Preparation of flaxseed oil emulsions using a homogenizer

Emulsion was subjected to homogenization using a homogenizer (Ultra Turrax T-25 digital, IKA-Werke GmbH & Co. KG, Germany) at speed of 15,000 for 2, 20,000 for 2 and 20,000 rpm for 4 min, respectively. Creaming stability (day 1 and day 7), accelerated emulsion stability, color, particle size (day 1), viscosity and microscopic imaging of the emulsions were determined.

Creaming stability

Creaming stability of an emulsion was determined according to the method of Gu et al. (2005). Emulsion (10 g) was transferred into a test tube, tightly sealed with a plastic cap and then stored at room temperature up to 90 days. Upon storage, emulsions that separated into two layers with opaque cream layer at the top and transparent serum layer at the bottom were measured at day 1 and day 7, respectively. The initial height of the emulsion (H_T) and the height of the serum layer (H_S) were measured. The extent of creaming was characterized by a creaming index which is defined by the formula shown as below:

$$\text{Creaming index(\%)} = H_S/H_T \times 100\%$$

Accelerated emulsion stability

Accelerated stability of an emulsion was determined according to the method of Huang et al. (2001) with some modifications. Freshly made emulsions were centrifuged after preparation using a micro-centrifuge (Hettich Instruments, U.S.A.). An emulsion (35 mL) was placed into a 50 mL centrifugal plastic tube and centrifuged at 7,500 g for 15 min at 25 °C. Centrifugation produced three separate layers which are aqueous layer (bottom), emulsion layer (middle) and oil layer (top). Only very unstable emulsions produced a

Fig. 1 Emulsifying capacity of emulsifiers in flaxseed oil. Values are expressed as means $\pm$ standard deviations. *ab Values with different superscript letters indicate significant difference at $p < 0.05$

significant oil layer. The initial height of the emulsion (H_T) and the height of the emulsion layer (H_e) were measured. Emulsion stability (ES) was calculated as a percentage with the formula shown as below:

$$ES (\%) = H_e / H_T \times 100\%$$

Particle size distribution

Particle size distribution of an emulsion was determined according to the method of Huang et al. (2001) with some modifications. The particle size distribution of an emulsion was measured using a Malvern Mastersizer 2000 particle size analyzer (Malvern Instruments Ltd, United Kingdom). The emulsion was measured as wet dispersions. One or few drops of emulsion were dispersed into 600 mL distilled water to obtain an obscuration of 0.2 %. A refractive index of 1.475 was used for flaxseed oil (Kentish et al. 2008). The particle size distribution of emulsion at day 1 and day 30, respectively was measured. The span is the width of the particle size distribution based on the 10, 50 and 90 % quantile. Specific surface area is the surface area per volume of particles. Span

and specific surface are were calculated automatically from the Malvern Mastersizer 2000.

Microscopic imaging analysis

Microscopic imaging analysis of an emulsion was determined according to the method of Ramin et al. (2009) with some modifications. The distribution and morphology of droplets of an emulsion was obtained using a light microscope (model BX51, Olympus Imaging & Audio Ltd, United Kingdom) equipped with a camera (model DS-Fi1c, Nikon Instruments Inc., U.S.A.). A drop of emulsion was placed on a glass slide and covered with a cover slip.

Viscosity

Viscosity of an emulsion was determined according to the method of İbanoğlu (2002) using a Brookfield Viscometer (RV DVII+Pro, Brookfield Engineering Laboratories, U.S.A.). Readings of torque of freshly made emulsions were taken at rotational speeds of 2.5, 5, 10, 20, 50 and 100 rpm using a spindle number 5 at room temperature after 1 min of shearing. A fixed volume (350 mL) of an emulsion in 600 mL

Table 1 Emulsion stability, creaming index and L^* , a^* , b^* values of emulsions produced using a mixer

Speed (rpm)	Average mixing time (min)	Emulsion stability (%)	Creaming index (%)		L^*	a^*	b^*
			Day 1	Day 7			
2,000	17.5	77.19 $\pm$ 1.51 ^b	-	-	72.31 $\pm$ 0.22 ^a	4.59 $\pm$ 0.14 ^a	32.45 $\pm$ 0.09 ^{ab}
2,000	35	86.40 $\pm$ 1.93 ^a	-	-	73.55 $\pm$ 0.67 ^a	4.47 $\pm$ 0.01 ^a	31.28 $\pm$ 0.89 ^b
1,000	29	83.71 $\pm$ 1.92 ^a	-	-	72.49 $\pm$ 1.49 ^a	4.91 $\pm$ 0.31 ^a	33.20 $\pm$ 0.09 ^a
1,000	58	85.83 $\pm$ 0.31 ^a	-	-	74.58 $\pm$ 0.13 ^a	4.72 $\pm$ 0.28 ^a	32.52 $\pm$ 0.07 ^{ab}

‘-’ indicates that the emulsion was not separated. L^* represents lightness (100=white; 0=black). a^* indicates redness when positive, greenness when negative. b^* indicates yellowness when positive, blueness when negative. Values are presented as means $\pm$ standard deviations. *ab Values with different superscript letters within a column indicate significant difference at $p < 0.05$

Fig. 2 Particle size distributions of emulsions mixed at 1,000 rpm

beaker was measured and the spindle depth was kept constant during each measurement. For interpretation of the results in terms of viscosity, the data were converted into shear stress–shear rate relation using numerical conversion values (Mitschka 1982) and the flow index (n) and apparent viscosities of the samples at each rotational speed were calculated.

Color

Color of an emulsion was determined according to the method of Chantrapornchai et al. (1998) using a colorimeter (ColorFlex EZ, Hunter Associates Laboratory, Inc., United States). The colorimeter was calibrated using black and white tiles. An emulsion sample was transferred into a cup and the readings in terms of L^* , a^* and b^* were taken. L^* represents the lightness and a^* and b^* are the color coordinates. Positive a^* and negative a^* represent the redness and greenness, respectively, while positive b^* represents yellowness and negative b^* represents blueness.

Statistical analyses

All data were interpreted by one-way analysis of variance (ANOVA) with Duncan's multiple-range test using SAS

software package (SAS Institute Inc., U. S.A.). Statistical significance was evaluated at $p < 0.05$ level.

Results and discussion

Emulsifying capacity of emulsifiers

Emulsifying capacity (EC) of an emulsifier depends on its ability to lower the interfacial tension at the oil–water interfaces (Zayas 1996). The EC of polysorbate 20, polysorbate 80, soy lecithin, soy protein isolate and whey protein isolate was measured based on the concentration of 1 % (w/w) emulsifier in this study (Fig. 1). Polysorbate 20, polysorbate 80 and soy lecithin are classified as surfactants. These surfactants showed significantly higher EC compared to the proteins (soy protein isolate and whey protein isolate) which was indicated by the higher volume of emulsified oil ($p < 0.05$). This was most likely due to their low molecular weight which results in rapid diffusion and absorption of the molecules at the interfaces. Compared to surfactants, proteins exist in large molecular weight globular structure. Furthermore, presence of large hydrophobic portion in the chemical structures of surfactants increases their surface activity.

Fig. 3 Particle size distributions of emulsions mixed at 2,000 rpm

Table 2 Mean particle size analysis of emulsions produced using a mixer at day 1

Speed (rpm)	Time (min)	Volume-weighted mean, $d_{4,3}$ (μm)	Span	Specific surface area, m^2/g
2,000	17.5	23.99 ± 1.34^c	7.29 ± 1.91^b	0.91 ± 0.26^a
2,000	35	27.45 ± 0.12^b	7.50 ± 1.16^b	0.87 ± 0.32^a
1,000	29	47.22 ± 1.99^a	16.45 ± 1.59^a	0.71 ± 0.07^a
1,000	58	24.14 ± 0.14^{bc}	7.93 ± 1.39^b	1.03 ± 0.05^a

*Values are presented as means $\pm$ standard deviations. ^{abc} Values with different superscript letters within a column indicate significant difference at $p < 0.05$

There was no significant difference in the EC of polysorbate 20, polysorbate 80 and soy lecithin. This is not in accordance with the study by Ruiz-Peña et al. (2010) who reported that polysorbate 80 had higher value of surface excess concentration at surface saturation compared to polysorbate 20. High value of surface excess concentration at surface saturation indicated high effectiveness of adsorption at surface, thereby giving high emulsifying capacity. In addition, small critical micelle concentration provides better emulsification ability. Study showed that critical micelle concentration of polysorbate 80 was lower than soy phospholipids and polysorbate 20 (Apenten and Zhu 1996; Das and Bhattacharyya 2006). Similarly, there was no significant difference in the EC of soy protein isolate and whey protein isolate which is not in accordance with the study of Dekanterewicz et al. (1987) who reported lower EC of soy protein isolate compared to that of whey protein concentrate. This may be due to the different measuring technique used. Dekanterewicz et al. (1987) measured the EC of proteins based on their water–oil absorption index which describes the relative hydrophilic-lipophilic character of proteins. In addition, Amundson and Sebranek (1990) found that the EC of sodium caseinate (salt of a type of milk protein) was lower than that of soy protein isolate but this investigation was based on different conditions. Furthermore, the oil used was corn oil which was a different oil compared to the oil used in this study (flaxseed oil).

Production of flaxseed oil emulsions using a mixer

The formation of a flaxseed oil emulsion using a mixer was obtained using a mixing speed of 2,000 rpm at 17.5 min (Table 1). A longer mixing time is needed to form an emulsion at a lower mixing speed of 1,000 rpm. A significantly higher accelerated emulsion stability of 86.40 % was obtained with a 2× longer mixing time at the mixing speed of 2,000 rpm ($p < 0.05$). Increased mechanical energy leads to rapid formation of large interfacial areas, thereby causing protein to absorb more rapidly and efficiently at the interfaces (Mira et al. 2003). All emulsions were stable against creaming up to 7 days (Table 1). Emulsions had a value of $L^* > 70$ and positive value of a^* and b^* , indicating that the emulsions were bright in appearance and were in the red-yellow color region in the L^* , a^* , b^* color coordinates.

The particle size distribution of emulsions produced using a mixer is shown in Fig. 2–3. The distribution curves of the emulsions were bimodal and skewed to the right. The peaks of the distribution were in the range of 0–150 μm . The mean particle size analysis of the emulsions at different mixing speeds and times is shown in Table 2. Span represents the polydispersity (i.e. width of particle size distribution) of particles in the emulsions. Emulsions mixed at 1,000 rpm (29 min) had a significantly higher span value compared to the other emulsions. This could imply that there was a wider range of particle sizes in the emulsions mixed at 1,000 rpm (29 min) compared to the other emulsions. There were no significance differences in specific surface area of particles in the emulsions. The specific surface area of particles in the emulsions was ranged from 0.71 to 1.03 m^2/g .

The emulsions mixed at 1,000 rpm (29 min) had the highest mean particle size compared to other emulsions (Table 2). However, it had high emulsion stability (Table 1). This is in contrast with the study of Ramin et al. (2009) who reported that smaller particle size of orange oil-in-water emulsions containing soy protein isolate and gum arabic had higher emulsion stability. This is most likely due to the use of different blend of emulsifiers and oil phase in this study.

Fig. 4 Microphotographs of emulsions mixed at 1,000 rpm for (a) 29 min; (b) 58 min (400×)

Fig. 5 Microphotographs of emulsions mixed at 2,000 rpm for (a) 17.5 min; (b) 35 min (400 $\times$)

Microphotographs of emulsions are shown in Fig. 4–5. The particles were not closely packed. However, a small surface-to-surface distance of particles was observed. This is an indicative of formation of large particles in the emulsions as a result of collision. The kinetic effect of particles caused disruption of the interfacial layer, consequently the particles merged to form larger particles.

All emulsions exhibited shear thinning behavior with an increase in apparent viscosity as shear rate decreased (Fig. 6). This indicates that the shear thinning behavior was not dependent on the mixing parameters but the presence of polysaccharide (gum arabic and xanthan gum) and whey protein isolate in the emulsions. Sharp reduction at lower shear rates, followed by smooth curve at higher shear rates were due to the deformation and reduction in aggregated particles as shear rate increased (Campanella et al. 1995).

Production of flaxseed oil emulsions using a homogenizer

All the emulsions homogenized at different speed and time was stable against creaming up to 7 days of storage (Table 3). There were no significant differences between the accelerated

emulsion stability of the emulsions homogenized at different speeds and times. This is in accordance with the study of Tornberg and Hermansson (1977) who reported similar behavior with stability–time and stability–intensity dependence of soybean oil emulsions with proteins-based emulsifiers mixed by an omni-mixer, Ultra-Turrax, ultrasonic or a valve homogenizer. Creaming stability does not seem to increase with an increase in emulsifying intensity or mixing time after a certain limit. There were also no significant difference in L^* , a^* , b^* values of the emulsions (Table 3). All the emulsions had an equal brightness ($L^* > 70$) and was in red-yellow color region in the L^* , a^* , b^* color coordinates (positive a^* and b^* values).

The particle size distribution of the emulsions produced using a homogenizer is shown in Fig. 7–8. The distribution curves were bimodal and skewed to the right except for emulsion homogenized at 15,000 rpm (2 min) which showed multimodal distribution. The peaks of the distributions were in the range of 0–150 μm (Fig. 7–8).

There were no significant differences in the mean particle sizes of the emulsions produced using a homogenizer (Table 4). This is not in accordance with the study of Ramin

Fig. 6 Flow behaviors of the emulsions produced using a mixer

Table 3 Evaluation of emulsion stability and L*, a*, b* values of emulsions produced at different homogenizing speeds and times

Speed (rpm)	Mixing time (min)	Emulsion stability (%)	Creaming index (%)		L*	a*	b*
			Day 1	Day 7			
20,000	2	83.73±2.24 ^a	-	-	74.19±0.09 ^a	4.88±0.40 ^a	32.07±0.05 ^a
20,000	4	87.84±0.61 ^a	-	-	75.09±0.13 ^a	4.61±0.06 ^a	30.44±1.25 ^a
15,000	2	85.39±0.45 ^a	-	-	74.41±1.16 ^a	4.39±0.26 ^a	31.53±1.20 ^a

‘-’ indicates that the emulsion was not separated. L* represents lightness (100=white; 0=black). a* indicates redness when positive, greenness when negative. b* indicates yellowness when positive, blueness when negative. Values are presented as means ± standard deviations. ^a Values with different superscript letters indicate significant difference at $p<0.05$

Fig. 7 Particle size distributions of emulsions homogenized at 20,000 rpm **Fig. 8** Particle size distributions of emulsions homogenized at 2 min**Table 4** Mean particle size analysis of emulsions produced using a homogenizer at day 1

Speed (rpm)	Time (min)	Volume-weighted mean, $d_{4,3}$ (μm)	Span	Specific surface area, m^2/g
20,000	2	30.39±3.42 ^a	7.39±0.616 ^a	0.71±0.02 ^a
20,000	4	24.10±1.26 ^a	7.76±1.284 ^a	0.83±0.03 ^a
15,000	2	31.04±2.879 ^a	9.38±4.046 ^a	0.78±0.186 ^a

*Values are presented as means ± standard deviations. ^a Values with different superscript letters within a column indicate significant difference at $p<0.05$

Fig. 9 Microphotographs of emulsions homogenized for 2 min at (a) 15,000 rpm; (b) 20,000 rpm (400 $\times$)

et al. (2009) who reported that the particle size of beverage emulsions containing soy protein isolate and gum arabic decreased with increasing speed of homogenization. This difference was probably due to the use of a combination of a mixer and a homogenizer (Ultra-Turrax) in their study. Furthermore, Spinelli et al. (2010) reported that an increase of processing time gave a reducing average particle size in oil in water nano emulsions. Different processing time and speed was used in

their study. Microphotographs of the emulsions are shown in Fig. 9–10. A small surface-to-surface distance of particles was observed, probably due to the kinetic effect of the particles.

The change in apparent viscosity as a function of shear rate for emulsions homogenized at different speeds and times is shown in Fig. 11. The emulsions had a similar apparent viscosity, probably because the degree of aggregation of particles was similar. An increase in the shear rate resulted in a decrease in the apparent viscosity. This indicates that the emulsion exhibited pseudoplastic or shear thinning behavior. The behavior was not influenced by different homogenizing speeds and times.

Fig. 10 Microphotographs of emulsions homogenized at 20,000 rpm for 4 min (400 $\times$)

Conclusion

Surfactants had higher emulsifying capacity than those of proteins in flaxseed oil. The emulsion stability of flaxseed oil emulsions produced using a mixer was dependent on the mixing time and speed where else the emulsion stability of

Fig. 11 Flow behaviors of emulsions produced using a homogenizer

flaxseed oil emulsions produced using a homogenizer was independent on the mixing time and speed. The mean particle size of the flaxseed oil emulsions prepared using a mixer showed a larger range ($23.99 \pm 1.34 \mu\text{m}$ to $47.22 \pm 1.99 \mu\text{m}$) as compared to those prepared using a homogenizer ($24.10 \pm 1.26 \mu\text{m}$ to $30.39 \pm 3.42 \mu\text{m}$). The particle size distribution, microstructure of the flaxseed oil emulsions demonstrated using microscopic imaging and their apparent viscosity prepared using the two mixing devices were quite similar. The flaxseed oil emulsions exhibited shear thinning (pseudoplastic) behavior with similar color characteristics.

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