



American Journal of
Food Technology

ISSN 1557-4571



Academic
Journals Inc.

www.academicjournals.com

Ultrasonic Emulsification of Whey Protein Isolate-Stabilized Nanoemulsions Containing Omega-3 Oil from Plant Seed

¹K. Chalothorn and ²W. Warisnoicharoen

¹Pharmaceutical Technology (International Program), Department of Pharmaceutics and Industrial Pharmacy, ²Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Road, Pathumwan, Bangkok 10330, Thailand

*Corresponding Author: Warangkana Warisnoicharoen, Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Road, Pathumwan, Bangkok 10330, Thailand
Tel: +66 2218 8332 Fax: + 66 2218 8332*

ABSTRACT

In this study, nanoemulsions containing omega-3 oil from *Echium plantagineum* seed and stabilized by Whey Protein Isolate (WPI) were prepared using ultrasonic emulsification technique. The ultrasonic process parameters (energy, amplitude and temperature) dealt with properties and stability of nanoemulsions were optimized. The size and size distribution of nanoemulsions decreased with increasing amount of WPI and lowering power amplitude. For WPI-stabilized nanoemulsions consisting of 5% (w/v) oil and 20% (w/v) WPI, the minimum droplet diameter (225.83 ± 1.52 nm) was obtained when optimum ultrasonic conditions (20% amplitude, energy of 1750 J and 45°C) were used. Thus, the amount of WPI and process parameters could possibly affect the properties of WPI molecules at the oil-water interface as well as emulsifying efficiency. The findings provide the novel food-grade nanoemulsions prepared by ultrasonic emulsification for further use in nanotechnology-based applications.

Key words: Nanoemulsions, whey protein isolate, *Echium plantagineum*, omega-3 fatty acids

INTRODUCTION

Among nanoparticulate systems, nanoemulsions are considered to be the one that are widely employed in food and nutritional purposes. Nanoemulsions are usually regarded as a system of oil droplets dispersed in continuous aqueous phase (oil-in-water) and stabilized by a membrane of emulsifier molecules. The size range of nanoemulsion is considerably covers 20-500 nm (Jafari *et al.*, 2006; Wulff-Perez *et al.*, 2009; Nair *et al.*, 2010). Nanoemulsions are predominantly served as nanocarriers in order to encapsulate the nutrients or food ingredients which are hydrophilic especially carotenoids (such as β -carotene) and plant polyphenols (such as curcumin) (Chu *et al.*, 2008; Wang *et al.*, 2008; Yin *et al.*, 2009; Huang *et al.*, 2010). Encapsulation of compounds inside nanoemulsions is capable of improvement in water solubility and prevention of degradation of the bioactive food components (De Vos *et al.*, 2010). Owing to the nanometric sizes, nanoemulsions are readily absorbed after orally taken and hence, greater bioavailability of the encapsulated compounds (Acosta, 2009; Huang *et al.*, 2010). The size properties and kinetic stability of nanoemulsions are controllable by several factors including choices of emulsifiers and

emulsification methods (McClements, 2004). The emulsifier of choices for food applications and nutraceuticals would be the natural type such as lecithin and protein owing to its classification as GRAS (generally recognized as safe) and high emulsifying properties. Recently, milk-derived WPI is attractive for nanoemulsion food formulation (Keowmaneechai and McClements, 2002). WPI contains globular proteins mostly β -lactoglobulin (LG), comprising 162 amino acids and molecular weight of 18.4 kDa and secondly α -lactalbumin (LB), comprising 123 amino acids and molecular weight of 14.2 kDa (McClements, 2004; Nicorescu *et al.*, 2008; He *et al.*, 2011). WPI structure possesses flexibility to be adsorbed at the oil-water interface to form nanoemulsion aggregates (He *et al.*, 2011). For the oil phase, triglyceride oils from plant or animal are generally used in the emulsion formulation (Hagi *et al.*, 2007). Lately, triglyceride oils rich in omega (ω)-3-fatty acids are proved to provide health benefits in terms of improved cellular immunity and anti-inflammatory effect (Wendel *et al.*, 2007). Apart from animal-derived fish oil, seed oil from plant *Echium plantagineum* (Boraginaceae) is also an abundant source of ω -3-fatty acids with benefits of less unpleasant smell compared to fish oil (Berti *et al.*, 2007; Kentish *et al.*, 2008).

As mentioned, emulsification methods involve in size, properties and stability of the nanoemulsions. Several high-energy emulsification methods are widely used to prepare nanoemulsions including high-pressure homogenization, microfluidization and ultrasonication (Solans *et al.*, 2005). Even extensive research has been focused on emulsification of WPI-stabilized nanoemulsions using the either high pressure homogenizer or microfluidizer, very little work was reported on ultrasonic emulsification of the nanoemulsions (Perrier-Cornet *et al.*, 2005; Lee and McClements, 2010; Qian and McClements, 2011). The ultrasonic method is based on the acoustic cavitation to disperse oil into a continuous phase where collapse of vapor cavities causes small droplet sizes (Canselier *et al.*, 2002). Emulsification by ultrasonic approach is sometimes less efficient than other high-energy based methods. However, it is more practicable with respect to production cost and equipment and reduced risk in over processing phenomenon which is subjected to droplet coalescence during emulsification (Jafari *et al.*, 2006, 2008). Furthermore, optimization of ultrasonic process for the specific formulation is substantial for cost effectiveness when scaled up (Hielscher, 2006). Therefore, the objective of this study was to investigate the ability of WPI emulsifier to produce nanoemulsion systems containing *E. plantagineum* seed oil. The effects of the processing parameters in particular, energy input, ultrasonic amplitude and temperature, on the droplet size and size distribution as well as the stability of the systems after storage have also been determined.

MATERIALS AND METHODS

Materials: Whey protein isolate (WPI, 90% protein) was purchased from Glanbia Nutritionals, Ireland. *E. plantagineum* seed oil (RevitElix™) and ethyleneglycol-monophenylether (EGP) were gifts from Croda, France. Glycerine was purchased from Sigma-Aldrich, USA. The purified water used in the formulation was obtained from an Elga apparatus (ElgaStat Option 3, Elga Ltd., Bucks, UK).

Preparation of nanoemulsions stabilized by WPI: In order to obtain a 10 mL nanoemulsion, a pre-emulsion was firstly prepared. A pre-emulsion was produced by mixing the aqueous phase, containing WPI, glycerin and water, with the oil phase, containing *E. plantagineum* seed oil and EGP. The oil concentrations of 5 and 10% (w/v) and WPI concentrations of 1-20% (w/v) were used

in the formulations. For all formulations, glycerin (2.5% (w/v)) was used as a wetting agent and EGP (0.7% (w/v)) was an antimicrobial agent. A mixture was stirred throughout using a high speed stirrer at room temperature. The pre-emulsion was then further emulsified using a 20 kHz ultrasonicator VCX 750 (Sonics and Materials Inc., Newtown, USA). An ultrasonic processor system was equipped with a power generator, a converter (Model CV 33), an ultrahigh intensity sonotrode (tapered horn microtip) with a diameter of 3 mm and an integrated temperature controller. The generator produced the power output of 750 watts and frequency of 20 kHz. The converter composed of Piezoelectric lead Zirconate Titanate (PZT) crystals which transduced the mechanical vibration to a sonotrode. The microtip sonotrode was made of titanium alloy Ti-6Al-4V and used to amplify ultrasonic cavitation into a 10 mL sample. The magnitude of amplitude was operated as a percentage of the maximum amplitude which was 40% (228 μm) for the microtip sonotrode. The process was run in a non-stop pulse mode until the final energy supply was reached. During ultrasonic emulsification, the horn microtip was immersed in the pre-emulsion and the cavitation parameters (amplitude, temperature, energy) were adjusted through the device software. The compositions and experimental parameters for ultrasonic emulsification are presented in Table 1 where the samples (formulations F1-F22) were able to be systematically compared. It was notably that formulations F5 and F6 were the same and alternatively represented as formulation (I) and formulations F8, F14, F16 and F21 were the same and represented as formulation (II).

Measurement of droplet size and zeta potential: Size and size distribution of the oil droplets were determined by the dynamic light scattering method using Zetasizer NanoZS (ZEN3600, Malvern, UK) equipped with 4 mW He-Ne laser (633 nm), automatic laser attenuator and avalanche photodiode detector. The measurement was performed at a scattering angle of 173° at 25°C . In order to eliminate multiple scattering effects, samples were diluted with deionized water to obtain a droplet concentration of less than 0.04% prior to a measurement. The size and size distribution of the emulsions were calculated by the cumulant method using the software provided with the instrument. The droplet size was analyzed and referred as the hydrodynamic diameter while the width of size distribution was reported as Polydispersity Index (PDI). The zeta potential analysis which determined the surface charges surrounding the droplets was determined by laser doppler electrophoresis method using the same light scattering apparatus. All experiments were reported as averages of three measurements.

Stability test of nanoemulsions: Nanoemulsions containing oil concentrations of 5% (w/v) (formulation F21) and 10% (w/v) (formulation F22) were kept at ambient temperature in light-protected and tightly sealed container. They were visually observed for any signs of instability such as creaming and oil separation and were evaluated for changes in size, size distribution and zeta potential after storage for 15, 30, 60 and 90 days using the previously described methods. All results were recorded as averages of three measurements.

RESULTS

Preparation of nanoemulsions by ultrasonic emulsification: The sample formulations F1-F22 (Table 1) were prepared and emulsified by ultrasonic cavitation. The hydrodynamic size and Polydispersity Index (PDI) of all nanoemulsions were evaluated. The effect of WPI concentrations (1-20% (w/v)) used in nanoemulsion formulations F1-F5 on droplet size and PDI is presented in Fig. 1a. The systems F1-F5 contained 5% (w/v) of oil and were prepared at the same

Table 1: Compositions (emulsifier and oil) and process parameters in ultrasonic emulsification of nanoemulsions

Formulation [#]	WPI (% w/v)	Oil (% w/v)	Amplitude (%)	Energy (J)	Temperature (°C)
F1	1				
F2	5				
F3	10	5	20	1250	45
F4	15				
F5 (I)	20				
F6 (I)				1250	
F7				1500	
F8 (II)	20	5	20	1750	45
F9				2000	
F10				2250	
F11					30
F12					35
F13	20	5	20	1750	40
F14 (II)					45
F15					50
F16 (II)			20		
F17			25		
F18	20	5	30	1750	45
F19			35		
F20			40		
F21 (II)	20	5	20	1750	45
F22	20	10	20	1750	45

[#]Formulations F5 and F6 were the same and alternatively labeled as (I), Formulations F8, F14, F16, F21 were the same and alternatively labeled as (II)

ultrasonic condition, 20% amplitude, energy of 1250 Joules (J) and at 25°C. From the results, the higher amount of WPI could bring about the smaller size of nanoemulsions. In addition, at higher concentrations of WPI, less in PDI values were observed indicating more homogeneity in size of nanoemulsions. The formulation containing 5% (w/v) oil and 20% (w/v) WPI was selected for further studies on the effect of process parameters (energy, amplitude and temperature) on size and size distribution of nanoemulsions. Nanoemulsion formulations F6-F10 were prepared at different supply energy from 1250-2250 J, 20% amplitude and 45°C. Various energy levels in ultrasonic emulsification seemed not to be directly related to the droplet sizes and could produce nanoemulsions with a size range of 225-320 nm and PDI between 0.2-0.3 (Fig. 1b). However, from the result the smallest size of nanoemulsion was seen when energy of 1750 J was supplied. Hence, other process parameters for producing nanoemulsions, namely temperature and amplitude were studied at 1750 J. Accordingly, the ultrasonic emulsifying temperatures varied from 30 to 55°C with an interval of 5°C were used in formulations F11-F15 while the % amplitudes were set at 20, 25, 30, 35 and 40% for formulations F16-F20, in orderly. Figure 1c-d illustrates the effects of temperature and amplitude, respectively, on the particle sizes and PDI. The temperatures of 40-45°C yielded the nanoemulsions with the particle sizes of less than 300 nm (Fig. 1c). In addition, the smallest size of 225.83±1.52 nm and least PDI of 0.24±0.01 were obtained at ultrasonic temperature of 45°C. It demonstrated that an increase in temperature caused the emulsions formed with the smaller sizes and less size distribution; however, when the temperature reached 50°C, the larger sizes and more polydispersity were observed. Conversely, an increase in amplitude level resulted in larger particle size and broader size distribution (Fig. 1d). In

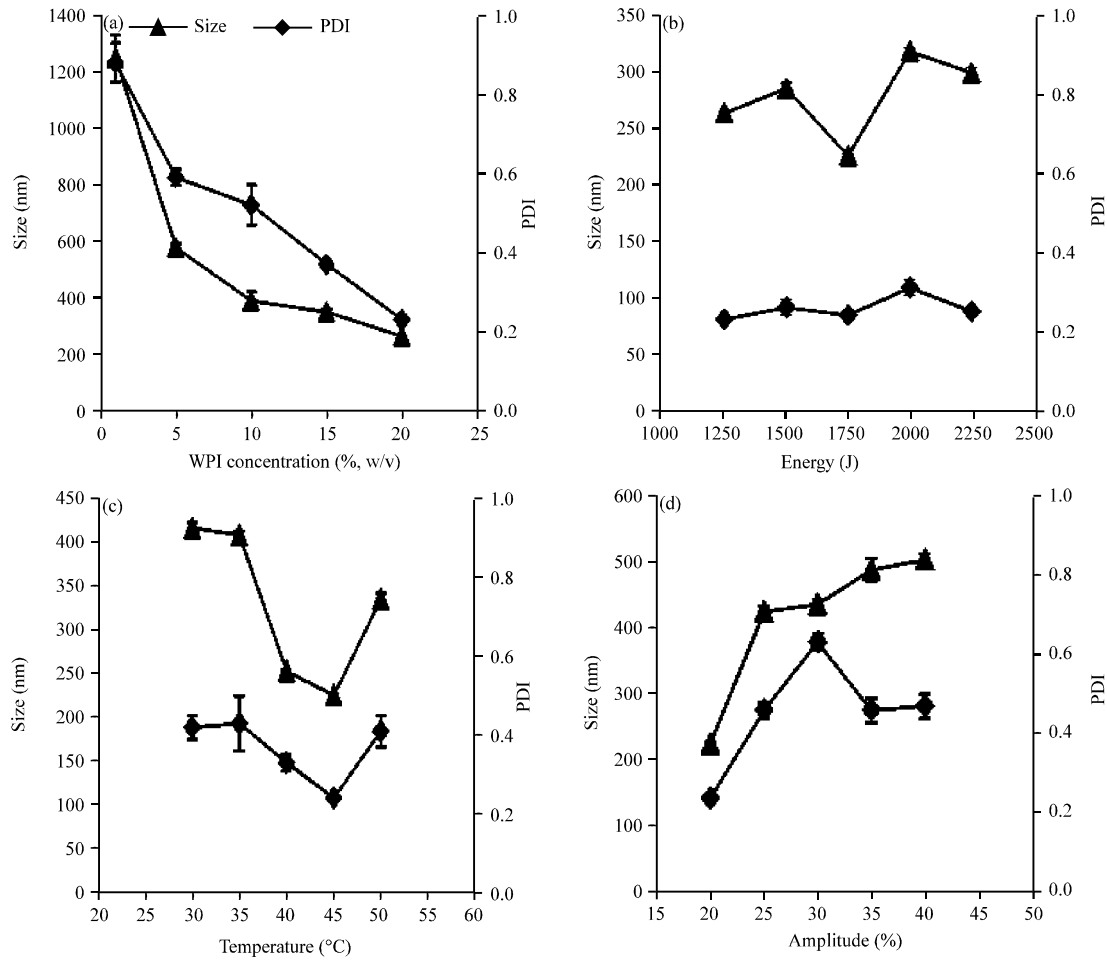


Fig. 1(a-d): Changes in size and PDI of WPI-stabilized nanoemulsions forming with 5% (w/v) oil and 20% (w/v) WPI as a function of (a) WPI concentration, or ultrasonic parameters (b) Energy (c) Temperature and (d) Amplitude. Each value is the Mean±SD, n = 3

comparison, amplitude of 20% was optimal to produce the nanosized emulsion. It was notably that all of the formulations studied had the zeta potential ranging from -30 to -40 mV (data not shown) which represented the negative charges of WPI-stabilized nanoemulsions. From the overall results, the optimized process parameters of ultrasonic emulsification as 20% amplitude, temperature of 45°C and energy of 1750 J were chosen for preparation of nanoemulsions for further stability study.

Stability test of nanoemulsions: Nanoemulsions containing either oil concentrations of 5% (w/v) (formulation F 21) or 10% (w/v) (formulation F 22) were prepared with the ultrasonic conditions as mentioned above. They were determined for any signs of instability and changes in particle size, size distribution and zeta potential after storage for 15, 30, 60 and 90 days compared to corresponding freshly prepared samples. It was mentioned that 2-fold higher in amount of oil phase led to larger sizes of newly made nanoemulsions, 225.83±1.52 nm and 271.30±2.49 nm for those

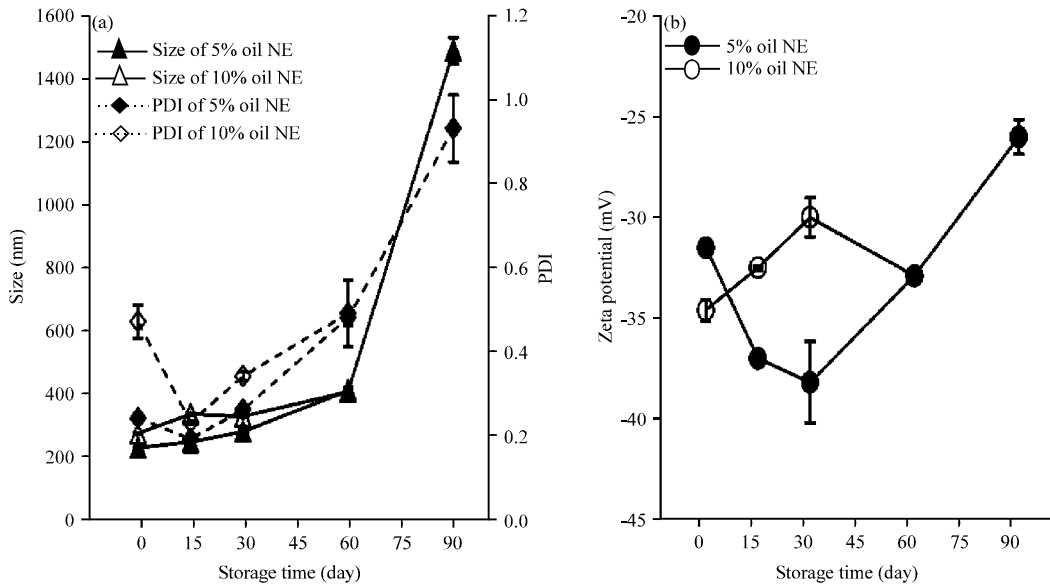


Fig. 2(a-b): Changes in stability; (a) Droplet size and PDI and (b) Zeta potential, of optimized WPI-stabilized nanoemulsions (NE) containing different oil concentrations as a function of storage time. Each value is the Mean \pm SD, n = 3

containing 5 and 10% (w/v) oil, in consecutively. From the results, no obvious instability of nanoemulsions containing 5% (w/v) oil was visually observed but the sizes of nanoemulsions tended to increase after being stored for 60 days (Fig. 2a). Unexpectedly, the sizes of 90 day old nanoemulsions containing 5% (w/v) oil abruptly increased to 1487 ± 41.78 nm. For nanoemulsions containing 10% oil, the sign of instability, oil separation in particular, was visually examined only in samples kept for 90 days. An increase in droplet sizes with longer time storage was also observed for 10% nanoemulsions (Fig. 2b). The higher PDI of both emulsions were obtained when increasing storage time and corresponded to the larger particle sizes (Fig. 2a). A sharp increase in PDI to 0.93 ± 0.08 was seen in 90 day old nanoemulsions containing 5% (w/v) oil indicating that the particles were non-monodispersed. In addition, the higher amount of zeta potential (normally -30 to -40 mV) would provide more electrostatic repulsive force between the droplets, thus resulting in more stability of the systems (He *et al.*, 2011). Regarding to the results, the zeta potentials between -30 to -40 mV were found in nanoemulsions containing either 5 or 10% (w/v) oil kept until 60 days (Fig. 2b). A decrease in zeta potential to -26.10 ± 0.85 mV was observed for 5% nanoemulsions determined after 90 days whereas the 10% nanoemulsions were definitely unstable. The changes in zeta potential of nanoemulsions after storage were correspondent to those of size and polydispersity in which the storage time could decline the stability.

DISCUSSION

The present study was the first report on preparation of WPI-stabilized nanoemulsions containing *E. plantagineum* seed oil. The nanoemulsions comprising of a food-grade emulsifier, WPI and the oil rich in omega-3 fatty acids would provide advantages for food, nutraceutical and other medicinal applications. The method of ultrasonic cavitation was used to emulsify the samples due to the lower cost of instrument and fewer occurrence of droplet coalescence as compared to other

high energy emulsification methods such as microfluidization (Jafari *et al.*, 2007). Since, the formation of emulsion in nano-scale was known to depend upon the compositions and the process of ultrasonic emulsification (Jafari *et al.*, 2008), the experimental work was required to determine the effect of such parameters.

For nanoemulsions prepared using the same condition of ultrasonic cavitation, varying quantity of compositions either WPI (F1-F5) or oil (F21-F22) led to differences in nanoparticle sizes. For WPI-stabilized nanoemulsions, smaller sizes were seen upon either increasing amount of WPI or lowering oil concentration. It has been known that the main protein components in WPI are β -lactoglobulin (LG) and α -lactalbumin (LB). These globular milk proteins are amphiphilic in nature, allowing them to adsorb at the oil-water interface and to reduce the interfacial tension in order to form colloidal aggregates (McClements, 2004; Nicorescu *et al.*, 2008; He *et al.*, 2011). The results indicated that at constant energy input of cavitation, droplet sizes of WPI-stabilized nanoemulsions were dependent on emulsifier concentrations. Increasing WPI concentration meant having sufficient emulsifier to completely adsorb at the oil-water interface of the droplet (McClements, 2004). Moreover, an increase in oil phase level was attributed to more interfacial surface area and larger droplet sizes (He *et al.*, 2011). The current finding agreed with other previous work in that sizes of protein-stabilized nanoemulsions reduced at higher protein concentration and less oil volume (He *et al.*, 2011). Moreover, the same trend in that narrower size distribution of WPI-stabilized nanoemulsions upon increasing WPI concentrations or decreasing oil concentrations was also observed.

The effect of parameters in ultrasonic cavitation, namely energy input, temperature and amplitude was systematically studied in nanoemulsions containing 5% oil and 20% WPI (F6-F20). Each parameter was determined at constant values of another two parameters. The smallest sizes of nanoemulsions (F6-F10) were obtained when supply energy of 1750 J was used and it was not possible to obtain any further decrease in particles size at energy beyond this point. In fact, when amount of emulsifier is sufficient or excess, the droplet size is controlled by energy input instead of emulsifier (McClements, 2004). Some studies suggested that there was optimum level of energy for producing the smaller emulsion droplet sizes (Jafari *et al.*, 2007). The energy beyond the optimum value would possibly cause the droplet re-coalescence and the bigger sizes of nanoparticles.

Based on the ultrasonic Energy (E) is a function of acoustic Power (P) and the time of exposure (T), $E = P \times T$ and different amount of power is controllable by varying %amplitudes (Hielscher, 2006). The effect of amplitude was determined for any changes in nanoparticle sizes and polydispersity. The result clearly showed that at constant energy (1750 J) the ultrasonic amplitude of 20% (150 watts) led to smaller sizes of nanoemulsions. It was unexpected since the higher amplitudes were reported to form smaller albumin microsphere (Han *et al.*, 2008). Unfortunately, however, the sizes of microsphere were found in broad distribution and the acoustic energy was inconsistent, making some uncertainty in explanation of amplitude effect on emulsion sizes. Recently, Tang *et al.* (2012) reported that for ultrasonically formed nanoemulsions stabilized by polyoxy-35-castor oil surfactant (Cremephore EL[®]), there was optimum power amplitude which gave maximum effect on emulsification. Importantly, the amplitude beyond the optimum level was able to generate the local turbulence and shear flow field around the microtip sonotrode; thereby facilitating the droplet coalescence and marked increase in size distribution (Tang *et al.*, 2012). Accordingly, the ultrasonic amplitude of 20% was probably optimum for preparation of nanoemulsions in this study. Besides, it was not impossible to explain that the lower amplitude

required longer emulsification time, thus allowing WPI to better adsorb at the interfacial area of droplet surface (Tang *et al.*, 2012). It was mentioned that the relationship between PDI and process parameters studied was noticeable when varying ultrasonic amplitudes in that the higher PDI (above 0.4) presented at higher amplitudes, 25-40%.

A reduction in particle sizes were found at raised ultrasonic temperature with an exception that the temperature was below 50°C. Generally, the emulsifying properties of LG and LB are affected by the thermal condition. The temperatures above thermal denaturation temperature (T_m) of proteins ($T_m \sim 70^\circ\text{C}$) cause protein unfolding which supports intermolecular aggregation by either via disulfide bridges or hydrophobic attraction, leading to protein denaturation and eventually destabilization of emulsions (McClements, 2004; Nicorescu *et al.*, 2008). Although, from the results, emulsifying temperatures were lower than the T_m and was unlikely to assume surface denaturation. However, some previous experiment showed an increase in particle sizes of WPI-stabilized β -carotene nanodispersions after heating at 60°C (Chu *et al.*, 2008). Alternatively, in view of ultrasonic emulsification, raise in temperature produces more ultrasonic nuclei and less pressure at the implosive collapse of bubbles; thereby diminishing the emulsifying efficiency (Tang *et al.*, 2012).

Using optimized ultrasonic process of 1750 J, 20% amplitude and 45°C, the smallest sizes of WPI-stabilized nanoemulsions, 225.83±1.52 nm (PDI = 0.24±0.01) and 271.30±2.49 nm (PDI = 0.47±0.04), were obtained for those containing 5 and 10% (w/v) oil, respectively. All formulations were stable and remained in nanometric size range of 200-400 nm unless the storage time was over 60 days. The 90-day-old nanoemulsions underwent a dramatic increase in sizes and less zeta potential (for 5% nanoemulsions) and instability (for 10% nanoemulsions). Truly, the globular proteins, LG and LB, require a period of time to gradually change their conformation after adsorption at an oil-water interface or so-called as surface denaturation (McClements, 2004). The extent of conformation change is the protein unfolding which promotes the droplet flocculation and destabilization. Moreover, the driven force for surface denaturation also includes the pH which is close to isoelectric point of protein, 5.2 for LG and 4.1 for LB (Koupantsis and Kiosseoglou, 2009; He *et al.*, 2011). However, it was not the case in the present work since all nanoemulsions had nearly neutral pH (pH~6.0-7.0) which was considerably avoidable in the occurrence of droplet coalescence. In addition, the pH values were unchanged after being kept for duration of experiment (data not shown). It could be seen that at neutral pH, the repulsive electrostatic interaction arisen from negative charges of WPI could diminish the intermolecular attraction from hydrophobic and/or disulfide bond formation between droplets. The (absolute) values of zeta potentials above 30 mV represent better stability and uniformity of the colloidal aggregates (He *et al.*, 2011). The results showed that nanoemulsions aged not over 60 days had the zeta potentials of at least -30 mV, indicating stability of the systems. In contrast, value of less than -30 mV was measured in nanoemulsions after storage for 90 days. It was plausible owing to the depletion of WPI molecules adsorbed around the droplet which caused less electrostatic repulsive force and droplet coalescence as seen from the results a significant increase in droplet size and extremely broad size distribution.

CONCLUSION

This study demonstrated that ultrasonic emulsification was the promising method for preparation of stable WPI-stabilized nanoemulsions incorporating omega-3 oil from plant seed. Not only emulsifier contents but also process parameters (energy input, amplitude and temperature)

had effects on emulsifying properties of WPI at the oil-water interfacial area and thus droplet size and size distribution of nanoemulsions. The reduction in droplet diameter could be controlled via the optimization of such parameters. The nanoemulsions obtained from this study could be additionally studied for their efficiency as nanocarriers in wide applications.

ACKNOWLEDGMENTS

This research was supported in part by the Faculty of Graduate Studies, Chulalongkorn University, the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (project code: HR1166I-55).

REFERENCES

- Acosta, E., 2009. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Curr. Opin. Colloid Interface Sci.*, 14: 3-15.
- Berti, M., B.L. Johnson, S. Dash, S. Fischer, R. Wilckens and F. Hevia, 2007. Echium: A Source of Stearidonic Acid Adapted to the Northern Great Plains in the US. In: *Issues in New Crops and New Uses*, Janick, J. and A. Whipkey (Eds.). American Society for Horticultural Science Press, Alexandria, pp: 120-125.
- Canselier, J.P., H. Delmas, A.M. Wilhelm and B. Abismail, 2002. Ultrasound emulsification: An overview. *J. Dispersion Sci. Technol.*, 23: 333-349.
- Chu, B.S., S. Ichikawa, S. Kanafusa and M. Nakajima, 2008. Stability of protein-stabilised α -carotene nanodispersions against heating, salts and pH. *J. Sci. Food Agric.*, 88: 1764-1769.
- De Vos, P., M.M. Faas, M. Spasojevic and J. Sikkema, 2010. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *Int. Dairy J.*, 20: 292-302.
- Hagi, A., M. Nakayama, Y. Miura and K. Yagasaki, 2007. Effects of a fish oil-based emulsion on rat hepatoma cell invasion in culture. *Nutrition*, 23: 871-877.
- Han, Y., D. Radziuk, D. Shchukin and H. Moehwald, 2008. Stability and size dependence of protein microspheres prepared by ultrasonication. *J. Mater. Chem.*, 18: 5162-5166.
- He, W., Y. Tan, Z. Tian, L. Chen, F. Hu and W. Wu, 2011. Food protein-stabilized nanoemulsions as potential delivery systems for poorly water-soluble drugs: Preparation, *in vitro* characterization and pharmacokinetics in rats. *Int. J. Nanomed.*, 6: 521-533.
- Hielscher, T., 2006. Ultrasonic production of nano-scale dispersions and emulsions. *Proceedings of the European Nanosystems Conference*, December 14-16, 2005, Grenoble, France, pp: 138-143.
- Huang, Q., H. Yu and Q. Ru, 2010. Bioavailability and delivery of nutraceuticals using nanotechnology. *J. Food Sci.*, 75: R50-R57.
- Jafari, S.M., Y. He and B. Bhandari, 2006. Nano-emulsion production by sonication and microfluidization: A comparison. *Int. J. Food Prop.*, 9: 475-485.
- Jafari, S.M., Y. He and B. Bhandari, 2007. Production of sub-micron emulsions by ultrasound and microfluidization techniques. *J. Food Eng.*, 82: 478-488.
- Jafari, S.M., E. Assadpoor, Y. He and B. Bhandari, 2008. Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloid*, 22: 1191-1202.
- Kentish, S., T.J. Wooster, M. Ashokkumar, S. Balachandran, R. Mawson and L. Simons, 2008. The use of ultrasonics for nanoemulsion preparation. *Innovative Food Sci. Emerg. Technol.*, 9: 170-175.
- Keowmaneechai, E. and D.J. McClements, 2002. Effect of CaCl_2 and KCl on physicochemical properties of model nutritional beverages based on whey protein stabilized oil-in-water emulsions. *J. Food Sci.*, 67: 665-671.

- Koupantsis, T. and V. Kiosseoglou, 2009. Whey protein-carboxymethylcellulose interaction in solution and in oil-in-water emulsion systems. Effect on emulsion stability. *Food Hydrocolloid*, 23: 1156-1163.
- Lee, S.J. and D.J. McClements, 2010. Fabrication of protein-stabilized nanoemulsions using a combined homogenization and amphiphilic solvent dissolution/evaporation approach. *Food Hydrocolloid*, 24: 560-569.
- McClements, D.J., 2004. Protein-stabilized emulsions. *Curr. Opin. Colloid Interface Sci.*, 9: 305-313.
- Nair, H.B., B. Sung, V.R. Yadav, R. Kannappan, M.M. Chaturvedi and B.B. Aggarwal, 2010. Delivery of antiinflammatory nutraceuticals by nanoparticles for the prevention and treatment of cancer. *Biochem. Pharmacol.*, 80: 1833-1843.
- Nicorescu, I., C. Loisel, C. Vial, A. Riaublanc, G. Djelveh, G. Cuvelier and J. Legrand, 2008. Combined effect of dynamic heat treatment and ionic strength on denaturation and aggregation of whey proteins-part I. *Food Res. Int.*, 41: 707-713.
- Perrier-Cornet, J.M., P. Marie and P. Gervais, 2005. Comparison of emulsification efficiency of protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill homogenization. *J. Food Eng.*, 66: 211-217.
- Qian, C. and D.J. McClements, 2011. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloid*, 25: 1000-1008.
- Solans, C., P. Izquierdo, J. Nolla, N. Azemar and M.J. Garcia-Celma, 2005. Nano-emulsions. *Curr. Opin. Colloid Interface Sci.*, 10: 102-110.
- Tang, S.Y., S. Manickam, T.K. Wei and B. Nashiru, 2012. Formulation development and optimization of a novel Cremophore EL-based nanoemulsion using ultrasound cavitation. *Ultrason. Sonochem.*, 19: 330-345.
- Wang, X., Y. Jiang, Y.W. Wang, M.T. Huang, C.T. Ho and Q. Huang, 2008. Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions. *Food Chem.*, 108: 419-424.
- Wendel, M., T. Rossela, S. Bergmannb, S. Ottoa and M. Ragallera *et al.*, 2007. Impact of total parenteral nutrition including omega-3 fatty acids on the regulation of plasma lipoproteins and glycemic control after major abdominal surgery. *e-SPEN Eur. e-J. Clin. Nutr. Metab.*, 2: 103-110.
- Wulff-Perez, M., A. Torcello-Gomez, M.J. Galvez-Ruiz and A. Martin-Rodriguez, 2009. Stability of emulsions for parenteral feeding: Preparation and characterization of o/w nanoemulsions with natural oils and Pluronic F68 as surfactant. *Food Hydrocolloid*, 23: 1096-1102.
- Yin, L.J., B.S. Chu, I. Kabayashi and M. Nakajima, 2009. Performance of selected emulsifiers and their combinations in the preparation of β -carotene nanodispersions. *Food Hydrocolloid*, 23: 1617-1622.