Production and Characterization of Beta-lactoglobulin/Alginate Nanoemulsion Containing Coenzyme Q₁₀: Impact of Heat Treatment and Alginate Concentrate

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Abstract

The aims of this research were to produce oil-in-water β -lactoglobulin/alginate (β -lg/Al) nanoemulsions loaded with coenzyme Q₁₀ and to investigate the combined effects of heating temperature and alginate concentration on the physico-chemical properties and encapsulation efficiency of β -lg/Al nanoemulsions. In β -lg/Al nanoemulsions production, various heating temperatures (60, 65, and 70°C) and alginate concentrations (0, 0.01, 0.03, and 0.05%) were used. A transmission electron microscopy was used to observe morphologies of β -lg/Al nanoemulsions. Droplet size and zeta-potential values of β -lg/Al nanoemulsions and encapsulation efficiency of coenzyme Q₁₀ were determined by electrophoretic light scattering spectrophotometer and HPLC, respectively. The spherically shaped β -lg/Al nanoemulsions with the size of 169 to 220 nm were successfully formed. The heat treatments from 60 to 70°C resulted in a significant ($p < 0.05$) increase in droplet size, polydispersity, zeta-potential value of β -lg/Al nanoemulsions, and encapsulation efficiency of coenzyme Q₁₀. As alginate concentration was increased from 0 to 0.05%, there was an increase in the polydispersity index of β -lg/Al nanoemulsions and encapsulation efficiency of coenzyme Q₁₀. This study demonstrates that heating temperature and alginate concentration had a major impact on the size, polydispersity, zeta-potential value and encapsulation efficiency of coenzyme Q₁₀ in β -lg/Al nanoemulsions.

Key words: β -lactoglobulin, alginate, nanoemulsion, coenzyme Q₁₀

Introduction

There is a growing interest in the application of nanoemulsions to dairy industry. Nanoemulsions are composed of small droplets, typically in the size of 20-200 nm (Solans *et al.*, 2005). Because of its small droplet size contributing to increased surface area available to interact with biological components, nanoemulsions can be used as an effective delivery system for bioactive components and has higher stability against coalescence and sedimentation compared with conventional emulsions (Bouchemal *et al.*, 2004; Solans *et al.*, 2005). Nanoemulsions can be manufactured with lower surfactant concentration (e.g., 4-8%) while conventional emulsions need a higher surfactant concentration (e.g., >20%) (Bouchemal *et al.*,

2004; Izquierdo *et al.*, 2002).

Coenzyme Q₁₀ (CoQ₁₀), also known as ubiquinone, has been found in virtually all cells of human body (Xia *et al.*, 2006). CoQ₁₀ is a lipid-soluble nutrient and contains many useful effects on human health since it functions as an antioxidant and can be used for energy production (Balakrishnan *et al.*, 2009; Crane, 2001). However, it is hard to use CoQ₁₀ for dairy food application due to its poor water solubility. The poor oral bioavailability and stability of CoQ₁₀ in food processing can be attributed to its low aqueous solubility (Balakrishnan *et al.*, 2009; Crane, 2001). To overcome this problem, lipid-based delivery systems, such as emulsions and liposomes, were thought to be a useful way for enhancing the solubility and bioavailability (Xia *et al.*, 2012).

When nanoemulsions can be practically used for dairy food applications, it should be developed with food-grade biopolymers (McClements *et al.*, 2007). In this study, β -lactoglobulin (β -lg) and alginate (Al) were used as food-grade biopolymers. β -lg, a small globular protein, consti-

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tutes about 50 to 60% of the amount of total whey protein in cow milk. β -lg has been widely used as a functional ingredient in food industry since it has GRAS (generally recognized as safe) status, high nutritional value, biodegradability, biocompatibility and ability to form gels and emulsions (Line *et al.*, 2005; Livney, 2010). However, β -lg, rigid globular protein, does not have considerable conformational changes at the oil and water interface in emulsion indicating that β -lg can be less surface active than flexible protein, such as casein (Damodaran, 1996; Schmidt *et al.*, 1984). When β -lg is heated above 60°C prior to emulsification, this globular protein undergoes partial denaturation, which can enhance the emulsifying properties and surface hydrophobicity of protein (Damodaran, 1996; Dannenberg and Kessler, 1988). It is widely believed that increasing entrapment efficiency of hydrophobic components in polymeric encapsulation systems can be attributed to an increase in hydrophobic interactions between biopolymers and hydrophobic components (Ishihara and Mizushima, 2010). Al, a non-toxic linear polysaccharide widely distributed in brown marine algae, consists of 1-4 linked β -D-mannuronic and α -L-guluronic acid unit (George and Abraham, 2006; Whistler and BeMiller, 1997). Sodium alginate has been extensively used as a stabilizer, thickener, and gelling agent in the food industry (Whistler and BeMiller, 1997; Yoo *et al.*, 2006). Sodium alginate can form a gel that has been called an “egg box” arrangement when sodium ions from glucuronic acid residue (G block) is exchanged with a divalent cation (George and Abraham, 2006; Whistler and BeMiller, 1997). Recently, it was reported that the formation of globular protein (β -lg) and polysaccharide (pectin) complex may confer better protection and stability for vitamin D₂ than single complex of vitamin D₂ and unprotected vitamin D₂ (Ron *et al.*, 2010; Zimet and Livney, 2009).

In this study, nanoemulsions (β -lg and Al food-grade biopolymer complex) were formed by the use of internal gelation method, which can be non-toxic and organic solvent free (Beaulieu *et al.*, 2002; Chen *et al.*, 2006). To prepare an effective food grade nanoemulsions to entrap CoQ₁₀, we hypothesized that heat treatment of β -lg, which may enhance the hydrophobicity, and surface coating of β -lg with Al, which can provide additional protection, may play an important role in the formation and physico-chemical properties of β -lg/Al nanoemulsions. As far as we know, the combined effects of heat treatment and Al concentration on the physico-chemical properties of β -lg/Al nanoemulsions made by internal gelation method have

not been reported. Therefore, the purposes of this study were to produce oil-in-water β -lg/Al complex nanoemulsions containing CoQ₁₀ and to investigate how manufacturing variables, which are heating temperatures and Al concentrations, affect the physico-chemical properties, such as morphology, droplet size, and zeta-potential, and encapsulation efficiency of CoQ₁₀.

Materials and Methods

Chemicals and reagents

β -lactoglobulin (β -lg), alginate (Al), calcium chloride (CaCl₂), polyoxyethylene (20) sorbitan monooleate (Tween 80), CoQ₁₀, fluorescent probe 1-anilino-naphthalene-8-sulfonate (ANS) were purchased from Sigma-Aldrich Inc. (St. Louis, USA).

Preparation of β -lg/Al nanoemulsions containing CoQ₁₀

β -lg/Al nanoemulsions containing CoQ₁₀ was prepared by a modified internal gelation method described in Beaulieu *et al.* (2002). β -lg solutions (0.98%, w/v) at pH 7.0 were pre-heated at 60, 65, 70°C for 10 min and then stored in a refrigerator about 5°C. Pre-heated β -lg solutions were then mixed with Al solutions to form β -lg/Al solutions. β -lg had a final concentration of 0.98% (w/v) while a final concentration of Al was 0, 0.01, 0.03, or 0.05% (w/v). Oil-in-water emulsion was prepared by mixing with 9 mL of β -lg/Al solutions containing tween 80 (5%, w/v) and 3 mL of grape seed oil containing CoQ₁₀ (0.5 mg/mL) at 600 rpm for 5 min using a homogenizer (Daihan scientific Co, Korea). This emulsion was then sonicated at preset ultrasonic powers using a probe-type ultrasonic sonicator (Bandelin Co, Germany): a first-stage power of 70 W, a second-stage power of 50 W, and a third-stage power of 30 W for 3 min, respectively. Forty eight microliters of 1 M CaCl₂ solution were added to 12 mL of sonicated emulsions (final concentration of CaCl₂: 4 mM) and vortexed for 5 min.

Morphological characteristics of β -lg/Al nanoemulsions

Morphological properties of β -lg/Al nanoemulsions were determined using a transmission electron microscopy (TEM) (FEI Tecnai 12, Philips, Netherlands). Twenty microliters of 10-fold diluted β -lg/Al nanoemulsions (or 10-fold dilution of β -lg/Al nanoemulsions) were deposited onto a 200 mesh copper grid coated with carbon. Samples were negatively stained with 2% uranyl acetate

solution for 15 s and then air-dried at room temperature. β -lg/Al nanoemulsions were examined at 120 kV.

Measurement of surface hydrophobicity of β -lg

Surface hydrophobicity (S_0) of β -lg was assessed by the modified method of Monahan *et al.* (1995). Thirty milligrams of β -lg were dispersed in 100 mL of 0.1 M citrate-phosphate buffer at pH 7.0. β -lg solutions were pre-heated at 60, 65, and 70°C for 10 min and cooled to room temperature. Pre-heated β -lg solutions were then diluted to 0.005, 0.01, 0.015, 0.02, and 0.025% (w/v), respectively. After dilution, 20 μ L of 8 mM ANS fluorescent probe was added to each diluted β -lg solutions (pH 7.0) and vortexed. The fluorescent intensities of reaction solutions were determined by the use of spectrofluorometer (Luminescence Spectrometer LS50 B, Perkin-Elmer Co, USA) with the excitation (390 nm) and emission (470 nm) wavelength. Both excitation and emission slit widths were set to 5 nm. The initial slope of relative fluorescence (R) values against β -lg concentration (% w/v) calculated by linear regression analysis was used as an index of β -lg surface hydrophobicity. The R value was assessed by following equation (Monahan *et al.*, 1995).

$$R = \frac{(F - F_0)}{F_0}$$

where F is the fluorescent intensity of β -lg solutions with ANS and F_0 is the fluorescent intensity of ANS solution without β -lg.

Emulsion droplet size and zeta-potential analysis

The electrophoretic light scattering spectrophotometer (ELS8000, Otsuka Electronics, Japan) was used to measure the droplet size and zeta-potential of β -lg/Al nanoemulsions. β -lg/Al nanoemulsions were diluted 10-fold with 1% (v/v) Tween 80 solution and transferred to an electrophoretic light scattering spectrophotometer. The droplet size and zeta-potential of nanoemulsions were determined at 20° and 90° scattering angles, respectively.

Encapsulation efficiency of CoQ₁₀

The encapsulation efficiency of CoQ₁₀ in β -lg/Al nanoemulsions was assessed using the modified method described by Kwon *et al.* (2002). β -lg/Al nanoemulsions containing CoQ₁₀ were ultra-centrifuged at 40,000 g for 1 h at 20°C using Optima XL-100K centrifuge (Beckman, USA). Supernatant (unencapsulated CoQ₁₀) was filtered through a 0.45 μ m syringe filter. CoQ₁₀ content in supernatant was measured using HPLC system consisting of a

Table 1. Experimental approach of β -lg/Al nanoemulsions

Manufacturing variables	Treatment
Heating temperature (°C)	60
	65
	70
Al concentration (% w/v)	0
	0.01
	0.03
	0.05

gradient pump (model 515, Waters Co., USA) with photodiode array detection (model 996, Waters Co., USA). The chromatographic separation of CoQ₁₀ was performed with a reverse phase C18 column (3.5 μ m, 4.6×150 mm, Xbridge C18, Waters Co., USA) at flow rate 1.2 mL/min. Methanol and ethanol (40:60) were used as mobile phase and detection wavelength was set at 275 nm. The amount of CoQ₁₀ in supernatant was calculated based on a standard calibration curve with a correlation coefficient (r) of 0.99 generated by plotting the peak area against CoQ₁₀ concentration (0.025 to 0.125 mg/mL, five levels). Encapsulation efficiency was calculated by following equation.

Encapsulation efficiency (%)

$$= \frac{\text{Total amount of CoQ}_{10} - \text{CoQ}_{10} \text{ in supernatant}}{\text{Total amount of CoQ}_{10}} \times 100$$

Statistical analysis

Effects of heating temperature and Al concentration on the droplet size, polydispersity, zeta-potential, and CoQ₁₀ encapsulation efficiency of β -lg/Al emulsions were studied using a two-factor (3×4) analysis of variance (ANOVA). Independent variables for this experiment were shown in Table 1. All statistical analyses were evaluated using the statistical analysis system (SAS Institute Inc., USA). Significance was established at $p < 0.05$. Each data was expressed as mean $\pm$ standard deviations and each experiment was performed in triplicate.

Results and Discussion

Formation and structure of β -lg/Al nanoemulsions

β -lg/Al nanoemulsions were formed by a modified internal gelation method with CaCl₂. Structures of nanoemulsions prepared with various heating temperatures and alginate concentrations are shown in Figs. 1 and 2. In micrographs obtained from TEM, spherically-shaped emulsions ranged in diameter from 169 to 220 nm were observed indicating that nanoemulsions are successfully formed

Fig. 1. Transmission electron micrographs of β -Ig/Al nanoemulsions treated at heating temperature 60 (A), 65 (B), or 70°C (C) for 10 min. Scale bar = 1 μ m. Black spot shown in micrographs presents the droplet of nanoemulsion.

Fig. 2. Transmission electron micrographs of β -Ig/Al nanoemulsions prepared with different Al concentration (0 (A), 0.01 (B), 0.03 (C), or 0.05 % (w/v) (D)) for 10 min. Scale bar = 1 μ m. Black spot shown in micrographs presents the droplet of nanoemulsion.

(Figs. 1 and 2). When pre-heated β -Ig solutions were mixed with Al solutions at pH 7.0 to form β -Ig/Al nanoemulsions, there were strong electrostatic repulsions

Fig. 3. Surface hydrophobicity of β -Ig heated at 60, 65, and 70°C for 10 min. Different letters on a column indicate significant differences ($p < 0.05$).

between β -Ig and Al since both β -Ig and Al are negatively charged at pH 7.0 (Harnsilawat *et al.*, 2006). When CaCl_2 was added to β -Ig and Al mixtures, electrostatic screening and ion bridges between Ca^{2+} and carboxyl groups of β -Ig and Al may decrease electrostatic repulsions between β -Ig and Al (Barbut and Foegeding, 1993; Chen and Subirade, 2006; George and Abraham, 2006). Ca^{2+} -mediated interactions between pre-heated β -Ig and Al occurred resulting in the formation of polymeric network, such as β -Ig/Al complex nanoemulsions (Chen and Subirade, 2006; George and Abraham, 2006; Hongsprabhas and Barbut, 1997).

Surface hydrophobicity of β -Ig

Impact of heat treatment on the surface hydrophobicity of β -Ig was investigated by the use of ANS probe method. The surface hydrophobicity of β -Ig was significantly ($p < 0.05$) increased with an increase in heat treatment from 60 to 70°C (Fig. 3). An increase in the surface hydrophobicity of β -Ig indicates that more hydrophobic residues present in the inside of native structure of β -Ig are exposed to aqueous environment during heat treatments since the hydrophobic residues on the protein surface can be attached by a fluorescence probe, ANS (Moro *et al.*, 2001). Therefore, heat treatment from 60 to 70°C resulted in a partial denaturation of β -Ig, which could lead to enhancing the surface hydrophobicity of β -Ig.

Droplet size and polydispersity of β -Ig/Al nanoemulsions

Two-factor ANOVA results (Table 2) present that heating temperature significantly ($p < 0.001$) affected the droplet size of β -Ig/Al nanoemulsions while Al concentration

had a no significant effect on the droplet size of nanoemulsions. As heating temperature increased from 60 to 70°C, the droplet size of β -lg/Al nanoemulsions was significantly ($p<0.05$) increased from 169.23 to 219.95 nm (Table 3). Keowmaneechai and McClements (2006) also reported that an increase in the droplet diameter of conventional oil-in water emulsions stabilized with whey protein isolate and CaCl_2 was observed with increasing heating temperature from 60 to 90°C. When β -lg was pre-heated from 60 to 70°C, a significant increase in the surface hydrophobicity of β -lg was observed (Fig. 3). Heat treatment of β -lg at $>55^\circ\text{C}$ also resulted in increasing free sulfhydryl groups exposed to aqueous environment (Lee *et al.*, 2008). An increase in surface hydrophobicity and exposed sulfhydryl groups may enhance interdroplet interactions between β -lg adsorbed at the fat globule interface and nonadsorbed β -lg in the continuous phase through hydrophobic attractions and intermolecular thiol-disulfide interactions (Euston *et al.*, 2000; Keowmaneechai and McClements, 2006; Raikos, 2010). It can increase the aggregation of nanoemulsion droplets resulting in an increase in the droplet size of β -lg/Al nanoemulsions with increasing heating temperature. No significant ($p<0.05$) differences in droplet size were observed in β -lg/Al nanoemulsions made with various alginate concen-

trations (Table 3). It indicates that Al concentration may not be a manufacturing variable to affect the aggregation of nanoemulsion droplets.

Both heating temperature ($p<0.001$) and Al concentration ($p<0.05$) had a significant effect on the polydispersity index value of β -lg/Al nanoemulsions (Table 2). It should be noted that heating temperature had a higher mean square value than that of alginate concentration indicating that heating temperature had a dominant effect on the polydispersity index value of β -lg/Al nanoemulsions (Table 2). Mean polydispersity index values of β -lg/Al nanoemulsions ranging from 0.11 to 0.26 were observed for all samples indicating that β -lg/Al nanoemulsions formed at pH 7.0 exhibited acceptable uniform (homogeneous) droplet distributions since the polydispersity index value was <0.3 (Dragicevic-Curic *et al.*, 2010; Yen *et al.*, 2010). Heat treatments from 60 to 70°C resulted in a significant increase in the polydispersity index value of β -lg/Al nanoemulsions (Table 3) indicating relatively broader droplet distributions were observed. The polydispersity index value of β -lg/Al nanoemulsions prepared with 0.05% Al concentration was higher than that of β -lg/Al nanoemulsions made with no Al concentration (Table 3). These results suggest that heat treatment and addition of Al in the formation of nanoemulsions, which may con-

Table 2. Analysis of variance for physic-chemical properties of β -lg/Al nanoemulsions

	Independent variables ¹⁾							
	Heating temperature			Al concentration			Heating temperature $\times$ Al concentration	
	df	MS		df	MS		df	MS
Droplet size (nm)	2	7741.83	***	3	331.60	NS	6	342.14
Polydispersity index	2	0.0738	***	3	0.0102	*	6	0.0030
Zeta-potential (mV)	2	21.06	*	3	2.88	NS	6	6.54
EE of CoQ ₁₀ (%) ²⁾	2	34.75	*	3	67.85	**	6	4.46

¹⁾Heating temperature $\times$ Al concentration = Interaction between heating temperature and Al concentration; df = degrees of freedom; MS = mean square

²⁾EE of CoQ₁₀ = Encapsulation efficiency of CoQ₁₀

*, **, ***, significantly different at $p<0.05$, $p<0.01$, and $p<0.001$, respectively

Table 3. Effects of heating temperature and Al concentration on the physicochemical properties of β -lg/Al nanoemulsions^{1), 2)}

	Heating temperature ($^\circ\text{C}$)				Al concentration (% w/v)				
	60	65	70	SEM ⁴⁾	0	0.01	0.03	0.05	SEM
Droplet size (nm)	169.23 ^c	192.20 ^b	219.95 ^a	3.976	188.71 ^a	200.70 ^a	188.68 ^a	197.08 ^a	4.591
Polydispersity index	0.11 ^b	0.14 ^b	0.26 ^a	0.016	0.13 ^b	0.16 ^{ab}	0.16 ^{ab}	0.21 ^a	0.019
Zeta-potential (mV)	-6.82 ^a	-7.36 ^{ab}	-8.77 ^b	0.485	-7.16 ^a	-8.42 ^a	-7.30 ^a	-7.73 ^a	0.560
EE of CoQ ₁₀ (%) ³⁾	75.38 ^b	77.50 ^{ab}	78.75 ^a	0.907	73.49 ^b	77.24 ^a	78.07 ^a	80.04 ^a	1.048

¹⁾Values with different letters in the same row denote significant differences ($p<0.05$).

²⁾All levels of one variable were used to assess means for levels of other variables.

³⁾EE of CoQ₁₀ = Encapsulation efficiency of CoQ₁₀

⁴⁾SEM: Standards error of means

tribute to the aggregation of nanoemulsion droplets, can be a manufacturing variable to affect homogenous droplet distributions observed in β -lg/Al nanoemulsions.

Zeta-potential of β -lg/Al nanoemulsions

Two-factor ANOVA results (Table 2) show that heating temperatures had a significant ($p < 0.05$) effect on the zeta-potential of β -lg/Al nanoemulsions. The zeta-potential values of all β -lg/Al nanoemulsions were between -6.82 mV and -8.77 mV indicating that β -lg/Al nanoemulsions had negative surface charges (Table 3). Since the isoelectric point (PI) of β -lg is around pH 5 and pKa of Al is around pH 3.5 (Harnsilawat *et al.*, 2006; Line *et al.*, 2005), it can be expected that β -lg/Al nanoemulsions formed at pH 7 had negative surface charges. The zeta-potential values of β -lg/Al nanoemulsions were significantly ($p < 0.05$) increased with an increase in heating temperature from 60 to 70°C (Table 3). Similar results were reported in β -lg dispersions heated at 85°C for 15 min (Schmitt *et al.*, 2009). As seen in Fig. 3, the heat treatment of β -lg from 60 to 70°C induced the partial unfolding of β -lg. Some negatively charged amino acid groups might be exposed to the surface of β -lg, which may contribute to an increase in the zeta-potential values of β -lg/Al nanoemulsions. However, the zeta-potential values of β -lg/Al nanoemulsions were not significantly ($p < 0.05$) affected by Al concentrations (Table 3).

Encapsulation efficiency of CoQ₁₀

Both heating temperature ($p < 0.05$) and Al concentration ($p < 0.01$) significantly affected the encapsulation efficiency of CoQ₁₀ in β -lg/Al nanoemulsions (Table 2). It was observed that alginate concentration was the pronounced factor influencing the encapsulation efficiency of CoQ₁₀ in β -lg/Al nanoemulsions as suggested by a higher mean square value (Table 2). There was a significant ($p < 0.05$) increase in the encapsulation efficiency of CoQ₁₀ from 75.38 to 78.75% as heating temperature was increased from 60 to 70°C (Table 3). An increase in the surface hydrophobicity of β -lg treated with increasing heating temperature from 60 to 70°C (Fig. 3) was due to the exposure of more hydrophobic groups to the surface of β -lg. Since β -lg had hydrophobic binding sites on its surface (Liang *et al.*, 2008), increasing heat treatment may provide more hydrophobic binding sites for CoQ₁₀, which may result in an increase in the encapsulation efficiency of CoQ₁₀. Furthermore, increasing free sulfhydryl groups exposed to aqueous environment during heat treatment (Lee *et al.*, 2008) may enhance the interfacial

polymerization of adsorbed β -lg through intermolecular thiol-disulfide interactions (Euston *et al.*, 2000; Keowmaneechai and McClements, 2006; Raikos, 2010). This may reduce the diffusion of CoQ₁₀ trapped in β -lg/Al nanoemulsions, which leads to increasing the encapsulation efficiency of CoQ₁₀.

The encapsulation efficiency of CoQ₁₀ in β -lg/Al nanoemulsions made with 0.01, 0.03, and 0.05% was significantly ($p < 0.05$) higher than that of β -lg/Al nanoemulsions made with no Al (Table 3). Ron *et al.* (2010) reported that the complex formation between β -lg and pectin provided additional protection against the escape of vitamin D₂ through enhancing immobilization effects. When β -lg solutions were mixed with Al solutions using CaCl₂ at pH 7.0, more junction zones were formed between β -lg and Al through Ca²⁺-mediated intermolecular interactions (Chen and Subirade, 2006; George and Abraham, 2006). Compared with nanoemulsions made without Al, the formation of associative β -lg and Al complex nanoemulsions to trap CoQ₁₀ could provide better immobilization for CoQ₁₀, which would decrease the diffusion of CoQ₁₀ resulting in increasing encapsulation efficiency of CoQ₁₀.

Conclusions

Spherically-shaped β -lg/Al complex nanoemulsions ranged in diameter from 150 to 200 nm were successfully produced. This study demonstrated that heating temperature and Al concentration were key manufacturing variables to influence the physico-chemical properties of β -lg/Al nanoemulsions. The use of heat treatment and Al can provide a useful tool to control the droplet size, surface charge, and encapsulation efficiency of CoQ₁₀ through enhancing surface hydrophobicity and immobilization effect.

Acknowledgements

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0013978; 2012R1A1A4A01013793) and by Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea (107072-3).

References

1. Balakrishnan, P., Lee, B. J., Oh, D. H., Kim, J. O., Lee, Y. I.,

- Kim, D. D., Jee, J. P., Lee, Y. B., Woo, J. S., Yong, C. S., and Choi, H. G. (2009) Enhanced oral bioavailability of Coenzyme Q10 by self-emulsifying drug delivery systems. *Int. J. Pharm.* **374**, 66-72.
2. Barbut, S. and Foegeding, E. A. (1993) Ca^{2+} -induced gelation of pre-heated whey protein isolate. *Food Sci.* **58**, 867-871.
3. Beaulieu, L., Savoie, L., Paquin, P., and Subirade, M. (2002) Elaboration and characterization of whey protein beads by an emulsification/cold gelation process: Application for the protection of retinol. *Biomacromolecule*. **3**, 239-248.
4. Bouchemal, K., Briancon, S., Perrier, E., and Fessi, H. (2004) Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. *Int. J. Pharm.* **280**, 241-251.
5. Chen, L., Remondetto, G. E., and Subirade, M. (2006) Food protein-based materials as nutraceutical delivery systems. *Trends Food Sci. Technol.* **17**, 272-283.
6. Chen, L. and Subirade, M. (2006) Alginate-whey protein granular microspheres as oral delivery vehicles for bioactive compounds. *Biomaterials* **27**, 4646-4654.
7. Crane, F. L. (2001) Biochemical functions of coenzyme Q₁₀. *J. Am. Coll. Nutr.* **20**, 591-598.
8. Damodaran, S. (1996) Amino acids, peptides and proteins. In: Food chemistry. Fennema, O. R. (3rd ed) Marcel Dekker, Inc., NY, pp. 321-429.
9. Dannenberg, F. and Kessler, H. G. (1988) Effect of denaturation of β -lactoglobulin on texture properties of set-style nonfat yoghurt. 1. Syneresis. *Milchwissenschaft* **43**, 632-635.
10. Dragicevic-Curic, N., Gräfe, S., Gitter, B., Winter, S., and Fahr, A. (2010) Surface charged temoporfin-loaded flexible vesicles: *In vitro* skin penetration studies and stability. *Int. J. Pharm.* **384**, 100-108.
11. Euston, S. R., Finnigan, S. R., and Hirst, R. L. (2000) Aggregation kinetics of heated whey protein-stabilized emulsions. *Food Hydrocolloid*. **14**, 155-161.
12. George, M. and Abraham, T. E. (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan - a review. *J. Control. Release* **114**, 1-14.
13. Harnsilawat, T., Pongsawatmanit, R., and McClements, D. J. (2006) Characterization of β -lactoglobulin-sodium alginate interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study. *Food Hydrocolloid*. **20**, 577-585.
14. Hongsprabhas, P. and Barbut, S. (1997) Protein and salt effects on Ca^{2+} -induced cold gelation of whey protein isolate. *J. Food Sci.* **62**, 382-385.
15. Ishihara, T. and Mizushima, T. (2010) Techniques for efficient entrapment of pharmaceuticals in biodegradable solid micro/nanoparticles. *Expert Opin. Drug Del.* **7**, 565-575.
16. Izquierdo, P., Esquena, J., Tadros, T. F., Dederen, C., Garcia, M. J. N., Azemar, and Solans, C. (2002) Formation and stability of nano emulsions prepared using the phase inversion temperature method. *Langmuir* **18**, 26-30.
17. Keowmaneechai, E. and McClements, D. J. (2006) Influence of EDTA and citrate on thermal stability of whey protein stabilized oil-in-water emulsions containing calcium chloride. *Food Res. Int.* **39**, 230-239.
18. Kwon, S. S., Nam, Y. S., Lee, J. S., Ku, B. S., Han, S. H., Lee, J. Y., and Chang, I. S. (2002) Preparation and characterization of coenzyme Q10-loaded PMMA nanoparticles by a new emulsification process based on microfluidization. *Coll. Surf. A: physicochem. eng. Aspects.* **210**, 95-104.
19. Lee, M. R., Nam, G. W., Choi, H. N., Yun, H. S., Kin, S. H., You, S. K., Park, D. J., and Lee W. J. (2008) Structure and chemical properties of beta-lactoglobulin nanoparticles. *J. Agric. Life Sci.* **42**, 31-36.
20. Liang, L., Tajmir-Riahi, H. A., and Subirade, M. (2008) Interaction of β -lactoglobulin with resveratrol and its biological implications. *Biomacromolecule*. **9**, 50-56.
21. Line, V. L. S., Remondetto, G. E., and Subirade, M. (2005) Cold gelation of β -lactoglobulin oil-in-water emulsions. *Food Hydrocolloid*. **19**, 269-278.
22. Livney, Y. D. (2010) Milk proteins as vehicles for bioactives. *Curr. Opin. Colloid Interface Sci.* **15**, 73-83.
23. McClements, D. J., Decker, E. A., and Weiss, J. (2007) Emulsion-based delivery systems for lipophilic bioactive components. *J. Food Sci.* **72**, R109-R124.
24. Monahan, F. J., German, J. B., and Kinsella, J. E. (1995) Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. *J. Agric. Food Chem.* **43**, 46-52.
25. Moro, A., Gatti, C., and Delorenzi, N. (2001) Hydrophobicity of whey protein concentrates measured by fluorescence quenching and its relation with surface functional properties. *J. Agric. Food Chem.* **49**, 4784-4789.
26. Raikos, V. (2010) Effect of heat treatment on milk protein functionality at emulsion interfaces. A review. *Food Hydrocolloid*. **24**, 259-265.
27. Ron, N., Zimet, P., Bargarum, J., and Livney, Y. D. (2010) Beta-lactoglobulin-polysaccharide complexes as nanovehicles for hydrophobic nutraceuticals in non-fat foods and clear beverages. *Int. Dairy J.* **20**, 686-693.
28. Schmitt, C., Bovay, C., Vuilliomenet, A. M., Rouvet, M., Bovoetto, L., Barbar, R., and Sanchez, C. (2009) Multiscale characterization of individualized β -lactoglobulin microgels formed upon heat treatment under narrow pH range conditions. *Langmuir* **25**, 7899-7909.
29. Schmidt, R. H., Packard, V. S., and Morris, H. A. (1984) Effect of processing on whey protein functionality. *J. Dairy Sci.* **67**, 2723-2733.
30. Solans, C., Izquierdo, P., and Nolla, J. (2005) Nano-emulsions. *Curr. Opin. Colloid Interface Sci.* **10**, 102-110.
31. Whistler, R. L. and BeMiller, J. N. (1997) Alginates. In: Carbohydrate chemistry for food scientists. Eagan press, MN, pp. 195-202.
32. Xia, F., Jin, H., Zhao, Y., and Guo, X. (2012) Preparation of coenzyme Q₁₀ liposomes using supercritical anti-solvent technique. *J. Microencapsul.* **29**, 21-29.
33. Xia, S., Xu, S., and Zhang, X. (2006) Optimization in the preparation of Coenzyme Q₁₀ nanoliposomes. *J. Agric. Food Chem.* **54**, 6358-6366.

-
34. Yen, F. L., Wu, T. H., Tzeng, C. W., Lin, L. T., and Lin, C. C. (2010) Curcumin nanoparticles improve the physicochemical properties of curcumin and effectively enhance its antioxidant and antihepatoma activities. *J. Agric. Food Chem.* **58**, 7376-7382.
35. Yoo, S. H., Song, Y. B., Chang, P. S., and Lee, H. G. (2006) Microencapsulation of α -tocopherol using sodium alginate and its controlled release properties. *Int. J. Biol. Macromol.* **38**, 25-30.
36. Zimet, P. and Livney, Y. D. (2009) Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for ω -3 polyunsaturated fatty acids. *Food Hydrocolloid.* **23**, 1120-1126.
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- (Received 2012.11.9/Revised 2013.1.31/Accepted 2013.2.6)