Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.09517

Optimized conditions to produce water-in-oil-in-water nanoemulsion and spray-dried nanocapsule of red ginseng extract

Ji-Young MIN[1], Sung-Il AHN[2], Yun-Kyung LEE[3], Hae-Soo KWAK[1], Yoon Hyuk CHANG[3]*

Abstract

The aim of this study was to optimize conditions for producing water-in-oil-in-water (W/O/W) nanoemulsion and spray-dried nanocapsule of red ginseng extract. Based on emulsion stability and average particle size analysis, optimum conditions to produce W/O/W nanoemulsion were: ratio of core to coating material, 3:7; primary emulsifier, 10% polyglycerol polyricinoleate; ratio of W/O phase to coating material, 1.5:8.5; secondary emulsifier, 10% polyoxyethylene sorbitan monolaurate; solvent, 20% ethyl acetate; secondary coating material, 10% maltodextrin (MD). Under these optimized conditions, particle size of spray-dried nanocapsules coated with MD of red ginseng ranged from 100 nm to 500 nm, based on response surface methodology with the highest yield (up to 99.7%). Results of the present study can be used to further optimize conditions to produce W/O/W nanoemulsion and spray-dried nanocapsule of red ginseng extract.

Keywords: red ginseng extract; nanoemulsion; spray-dried nanocapsule; response surface methodology.

Practical Application: W/O/W nanoencapsules of red ginseng extract can have practical application as ingredient in beverages.

1 Introduction

Nanoemulsion system, one of the fast-growing techniques in food and pharmaceutical industries, is utilized to encapsulate, protect and deliver the functional ingredients for health benefits (McClements & Rao, 2011). This technology has been recognized as a prospective delivery system for bio-functional products. Having remarkably lower droplet sizes (<200 nm), nanoemulsions have more benefits than conventional emulsions, such as improved stability against aggregation of particle and gravitational separation, enhanced optical property and 46 superior bioavailability of nutraceutical components (Tadros et al., 2004; Acosta, 2009). Nanoemulsion generally consists of a heterogeneous system with lipid and aqueous phase which is stabilized by using emulsifiers (Sharma et al., 2010).

Among the methods for nanoemulsion preparation, a solvent evaporation method is most popular because it may produce droplets which ranged from a few to hundreds of nanometer by disrupting the oil and aqueous phases. This method involves the homogenization of an organic phase containing lipid and an organic solvent with an aqueous phase containing water and an emulsifier, which followed by the evaporation of the organic solvent from the lipid droplets which occurs the droplets to shrink in size (Lee & McClements, 2010). The solvent evaporation technique has already been successfully conducted to manufacture ß-carotene nanoemulsions as food grade ingredients (Tan & Nakajima, 2005; Chu et al., 2007). Ethyl acetate is usually used as a solvent for the solvent evaporation method, and Food and Drug Administration of USA declares ethyl acetate as being generally recognized as safe (GRAS) for application in foods and beverages 59 (Lee & McClements, 2010).

Water-in-oil (W/O) emulsion is practiced in the food industry as a method of microencapsulation. However, W/O emulsion is not stable during storage, and it cannot be converted into a powder form (Benichou et al., 2001; Gharsallaoui et al., 2007). Therefore, in the present study used the water-in-oil-in-water (W/O/W) emulsion because it can be a longer storage life and dried into a powder form. Additionally, W/O/W emulsion can easily be increased with bioavailability of red ginseng extract (Matos et al., 2014).

In general, spay drying is economical and flexible, readily available equipment and highly qualified for W/O/W emulsion. The shape and type of capsule formed are influenced by procedure of emulsification with core and coating materials and drying process (Rosenberg et al., 1990). Recently, Pérez-Masiá et al. (2015) reported that spray drying is very efficient for the production of nanocapsule of folic acid in food hydrocolloids.

Ginseng has been used as a traditional medicine in the East Asian countries for several centuries (Kang et al., 2006) and is one of the most widely used herbal medicines in the world until now (Ernst, 2010).

Red ginseng, which is prepared by steaming and drying of fresh ginseng, is claimed to possess more bioactive substances than

Received 15 Mar., 2017
Accepted 06 Feb., 2018
[1] *Department of Food Science and Technology, Sejong University, Seoul, 05006, Korea*
[2] *Program of Animal Products and Food Science, College of Animal Life Sciences, Kangwon National University, Kangwon, 24341, Korea*
[3] *Department of Food and Nutrition, KyungHee University, Seoul, 02447, Korea*
*Corresponding author: yhchang@khu.ac.kr

both fresh and white ginsengs because of the chemical changes that occur during processing (Park, 1996). It have a number of active ingredients, including anti-inflammatory (Song et al., 2009), anticancer (Chae et al., 2009), antitumor (Mochizuki et al., 1995), antidiabetic (Ni et al., 2010), antihypertensive (Stavro et al., 2005), antifatigue (Lee et al., 2005), and antioxidative effects (Liu et al., 2003). Even though the presence of a number of active ingredients in red ginseng extract is noteworthy, the level of bioavailability of those substances needs to be further improved to be utilized for human health benefit.

Recent reports suggested that the formulation of nanoemulsion and powdered nanocapsule can remarkably improve the bioavailability of the functional ingredients (Ragelle et al., 2012; Zhao et al., 2013). Furthermore, Ganesan et al. (2015) reported that nano bioactive compounds from red ginseng are effective to chronic diseases since it has positive role in antidiabetic, aphrodisiac, cardiovascular and protective role in neurodegenerative diseases. Therefore, the production of nanoemulsion and spray-dried nanocapsule of red ginseng extract might be an effective way to enhance the bioavailability of its active ingredients. However, there is extremely limited information in the literature on W/O/W nanoemulsion and spray-dried nanocapsule of red ginseng extract, as far as the authors are aware. Thus, the objective of the present study was to establish the optimum conditions for the production of W/O/W nanoemulsion and spray-dried nanocapsule of red ginseng extract using the high energy emulsification-evaporation method.

2 Materials and methods

2.1 Materials

Red ginseng extract as a core material was provided by Korea Bio Red Ginseng. Co., Ltd. (Geumsan, Korea). Medium-chain triglyceride (MCT), the primary coating material, was purchased from Wellga Co., Ltd. (Seongnam, Korea). As the secondary coating materials, maltodextrin (MD, DE 18, Samyang Genex, Seoul, Korea), arabic gum (AG, Samchun Pure Chemical Co., Ltd., Pyeongtaek, Korea) or whey protein concentrate (WPC, 80% protein content, Davisco Foods International, Eden Prairie, MN, USA) were used. As the emulsifiers, polyglycerol polyricinoleate (PGPR, HLB 0.6) and polyoxyethylene sorbitan monolaurate (PSML, HLB 16.7) (purity 95.0%) were provided from Il-Shin Co., Ltd. (Seoul, Korea). Other emulsifiers, such as polyglycerol fatty acid esters (PFAE, HLB 3, 4, 13 and 14.5) and sorbitan monooleate (HLB 4.3) (purity 95.0%) were obtained from Nam-Young Co., Ltd. (Seoul, Korea). Oil Red O was used as a marker compound and was obtained from Sigma–Aldrich (St. Louis, MO, USA). All chemicals were of reagent grade unless otherwise specified.

2.2 W/O/W nanoencapsulation of red ginseng extract

Red ginseng extract (the core material) was dissolved in distilled water (0.8 g/mL) and filtered through membrane paper (Whatman No. 4), and 0.02% sodium benzoate was added to the mixture for preventing the degradation of red ginseng extract.

The primary emulsion (W/O) of red ginseng extract was made with MCT as the primary coating material. The ratios of core to coating materials were 1:9, 2:8, 3:7 and 4:6 and various emulsifiers were used such as PGPR (HLB 0.6), PFAE (HLB 4) and sorbitan monooleate (HLB 4.3) with various concentrations (2, 4, 6, 8, and 10%). The primary coating material (MCT) was mixed with the emulsifiers at 400 rpm and 50 °C for 30 min. Then, core material and ethyl acetate (as a solvent in coating material and surfactant mixture) were mixed with coating materials. The core material to ethyl acetate with different ratios (9:1, 8:2, 5:5, 3:7, and 1:9, w/w) were prepared by mixing them using a magnetic stirrer. The mixture was homogenized by a high speed homogenizer (Matsushita Electric Industrial Co. Ltd., Tokyo, Japan) at 12,000 rpm for 5 min to produce the W/O emulsion.

The secondary emulsion (W/O/W) was prepared with W/O emulsions and various concentrations (10, 15, 20, 25 and 30%) of secondary coating materials, such as MD, WPC and AG. The ratios of W/O emulsion to coating materials were 1:7, 1:7.5, 1:8 and 1:8.5, and various emulsifiers were also used such as. PFAE (HLB 13), Tween 60 (HLB 14.9) and PSML (HLB 16.7) with various concentrations (2, 4, 6, 8 and 10%). The secondary coating materials were mixed with emulsifiers at 400 rpm and 50 °C for 30 min with a hotplate stirrer, and then W/O emulsions were added. The mixture was homogenized to form the W/O/W nanoemulsions by a high speed homogenizer at 12,000 rpm for 5 min.

Finally, ethyl acetate was evaporated by a rotary evaporator (Eyela, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) at 50 °C for 30 min. To produce spray-dried nanocapsule of red ginseng extract, W/O/W emulsions were spray-dried using a two-way, nozzle-spray dryer (Eyela SD-1000, drying capacity 1,500 mL/h; Tokyo Rikakikai Co. Ltd., Tokyo, Japan). Inlet- and outlet-air temperatures were set at 170 and 85 °C, respectively, and rotary atomizer was fixed at 14 × 10 kPa and blower rate were 0.60 ± 0.2 m^3/min. Finally, the dried powder was collected and stored in frosted airtight containers at 4 °C until further analysis.

2.3 Emulsion stability index (ESI)

The volumetric method was used to measure the emulsion stability index (ESI) for W/O/W nanoemulsions according to the procedure of Chang et al. (1994). The emulsion was stored at 100 °C for 2 h and the volume of the separation layer was calculated. All samples were measured in triplicate and ESI was calculated by the following Equation (1):

$$ESI\ (\%) = \left[1 - \left(volume\ of\ separation\ layer\ /\ total\ volume\ of\ emulsion\right)\right] \times 100 \quad (1)$$

2.4 Optimization of the conditions for producing spray-dried nanocapsule of red ginseng extract by response surface methodology (RSM)

Response surface methodology (RSM) was conducted to optimize the conditions for producing the spray-dried nanocapsules of red ginseng extract by faced central composite design (FCCD) with 4 variables. The variables and the ranges were the ratio of core material (red ginseng extract) to coating material (MCT) (X_1) from 2:8 to 3:7, PGPR (HLB 0.6) concentration (X_2) from 6 to 10%, ratio of W/O emulsion to coating material (MD) (X_3)

from 1:16 to 1:20, and PSML (HLB 16.7) concentration (X_4) from 6 to 10% (Table 1).

Thirty one experimental designs were produced with 4 factors and 3 coded levels by the principal of RSM using MINITAB Release 16 (Minitab Inc., State college, PA, USA). The encapsulation yield and particle size of the spray-dried nanocapsules were used following Equation (2):

$$Y = \beta_{k0} + \sum_{i=1}^{1} \beta k_i x_i + \sum_{i=1}^{1} \beta k_{ji} x_i^2 + \sum_{i=1}^{4} \sum_{j=1+1}^{1} \beta k_{ij} x_i x_j \quad (2)$$

where the encapsulation yield and particle size of the spray-dried nanocapsules are observed response value, βk_0, βk_i, βk_{ji}, and βk_{ij} are constant coefficients of intercept, linear, quadratic and interaction terms, respectively. X_i *and* X_j are uncoded independent variables (ratio of coating materials to core material and emulsifier concentration).

2.5 Encapsulation yield

Encapsulation efficiency (EY) was determined using Oil Red O as a marker compound by UV–visible spectrophotometry (Beckman coulter, inc., Fullerton, CA, USA). Prior to the encapsulation, 0.02% of Oil Red O was dissolved in MCT. The EY was measured by suspending samples (5 ml) in 10 mL of hexane (Baik et al., 2004). The suspensions were then mixed in a magnetic stirrer (HSD 150-03P; Misung Scientific Co., Ltd, Yangju, Korea) at 250 rpm for 5 min. The resulting suspensions were centrifuged at 3,000 rpm and 20 °C for 10 min and supernatants were collected for UV analysis at 500 nm wave length. The EY was determined by following Equation (3):

$$EE(\%) = \frac{B - A}{B} \times 100 \quad (3)$$

A: Absorbance of Oil Red O isolated from noncapsulated layer

B: Absorbance of Oil Red O added in hydrogenated MCT

Table 1. Factors for independent variables at different coded levels in developing experimental data.

Factors	Symbol	Coded levels		
		-1	0	1
W/O ratio	X_1	2: 8	2.5: 7.5	3: 7
1st emulsifier (%)	X_2	6	8	10
W/O/W ratio	X_3	1: 16	1: 18	1: 20
2nd emulsifier (%)	X_4	6	8	10

2.6 Particle size distribution

Particles size distribution of W/O/W nanoemulsions of red ginseng extract was determined by a particle size analyser (Delsa@ Nano C, Beckman Coulter, Inc., Fullerton, CA, USA) which employed laser diffraction for the size measurement. For the size measurement of W/O/W nanoemulsions, one gram of sample was prepared by mixing with 10 mL of deionized water at a ratio of 1: 10 (w/v) at 25 °C for 30 second. The sample was poured in a cuvette, and was measured at 25 °C with fixed scattering angle at 165°.

The spray-dried nanocapsules was also determined by a particle size analyser. The particle size of spray-dried nanocapsules was prepared by mixing the powder with 10 mL of deionized water at a ratio of 1: 100 (w/v) and stirring on a magnetic stirrer (Wisestir MS-MP, DAIHAN Scientific, Seoul, Korea) at 25 °C for 10 min. The sample of suspension was poured in a cuvette, and its particle size of spray-dried nanocapsules was measured at 25 °C with fixed scattering angle at 165°.

2.7 Statistical analysis

Analysis of variance (ANOVA) and Duncan's multiple tests were performed to analyse the differences between groups. Data was expressed as mean ± SD and statistical significance was set at $p<0.05$. All statistical analyses were done using SAS 9.0 (SAS Institute Inc., NC, USA) and the graphical representations were performed using Sigmaplot 10.0 (Systat Software Inc., San Jose, CA, USA).

3 Results and discussion

3.1 Optimization of the conditions for producing W/O nanoemulsion of red ginseng extract

ESI was determined to optimize W/O nanoemulsion by emulsifiers which have low HLB-value (HLB 0.6, 3.0, 4.0 and 4.3) and ratios of core to coating materials (1:9, 2:8, 3:7, and 4:6) (Table 2). Generally speaking, emulsifiers with HLB value lower than 10 are predominantly hydrophobic and it is good for W/O emulsification (Florence & Atwood, 1998; Abalos et al., 2004). In the present study, the ESI value of PGPR (HLB 0.6) was higher than those of PFAE (HLB 3.0 and 4.0) and sorbitan monooleate (HLB 4.3) at all the ratios of core and coating materials. PGPR (HLB 0.6) with the ratios of 1:9 to 3:7 in the core and coating materials showed more than 97% ESI and highly stable, but ESI was significantly decreased thereafter. These findings were in accordance with Benichou et al. (2001), who indicated that PGPR is known to be one of the most effective emulsifiers

Table 2. Emulsion stability index (ESI) of red ginseng extract W/O emulsion coated with medium chain triglyceride in various ratios of water to oil phase and HLB values of emulsifiers [Polyglycerol polyricinoleate (HLB 0.6), polyglycerol fatty acid esters (HLB 3.0, 4.0), sorbitan monooleate (HLB 4.3)].

HLB	Ratio of core to coating material			
	1:9	2:8	3:7	4:6
0.6	97.8 ± 0.3^{aA}	96.8 ± 0.4^{bA}	97.3 ± 0.5^{aA}	87 ± 1^{cA}
3.0	92.9 ± 0.6^{aB}	85.3 ± 0.5^{bB}	77 ± 1^{cB}	67 ± 2^{dB}
4.0	91.3 ± 0.7^{aC}	84.2 ± 0.9^{bC}	72 ± 2^{cC}	62 ± 1^{dC}
4.3	87 ± 2^{aD}	63.8 ± 0.3^{bD}	59 ± 1^{cD}	51 ± 3^{dD}

Values with different letters within the same column (A-D) and raw (a-d) differ significantly ($p<0.05$).

for W/O emulsions and 30% water phase was the optimum amount of PGPR required to obtain well-balanced emulsions. Based on the results in the present study, the core-to-coating material ratio of 3:7 was selected because it contains higher amounts of core material compared to 1:9 and 2:8, and PGPR was chosen as the primary emulsifier for subsequent studies.

After optimizing the ratio of core to coating material (3:7), the optimum concentration of PGPR was established by the average particle size using different concentrations (2, 4, 6, 8, and 10%) of PGPR in primary emulsion during incubation for 2 h at 100 °C. Increasing the concentrations of PGPR from 2 and 10% was significantly decreased the average particle sizes of the W/O nanoemulsions from 276 to 168 nm, respectively (data are not shown). Similarly, Matos et al. (2014) reported that the mean droplet sizes of W/O/W emulsions containing trans-resveratrol were decreased when the addition of PGPR was elevated. Therefore, it was found in the present study that the optimum concentration of the primary emulsifier (PGPR) for the production of W/O nanoemulsion was 10%, since the smallest particle size (168 nm) was obtained among all the samples.

3.2 Optimization of the conditions for producing W/O/W nanoemulsion of red ginseng extract

Above of all, to optimize the conditions for producing W/O/W nanoemulsion of red ginseng extract, the effects of the different ratios (1:9, 1.5:8.5, 2:8, 2.5:7.5 and 3:7) of W/O nanoemulsion to the second coating material (10% MD) and the different HLB-valued secondary emulsifiers (HLB 13.0, 14.5 and 16.7) on ESI of W/O/W nanoemulsions were investigated (Table 3). Emulsifiers with HLB value higher than 10 are predominantly hydrophilic and favor the formation of O/W emulsion. Tan & Nakajima (2005) noted that emulsifiers of high-HLB value can make and stabilize the particles more efficiently in the O/W emulsion. Also, the HLB value of emulsifier is high, stability of the emulsion increase in W/O/W emulsification (Abalos et al., 2004). In the present study, it was found that PSML (HLB 16.7) was the most effective in respect of the ESI values of the nanoemulsions among different secondary emulsifiers. In addition, the highest ESI values of nanoemulsions were about 99% with the ratios of 1:9 and 1.5:8.5 in PSML added samples.

After selecting the optimum ratio of core and coating materials (1.5:8.5), the optimum concentration of PSML was investigated by the average particle size using different concentrations (2, 4, 6, 8 and 10%). The elevation in the concentration of PSML from 2 to 10% significantly decreased the average particle sizes of W/O/W nanoemulsions from 784 to 241 nm ($p<0.05$) (data are not shown). Yang et al. (2013) also observed that tinier droplets can be produced at higher concentrations of Tween 80 (HLB 15.0). Also, Van der Graaf et al. (2005) reported that high concentration of emulsifier can improve the emulsion stability. Therefore, it is indicated in the present study that 10% concentration of PSML can be sufficiently effective to produce W/O/W nanoemulsion of red ginseng extract.

The optimum concentration of ethyl acetate (as a solvent in coating material and surfactant mixture) was determined by measuring the average particle sizes of W/O/W nanoemulsions (data not shown). The average particle sizes of the W/O/W nanoemulsions significantly reduced from 253 to 198 nm with increasing the concentrations of ethyl acetate from 5 to 20%, respectively ($p<0.05$). The result was consistent with Chattopadhyay et al. (2006), who reported that the increase in the ethyl acetate concentration caused a decrease in the particle sizes of poly (lactic/glycolic) acids. Based on the results obtained from the present study on the particle size of W/O/W nanoemulsions of red ginseng extract, the highest concentration (20%) of ethyl acetate was determined as the optimum solvent concentration.

ESI was carried out to optimize the concentration of MD, the secondary coating material, for W/O/W nanoemulsions of red ginseng extract during incubation at 100 °C for 2 h (data not shown). It was revealed that the ESI value of the nanoemusion coated with 10% MD was significantly greater than those coated with 15, 20, 25 and 30% MD ($p<0.05$). Klinkesorn et al. (2004) also reported that there was no evidence of creaming in emulsions for less than 13% MD, but the creaming rate was rapidly increased at 15% MD with emulsion separation being observed in less than 2 h. Thus, the concentration of 10% was considered as the optimum concentration of MD (the secondary coating material) for W/O/W nanoemulsion of red ginseng extract in the present study.

Finally, to select the optimum secondary coating material for W/OW nanoemulsion of red ginseng extract, the different secondary coating materials such as MD, WPC and AG at 10% concentrations were employed (data not shown). It was found that the ESI value (99%) of the nanoemulsion coated with 10% MD was the highest, followed by 10% WPC and 10% AG. In particular, the lower ESI value of nanoemulsion coated with AG can be probably due to the higher viscosity of AG, subsequently leading to the breakdown of the nanoemulsion. Gharsallaoui et al. (2007) noted that MD (DE 18.5) was considered as the most suitable coating material for microencapsulation of food components since the emulsion coated with MD showed

Table 3. Effects of HLB-value of emulsifiers [Polyglycerol fatty acid esters (HLB 13.0, 14.5), polyoxyethylene sorbitan monolaurate (HLB 16.7)] and mixing ratio of W/O to outer aqueous phase on emulsion stability index (ESI) of W/O/W emulsion cored with W/O emulsion and coated with 10% maltodextrin (DE 18).

HLB	Ratio of core to coating material				
	1.0:9.0	1.5:8.5	2.0:8.0	2.5:7.5	3.0:7.0
13.0	92.9 ± 0.3^{aB}	85.7 ± 0.4^{bC}	82.1 ± 0.2^{cB}	75.0 ± 0.4^{dC}	70.6 ± 0.3^{eC}
14.5	99.48 ± 0.06^{aA}	98.7 ± 0.1^{aB}	97.32 ± 0.06^{bA}	95.67 ± 0.04^{cB}	94.93 ± 0.05^{dB}
16.7	99.74 ± 0.04^{aA}	99.59 ± 0.04^{aA}	97.26 ± 0.03^{bA}	96.40 ± 0.03^{cA}	95.1 ± 0.1^{dA}

Values with different letters within the same column (A-C) and raw (a-d) differ significantly ($p<0.05$).

the good solubility and the rapid reconstruction of the emulsion in water. Therefore, it was suggested in this study that MD was the optimal secondary coating material for the production of W/O/W nanoemulsion of red ginseng extract.

3.3 Optimization of the conditions for producing spray-dried nanocapsule of red ginseng extract by RSM

To optimize the W/O/W nanoencapsulation of red ginseng extract, a four-factor FCCD was adopted on the basis of coded level obtained from four independent variables (Table 4). The encapsulation yield (Y_1) and average particle size (Y_2) were optimized using statistical experimental design on the basis of coded level from four independent variables, such as the ratio of core to coating materials (X_1), concentration of primary emulsifier (PGPR, X_2), ratio of W/O emulsion to secondary coating materials (X_3) and concentration of secondary emulsifier (PSML, X_4). The mathematical relationship in the form of regression equation was given below in terms of coded factors:

$$Y_1(\%) = 61.9152 + 0.0089X_1 + 0.4003X_2 + 0.0003X_3 + 7.1737X_4 - 0.3757X_4^2$$

$$Y_2(nm) = 1037.68 - 0.07X_1 - 34.97X_2 - 13.21X_3 - 137.83X_4 + 4.10X_4^2 + 3.27X_2X_4 + 1.49X_3X_4$$

The regression equation of Y_1 and Y_2 is the predicted dependent response and X_1, X_2, X_3 and X_4 were the coded values of factors. The values of the coefficients and the analysis of variance (ANOVA) to evaluate the significant of the coefficients calculated for the yield (Y_1) and average particle size (Y_2) of the quadric polynomial models are presented in Table 4.

The effect of factor X_2, X_4, X_4 and X_2X_4 were revealed to be highly significant (p<0.05) on the yield of encapsulation (Y_1). For the average particle size (Y_2), it was found that X_2, X_4, X_4, X_2X_4 and X_3X_4 significantly affect the particle size of spray-dried nanocapsule of red ginseng extract. Reliabilities of the variation of the microencapsulation yield and size were presented by the model as 98.14 and 97.51%, respectively.

The response surface plots of spray-dried nanocapsules of red ginseng extract for encapsulation yield and the average particle size are illustrated in Figure 1. Increasing the first emulsifier and second emulsifier led to the increase in the microencapsulation yield. For instance, 10% of the first emulsifier and 10% of the second emulsifier provided the highest encapsulation yield (>99.40%) (Figure 1A). Also, the highest concentration of the primary emulsifier (10%) and the secondary emulsifier (10%) led to the smallest particle size of below 80 nm (Figure 1B). In the study, as the amount of emulsifier increased, the particle size increased.

As indicated in Figure 2, the optimum condition of factor settings to achieve desired response was confirmed to be the W/O ratio (3:7), first emulsifier concentration (10% PGPR), W/O/W ratio (1:16) and second emulsifier concentration (10% PSML) with a desirable encapsulation yield of 99.2996% and particle size of 61.3206 nm.

3.4 Size distribution

The particle sizes of W/O/W nanomulsions and spray-dried nanocapsules prepared with different secondary coating materials (MD, WPC and AG) are showed in Figure 3 and 4. Prior to the spray drying process, the particle size of W/O/W nanoemulsions coated with MD showed the only nano-size among all the samples (Figure 3). For the particle size of spray-dried nanocapsules coated with MD, WPC and AG ranged from 100 to 500 nm, 100 nm to 1 μm, and 300 nm to 3 μm, respectively (Figure 4).

Table 4. Analysis of variance of the regression coefficient calculated for the yield (Y_1) and average particle size (Y_2).

Term	Yield (Y_1)				Particle size (Y_2)			
	Coefficient	Standard error	T-value	P-value	Coefficient	Standard error	T-value	P-value
Constant	57.8118	17.6470	3.276	0.005	1402.16	346.618	4.045	0.001
Linear								
X_1	-4.0797	5.3345	-0.765	0.456	-52.23	104.779	-0.499	0.625
X_2	0.7246	1.0405	0.696	0.496	-59.83	20.438	-1.028	0.319
X_3	-0.1431	2.2324	-0.064	0.950	-116.47	22.078	-5.275	0.000
X_4	9.4610	1.1241	8.417	0.000	11.50	19.202	0.599	0.558
Quadratic								
X_1^2	0.9300	0.9776	0.951	0.356	11.50	19.202	0.599	0.558
X_2^2	0.0407	0.0547	0.745	0.467	0.81	1.075	0.755	0.461
X_3^2	-0.0081	0.0611	-0.133	0.896	0.88	1.200	0.737	0.472
X_4^2	-0.4394	0.0611	-7.191	0.000	2.38	1.200	1.987	0.064
Interaction								
$X_1 X_2$	-0.0956	0.1001	-0.955	0.354	0.82	1.966	0.415	0.684
$X_1 X_3$	0.0494	0.1001	0.493	0.628	-1.74	1.966	-0.884	0.390
$X_1 X_4$	-0.0856	0.1001	-0.856	0.405	2.43	1.966	1.237	0.234
$X_2 X_3$	0.0283	0.0250	1.130	0.275	0.54	0.491	1.108	0.284
$X_2 X_4$	-0.1561	0.0250	-6.238	0.000	3.27	0.491	6.646	0.000
$X_3 X_4$	0.0108	0.0250	0.431	0.672	1.49	0.491	3.036	0.008
r^2				0.9814				0.9751

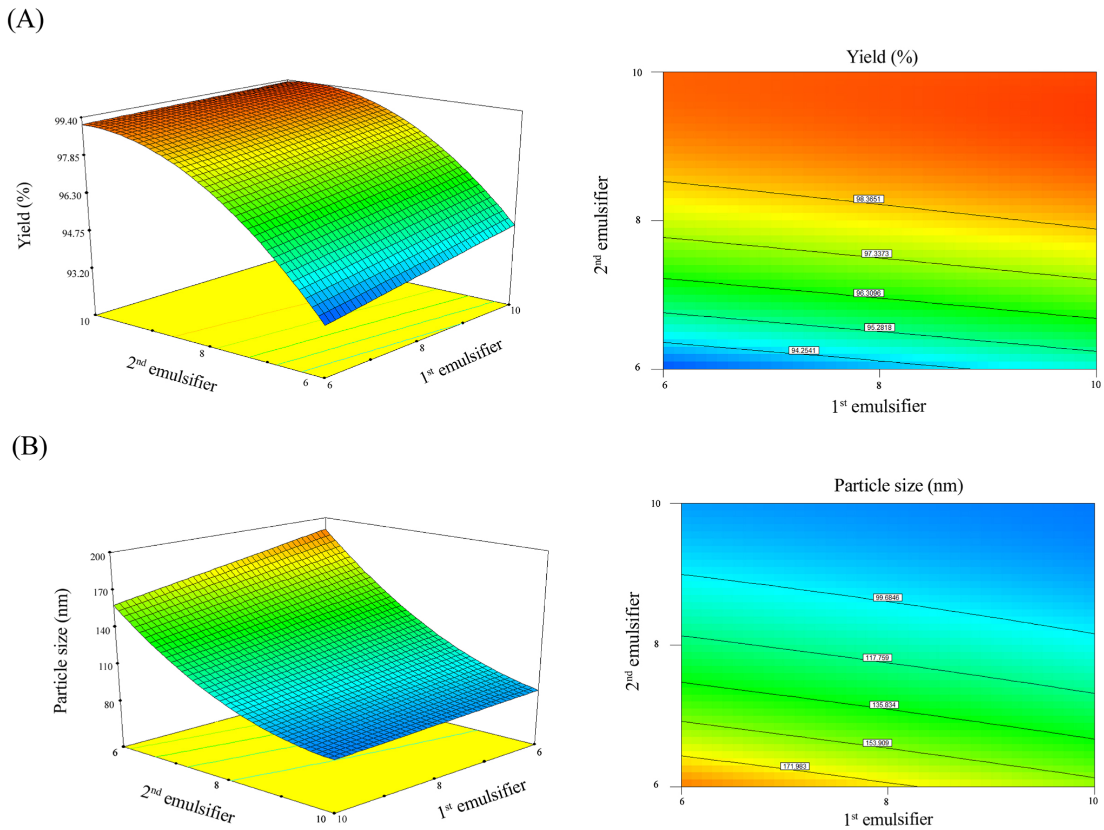

Figure 1. Response surface plots for the effects of variables on the encapsulated yield (%) (A) and the average particle size (nm) (B) of spray-dried nanocapsules of red ginseng extract.

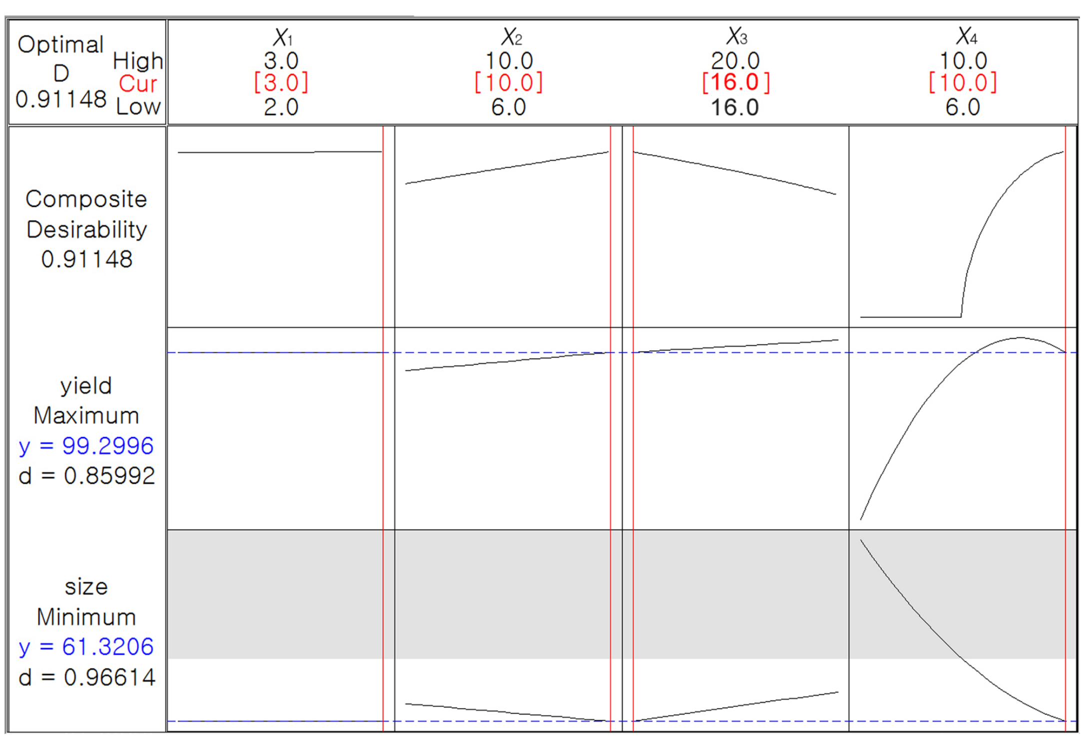

Figure 2. Optimum conditions of selective spray-dried nanocapsules of red ginseng extract by response surface methodology (RSM). X_1: W/O ratio; X_2: 1st emulsifier; X_3: W/O/W ratio; X_4: 2nd emulsifier; Y_1: encapsulation yield; Y_2: average particle size.

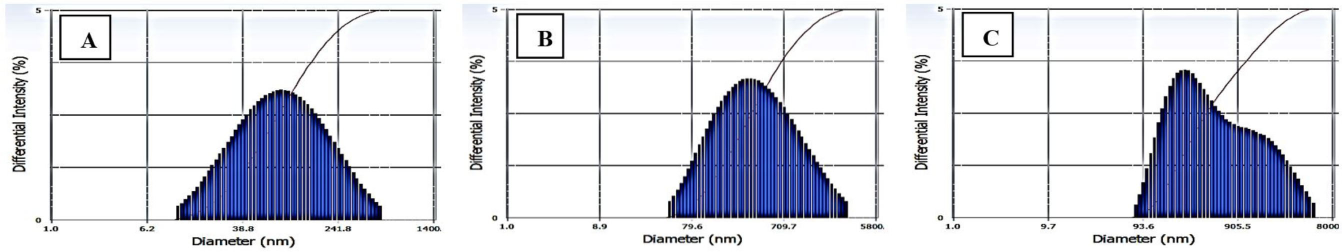

Figure 3. Particle size distributions for W/O/W nanoemulsions coated with different secondary coating materials. A. 10% maltodextrin (MD, DE 18), B. 10% whey protein concentrates (WPC), C. 10% arabic gum (AG).

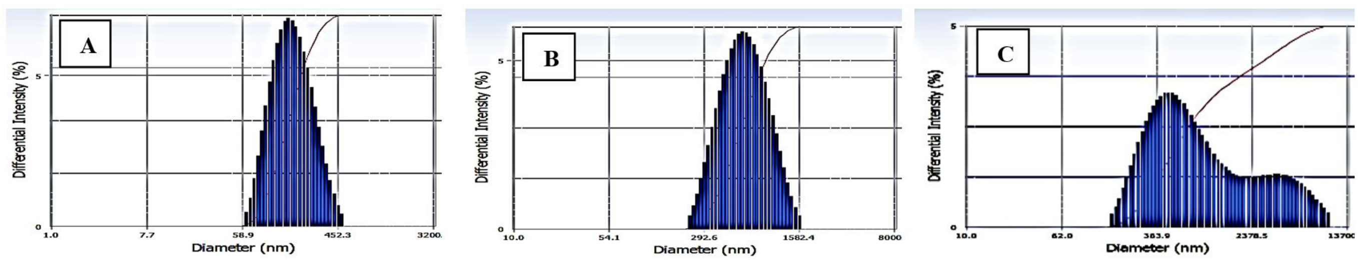

Figure 4. Particle size distributions for spray-dried nanocapsules coated with different secondary coating materials. A. 10% maltodextrin (MD, DE 18), B. 10% whey protein concentrates (WPC), C. 10% arabic gum (AG).

Therefore, this study showed that MD could be the most efficient coating material to spray-dried nanocapsules with the nano-size. The capsules coated with MD had the increase the stability of the core ingredient compared with that coated with gum arabic, because of the smaller sizes of capsules coated with MD (Barbosa et al., 2005). Based on the result from this, it is considered that W/O/W nanomulsions and spray-dried nanocapsules coated with MD could have better dispersibility than with WPC or AG.

4 Conclusion

The present research revealed the optimum conditions for the preparation of W/O/W nanoemulsion and spray-dried nanocapsules of red ginseng extract. The optimum conditions of W/O/W nanoemulsion were chosen as the ratio of core to coating material (3:7), primary emulsifier concentration (10%), the ratio of W/O phase to coating material (1.5:8.5), secondary emulsifier concentration (10%), solvent concentration (20%) and secondary coating material (10%, MD). The optimum conditions of spray-dried nanocapsules conducted by RSM were the ratio of core material to coating material (3:7), the concentration of primary emulsifier (10%), the ratio of W/O emulsion to secondary coating material (1:16), the concentration of secondary emulsifier (10%) and 10% MD concentration. In the optimum condition, the encapsulation yield of spray-dried nanocapsule were 99.3%, and the particle sizes was from 100 and 500 nm. Based on the results, the present study could offer a useful information and guideline on optimizing the conditions for producing W/O/W nanoemulsion and spray-dried nanocapsule of red ginseng extract.

References

Abalos, A., Vinãs, M., Sabaté, J., Manresa, M. A., & Solanas, A. M. (2004). Enhanced biodegradation of casablanca crude oil by a microbial consortium in presence of a rhamnolipid produced by *Pseudomonas aeruginosa* AT10. *Biodegradation*, 15(4), 249-260. http://dx.doi.org/10.1023/B:BIOD.0000042915.28757.fb. PMid:15473554.

Acosta, E. (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Current Opinion in Colloid & Interface Science*, 14(1), 3-15. http://dx.doi.org/10.1016/j.cocis.2008.01.002.

Baik, M. Y., Suhendro, L., Nawar, W. W., McClements, D. J., Decker, E. A., & Chinachoti, P. (2004). Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. *Journal of the American Oil Chemists' Society*, 81(4), 355-360. http://dx.doi.org/10.1007/s11746-004-0906-7.

Barbosa, M. I. M. J., Borsarelli, C. D., & Mercadante, A. Z. (2005). Light stability of spray-dried bixin encapsulated with different edible polysaccharide preparations. *Food Research International*, 38(8-9), 989-994. http://dx.doi.org/10.1016/j.foodres.2005.02.018.

Benichou, A., Aserin, A., & Garti, N. (2001). Polyols, high pressure, and refractive indices equalization for improved stability of W/O emulsions for food applications. *Journal of Dispersion Science and Technology*, 22(2-3), 269-280. http://dx.doi.org/10.1081/DIS-100105214.

Chae, S., Kang, K. A., Chang, W. Y., Kim, M. J., Lee, S. J., Lee, Y. S., Kim, H. S., Kim, D. H., & Hyun, J. W. (2009). Effect of compound K, a metabolite of ginseng saponin, combined with γ-ray radiation in human lung cancer cells in vitro and in vivo. *Journal of Agricultural and Food Chemistry*, 57(13), 5777-5782. http://dx.doi.org/10.1021/jf900331g. PMid:19526988.

Chang, P. S., Shin, M. G., & Lee, W. M. (1994). Relationship between emulsion stability index and HLB value of emulsifier in the analysis of W/O emulsion stability. *Analytical Sci & Technol*, 7, 237-243.

Chattopadhyay, P., Huff, R., & Shekunov, B. Y. (2006). Drug encapsulation using supercritical fluid extraction of emulsions. *Journal of Pharmaceutical Sciences*, 95(3), 667-679. http://dx.doi.org/10.1002/jps.20555. PMid:16447174.

Chu, B. S., Ichikawa, S., Kanafusa, S., & Nakajima, M. (2007). Preparation of protein-stabilized beta-carotene nanodispersions by emulsification-evaporation method. *Journal of the American Oil Chemists' Society*, 84(11), 1053-1062. http://dx.doi.org/10.1007/s11746-007-1132-7.

Ernst, E. (2010). Panax ginseng: an overview of the clinical evidence. *Journal of Ginseng Research*, 34(4), 259-263. http://dx.doi.org/10.5142/jgr.2010.34.4.259.

Florence, A. T., & Atwood, D. (1998). Emulsions. In A. T Florence & D. Atwood (Eds.), *Physicochemical principles of pharmacy* (pp. 252-307). New York: Palgrave.

Ganesan, P., Ko, H. M., Kim, I. S., & Choi, D. K. (2015). Recent trends of nano bioactive compounds from ginseng for its possible preventive role in chronic disease models. *RSC Advances*, 5(119), 98634-98642. http://dx.doi.org/10.1039/C5RA20559J.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, 40(9), 1107-1121. http://dx.doi.org/10.1016/j.foodres.2007.07.004.

Kang, K. S., Yokozawa, T., Kim, H. Y., & Park, J. H. (2006). Study on the nitric oxide scavenging effects of ginseng and its compounds. *Journal of Agricultural and Food Chemistry*, 54(7), 2558-2562. http://dx.doi.org/10.1021/jf0529520. PMid:16569043.

Klinkesorn, U., Sophanodora, P., Chinachoti, P., & McClements, D. J. (2004). Stability and rheology of corn oil-in-water emulsions containing maltodextrin. *Food Research International*, 37(9), 851-859. http://dx.doi.org/10.1016/j.foodres.2004.05.001.

Lee, S. J., & McClements, D. J. (2010). Fabrication of protein-stabilized nanoemulsions using a combined homogenization and amphiphilic solvent dissolution/evaporation approach. *Food Hydrocolloids*, 24(6-7), 560-569. http://dx.doi.org/10.1016/j.foodhyd.2010.02.002.

Lee, T. K., Johnke, R. M., Allison, R. R., O'brien, K. F., & Dobbs, L. J. Jr (2005). Radioprotective potential of ginseng. *Mutagenesis*, 20(4), 237-243. http://dx.doi.org/10.1093/mutage/gei041. PMid:15956041.

Liu, Z. Q., Luo, X. Y., Liu, G. Z., Chen, Y. P., Wang, Z. C., & Sun, Y. X. (2003). In vitro study of the relationship between the structure of ginsenoside and its antioxidative or prooxidative activity in free radical induced hemolysis of human erythrocytes. *Journal of Agricultural and Food Chemistry*, 51(9), 2555-2558. http://dx.doi.org/10.1021/jf026228i. PMid:12696936.

Matos, M., Gutiérrez, G., Coca, J., & Pazos, C. (2014). Preparation of water-in-oil-in-water (W1/O/W2) double emulsions containing trans-resveratrol. *Colloids and Surfaces. A, Physicochemical and Engineering Aspects*, 442, 69-79. http://dx.doi.org/10.1016/j.colsurfa.2013.05.065.

McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition*, 51(4), 285-330. http://dx.doi.org/10.1080/10408398.2011.559558. PMid:21432697.

Mochizuki, M., Yoo, Y. C., Matsuzawa, K., Sato, K., Saiki, I., Tono-oka, S., Samukawa, K., & Azuma, I. (1995). Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20 (R)-and 20 (S)-ginsenoside-Rg3, of red ginseng. *Biological & Pharmaceutical Bulletin*, 18(9), 1197-1202. http://dx.doi.org/10.1248/bpb.18.1197. PMid:8845804.

Ni, H. X., Yu, N. J., & Yang, X. H. (2010). The study of ginsenoside on PPARγ expression of mononuclear macrophage in type 2 diabetes. *Molecular Biology Reports*, 37(6), 2975-2979. http://dx.doi.org/10.1007/s11033-009-9864-0. PMid:19821054.

Park, J. D. (1996). Recent studies on the chemical constituents of Korean ginseng (Panax ginseng C.A. Meyer). *Kor J Ginseng Sci*, 20, 389-415.

Pérez-Masiá, R., López-Nicolás, R., Periago, M. J., Ros, G., Lagaron, J. M., & López-Rubio, A. (2015). Encapsulation of folic acid in food hydrocolloids through nanospray drying and electrospraying for nutraceutical applications. *Food Chemistry*, 168, 124-133. http://dx.doi.org/10.1016/j.foodchem.2014.07.051. PMid:25172691.

Ragelle, H., Crauste-Manciet, S., Seguin, J., Brossard, D., Scherman, D., Arnaud, P., & Chabot, G. G. (2012). Nanoemulsion formulation of fisetin improves bioavailability and antitumour activity in mice. *International Journal of Pharmaceutics*, 427(2), 452-459. http://dx.doi.org/10.1016/j.ijpharm.2012.02.025. PMid:22387278.

Rosenberg, M., Kopelman, I. J., & Talmon, Y. (1990). Factors affecting retention in spray-drying microencapsulation of volatile materials. *Journal of Agricultural and Food Chemistry*, 38(5), 1288-1294. http://dx.doi.org/10.1021/jf00095a030.

Sharma, N., Bansal, M., Visht, S., Sharma, P. K., & Kulkarni, G. T. (2010). Nanoemulsion: a new concept of delivery system. *Chron Young Scientists*, 1, 2-6.

Song, X., Bao, S., Wu, L., & Hu, S. (2009). Ginseng stem–leaf saponins (GSLS) and mineral oil act synergistically to enhance the immune responses to vaccination against foot-and-mouth disease in mice. *Vaccine*, 27(1), 51-55. http://dx.doi.org/10.1016/j.vaccine.2008.10.030. PMid:18973783.

Stavro, P. M., Woo, M., Heim, T. F., Leiter, L. A., & Vuksan, V. (2005). North American ginseng exerts a neutral effect on blood pressure in individuals with hypertension. *Hypertension*, 46(2), 406-411. http://dx.doi.org/10.1161/01.HYP.0000173424.77483.1e. PMid:15998708.

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 108-109, 303-318. http://dx.doi.org/10.1016/j.cis.2003.10.023. PMid:15072948.

Tan, C. P., & Nakajima, M. (2005). Effect of polyglycerol esters of fatty acids on physicochemical properties and stability of beta-carotene nanodispersions prepared by emulsification/evaporation method. *Journal of the Science of Food and Agriculture*, 85(1), 121-126. http://dx.doi.org/10.1002/jsfa.1947.

Van der Graaf, S., Schroen, C. G. P. H., & Boom, R. M. (2005). Preparation of double emulsions by membrane emulsification-a review. *Journal of Membrane Science*, 251(1-2), 7-15. http://dx.doi.org/10.1016/j.memsci.2004.12.013.

Yang, Y., Leser, M. E., Sher, A. A., & McClements, D. J. (2013). Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale®). *Food Hydrocolloids*, 30(2), 589-596. http://dx.doi.org/10.1016/j.foodhyd.2012.08.008.

Zhao, L., Wei, Y., Huang, Y., He, B., Zhou, Y., & Fu, J. (2013). Nanoemulsion improves the oral bioavailability of baicalin in rats: *in vitro* and *in vivo* evaluation. *International Journal of Nanomedicine*, 8, 3769-3779. http://dx.doi.org/10.2147/IJN.S51578. PMid:24124365.