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Effect of soy proteins and emulsification-evaporation process on physical stability of lycopene emulsions

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Abstract
Soy protein concentrate (SPC) was explored as an emulsifier in preparation of rice bran oil based lycopene emulsions. The SPC concentration and high pressure homogenization variables (pressure and number of passes) were optimized using response surface design obtained through Design-Expert Software. Twenty experimental combinations of SPC (0.25 to 1.25 %, w/v); homogenization pressure (10,000 to 30,000 psi) and number of passes (1 to 5) were carried out. The effect of process variables on particle size, polydispersity index and zeta (ζ) potential of lycopene emulsions was studied. The results obtained using analysis of variance and relative standard deviations of responses suggested that the maximum lycopene emulsification efficiency (85.85 ± 3.501 %), minimum particle size (346.90 ± 22.870 nm) and polydispersity index (0.27 ± 0.041) could be achieved at optimum conditions of 0.89 % (w/v) SPC at 25,000 psi in 4 passes through high pressure homogenizer. The emulsions prepared at optimum condition showed good physical stability in terms of ζ-potential (-42.32 ± 1.656 mV) with overall desirability of 0.8175.

Keywords: rice bran oil, particle size, zeta potential, optimization.

Introduction
Soy proteins are widely used in during formation of various protein-based food systems. Soy proteins posses good emulsifying ability and has high nutritional value. It provides a good balance in amino acid composition, since all the essential amino acids are present in it. In addition, it has physiologically active components which help to lower the cholesterol and reduce the risk of cardiovascular diseases [1]. Soy proteins are also found to inhibit lipid peroxidation when used in food matrix [2].

Tension-activity character of protein allows their fundamental use in dispersed systems formations such as foams and emulsions. Proteins act as good emulsifiers due to the presence of both hydrophilic and hydrophobic parts in its molecular structure [3]. Soy proteins get readily adsorb at the oil-water interface forming a thin film around the oil droplet which acts as a barrier against coalescence and flocculation of dispersed phase in continuous system. This reduces the interfacial tension between dispersed and continuous phases and thus facilitates formation of stable oil-in-water (O/W) emulsions, when protein dispersion is mixed with oil [4, 5].

High pressure homogenization is mechanical method for preparation of emulsions. In this, the shear forces developed during homogenization helps to break oil phase into small droplets. In high pressure homogenization – evaporation (HPHE) process, the solvent phase used in making an emulsion is evaporated to get food grade emulsions. Studies on use of soy proteins (concentrates as well as isolates) have been carried out to check its efficacy against destabilization of emulsions. However, the literature on formation of soy protein emulsions through mechanical processing e.g. high pressure homogenization, microfluidization, ultrasonication, etc. is very few. Wan *et al.* (2013) [4] observed the competitive adsorption of stevioside micelles along with soy protein isolates (SPI) at the interfacial layer of emulsion. The mixed layer formed on interface contributed to lower the particle size and improved the physical stability of emulsions. Roesch and Corredig (2003) [6] noted bimodal distribution of particles in O/W emulsions prepared using soy protein concentrate (SPC) and heating/HPH process. Fernandez-Avila and Trujillo (2016) [5] found that O/W emulsions prepared with 4 %

(w/v) of SPI + 20 % (v/v) soybean oil and homogenized at 100 and 200 MPa exhibited the most oxidative stability.

Lycopene, a known natural antioxidant is susceptible to oxidative loss when exposed to light, temperature or oxygen due to presence of unsaturated bonds in its molecular structure [7]. The degradation of lycopene can be delayed to some extent, through increasing its solubility and hence the stability. Emulsification of lipophilic compounds such as lycopene is found to improve their stability. It also aids in bio-availability of lipophilic lycopene as reported by Ha *et al.* (2015) [8]. The combined effect of soy proteins and homogenization process may improve the stability of lycopene in O/W emulsions. Therefore, an investigation on use of soy protein concentrate as an emulsifier in lycopene emulsions prepared through HPHE process was carried out. Rice bran oil, a rich source of gamma oryzanol with proven antioxidants properties (Juliano *et al.*, 2005) [9] was selected as an oil phase for preparation of emulsions.

2. Materials and methods

Soy protein concentrate and was purchased from local supermarket, New Delhi, India. Physically refined rice bran oil was procured from Shiv Sales Corporation, New Delhi, India. Double distilled and deionized water, prepared in our laboratory (Sartorius Stedim Biotech, Germany) was used for the preparation of all solutions and emulsions. Papaya fruits used for lycopene extraction were obtained from the Institute's (ICAR-Indian Agricultural Research Institute, New Delhi) fruit orchard.

2.1. Formation of lycopene emulsions

The stock solution was prepared by dissolving SPC in hot water (50-60 °C) while agitating (Magnetic stirrer, MT2A, Amacon, USA) for 2 h. To it, 0.02 % (w/v) of sodium azide was added as an antimicrobial agent. The solution was prepared 24 h before use for complete hydration of proteins and was kept at 4 °C.

Lycopene extract from red papaya obtained using supercritical CO_2 extraction (≈ 40 mg of lycopene concentration per 100 ml ethanol) was used as a lycopene source without further purification [10]. Pre-emulsions obtained through addition of ethanol lycopene slowly to emulsifier solution (1:1 ratio) while homogenizing (IKA T25 D Ultra-turrax, Germany) at 10,000 rpm for 3 min were passed through high pressure homogenizer (SPCH-10 Stansted Fluid Power, England) at different combination of pressure and number of passes as given in Table 1. Then ethanol from emulsions was evaporated in rotary vacuum evaporator (Hei-VAP, Heidolph, Germany) at 175 mbar and 40 °C under reduced light conditions. Chemical stability (lycopene emulsification efficiency) and physical stability (particle size, polydispersity index and zeta potential) of prepared emulsions were evaluated thereafter.

2.2. Determination of particle size (PS), polydispersity index (PI) and zeta (ζ) potential

Average particle size (PS), polydispersity index (PI) and zeta (ζ) potential of lycopene emulsions were determined to study physical stability of emulsions. The measurements were made using a commercial particle size analyzer (SZ-100 Nano Partica, Horiba Scientific Instruments, Japan) based on dynamic light scattering principle at 25 °C with a scattering angle of 173°. To measure size and PI, 3 ml of lycopene emulsion were transferred to an optical-grade cell immediately after preparation and analyzed for intensity distribution. Zeta potential (surface charge) of lycopene nanoemulsions was assessed by determining the droplet electrophoretic light scattering at 25°C. For this, emulsions were filled in dip cell through use of syringe and measurements were performed at least in duplicate for each sample.

2.3. Determination of lycopene emulsification efficiency (LEE)

Lycopene concentration in emulsion was determined by dissolving 5 ml of emulsion in 20 ml of hexane: acetone: ethanol (2:1:1) containing 0.05 % BHT. The mixture was agitated for 5 min using vortex shaker, shaken at 200 rpm for 20 min (Orbital shaker, Horiba Scientific) at 4 °C and centrifuged at 10,000 rpm for 10 min. The upper hexane layer was separated and readings were taken in spectrophotometer (Jasco V–670 UV–VIS–NIR spectrophotometer, Japan) at 503 nm against hexane as a blank (Kong and Ismail, 2011) [11]. Lycopene emulsification efficiencies were calculated as the quantity of lycopene present in the emulsion compared to the lycopene initially used to produce them.

2.4. Statistical analysis

The data was analysed by fitting of a second order polynomial models into each of the dependent variables (lycopene emulsification efficiency, particle size, polydispersity index and zeta potential) to rich the optimum response (Design-Expert software version 9.0.6, StatEase Inc., Minneapolis, MN). Optimum was decided based on insignificant lack of fit ($p>0.05$), significant model fit ($p<0.05$), with minimum standard error of the response.

Table1: Central Composite Design used for optimization of SPC concentration and HPH parameters for preparation of lycopene emulsions.

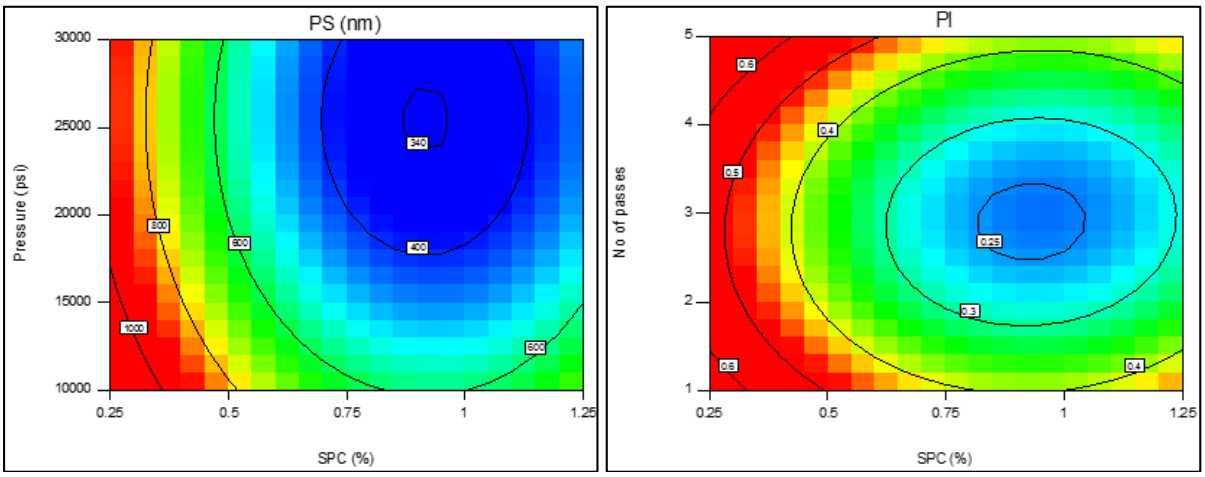
Variables	Coded X_i	Coded levels				
		-α	-1	0	1	+α
SPC (%, w/v)	X_1	0.25	0.5	0.75	1	1.25
Pressure (psi)	X_2	10,000	15,000	20,000	25,000	30,000
Number of passes	X_3	1	2	3	4	5

3. Results and Discussion

3.1. Effect on particle size

Particle size and its distribution in emulsions are related to physical stability of emulsions. Lower the particle size more are the chances of aggregation of particles in emulsion system. But, the bioavailability of a particular compound in emulsion is generally increased with decrease in particles size as suggested by Ha *et al.* (2015) [8]. Hence we have studied the effect of soy protein concentrates as an emulsifier and HPH conditions on particle size of rice bran oil based lycopene emulsions. Particle size of emulsions was significantly affected ($p<0.01$) by concentration of SPC, homogenization pressure as well as their quadratics (Fig.1a). The minimum particle size (<340 nm) was observed for 0.85 to 1 % SPC concentration, with higher values in both directions, suggesting quadratic effect of SPC on PS of emulsions. The reduction in PS with rise in homogenization pressure could be attributed to increase in cavitation and shear forces (Mehmood, 2015) [12] which help to disrupt oil particles into smaller sizes. The linear and quadratic effect of number of passes was also found on PS (Table 2) of emulsions. Particle size decreased up to 4 passes through homogenizer and increased thereafter up to 5 passes. The over-processing may have resulted in coalescence of oil droplets and hence

increases in PS of emulsions after certain limit of process variables [13]. The optimum solution at 0.89 % SPC + 25,000 psi + 4 passes showed minimum particle size of lycopene emulsions as 346.90 ± 22.870 nm.



(a) (b)

Fig 1: Contour plots for significant effect of independent variables on (a) particle size and (b) polydispersity index of lycopene emulsions

3.2. Effect on polydispersity index

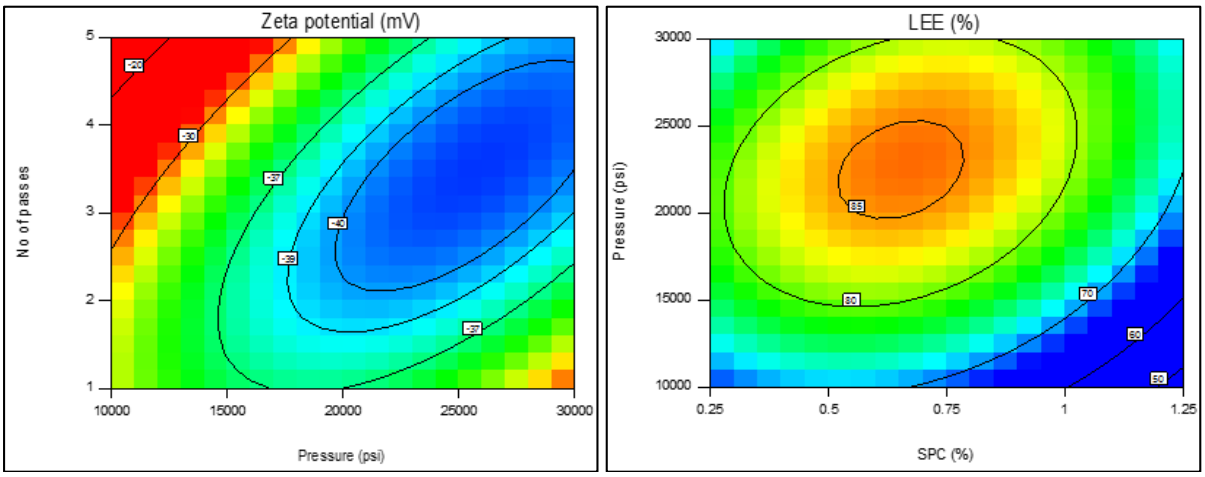
The polydispersity index indicates the variation in particle sizes in emulsions. The lower the PI lower is the difference between the particle sizes of emulsions and higher is the physical stability of emulsions as suggested by Gué *et al.* (2016) [14]. Therefore, the effect of process variables on PI was evaluated. The analysis of data showed significant effect ($p<0.01$) of %SPC and its quadratic on PI (Fig. 1b). Similar to PS, PI was first decreased with rise in SPC concentration up to 0.85 % with further decrease above 1 % emulsifier level in emulsion. However, all the emulsions showed bimodal distribution of particles in emulsions. The highly significant quadratic effect ($p<0.01$) of number of homogenization passes on PI was observed (Table 2). Jafari *et al.* (2007) [13] detected re-coalescence of oil droplets due to over-processing. The similar results of increase in number of passes beyond certain limit on increase in PI were observed here. Polydispersity index of 0.27 ± 0.041 obtained at optimum conditions of 0.89 % SPC + 25,000 psi + 4 passes, indicated that the prepared emulsions were heterogeneous system [14]. This was supported by bimodal particle distribution of emulsions. These findings are in agreement with Roesch and Corredig (2003) [6], who also observed bimodal distribution of particles in O/W emulsion prepared using SPC and HPH process.

3.3. Effect on zeta potential

The particle charge (ζ-potential) of emulsions was predominantly affected ($p\leq0.01$) by pressure and its interaction with number of passes (Table 2). The quadratic of pressure as well as number of homogenization passes had significant effect ($p<0.01$) on ζ-potential of emulsions as shown in Fig.2a. The highest zeta potential values for emulsions were found between 20,000 to 30,000 psi pressure and 2 to 4 cycles. All the emulsions showed high negative particle surface charge, more than -30 mV, which was vital for stable emulsion formulations [15]. The possible reason for this could be adsorption of soy proteins on surface of RBO droplets in a thin layer, generating ample electrostatic repulsive forces at neutral pH [16]. ζ-potential of -42.32 ± 1.656 mV was attained at optimum combination. Therefore, we it could be said that the rice bran oil based lycopene emulsions prepared using soy proteins had very good electrostatic stability.

3.4. Effect on lycopene emulsification efficiency

The effect of processing conditions on stability of lycopene in terms of emulsification efficiency was determined. LEE was significantly ($p<0.05$) affected by linear terms of all the three independent variables (%SPC, homogenization pressure & number of passes through homogenizer). The interaction of pressure with number of homogenization passes also had significant effect ($p<0.05$) on LEE. The LEE improved from 70 to 95 %, with simultaneous rise in homogenization pressure and number of passes from 10,000 to 30,000 psi and 1 to 5 respectively (Table 2). This suggested the positive effect of homogenization process on uniform dispersion of lycopene rich rice bran oil droplets in emulsion. However, when SPC concentration in emulsion was raised from 0.25 % to 0.75 %, the significant ($p<0.01$) increase in emulsification of lycopene was observed at pressure ranging from 20,000 to 25,000 psi (Fig.2b). The further increase in SPC and level in emulsions reduced the LEE. This could be due to lower solubility of soy protein concentrates in aqueous phase after above concentration and coagulation during homogenization due to over processing and heat generation [13, 5]. Homogenization pressure showed quadratic effect on LEE of emulsions (Fig.2b). Lycopene emulsification efficiency of 85.85 ± 3.501 % was achieved at optimum conditions of 0.89 % SPC concentration and 25,000 psi pressure in 4 passes.



(a) (b)

Fig 2: Contour plots for significant effect of independent variables on (a) zeta potential (b) lycopene emulsification efficiency

3.5. Optimization of emulsification-evaporation conditions

The optimum conditions showed that the lycopene emulsions prepared using lycopene-rice bran oil as an oil phase and soy protein concentrates as an emulsifier through emulsification-evaporation method had emulsification efficiency of 85.85 ± 3.501 %, ζ-potential of -42.32 ± 1.656 mV with minimum particle size of 346.9 ± 22.87 nm and polydispersity index of 0.27 ± 0.041 at 0.80 %, w/v soy protein concentrate at 21,000 psi homogenization pressure and 3 passes. Analysis of variance (Table 2) showed that the quadratic models obtained for each of the independent variables had insignificant lack of fit ($p>0.05$), highly significant model fit ($p<0.01$) and good regression coefficients ($R^2>0.8$). Hence, the developed second order polynomial models could be used to predict various properties of rice bran oil based lycopene emulsions.

Table 2: ANOVA for regression coefficients of the fitted second order polynomial models for the properties of lycopene emulsion

Response	PS (nm)	PI	ζ-potential (mV)	LEE (%)
Intercept	404.34***	0.26***	-39.97***	84.11***
X_1	-109.9375***	-0.0556***	-0.025	-7.6218***
X_2	-58.0625***	-0.0219**	-2.6625***	2.1919**
X_3	-35.6875***	0.0106	0.9625**	4.7944***
$X_1 X_2$	0.125	0.0263*	-2.05***	3.7238***
$X_1 X_3$	3.625	-0.0038	-0.225	2.8763**
$X_2 X_3$	9.875	0.0113	-1.85***	3.0588**
X_1^2	82.7955***	0.0385***	0.2898	-0.2957
X_2^2	26.5455***	0.0123	1.5898***	-2.9157***
X_3^2	82.1705***	0.0423***	1.2273***	-0.5469
Lack of fit (P value)	0.1154	0.7291	0.6520	0.2325
Model F ratio (P value)	148.4602 (<0.0001)	11.4707 (0.0023)	14.1408 (0.0001)	21.2022 (<0.0001)
R^2	0.9926	0.9117	0.9271	0.9502
Adj. R^2	0.9856	0.8322	0.8616	0.9054

4. Conclusion

It can be concluded from the study that the lycopene emulsions prepared at optimum combination of soy protein concentrate and HPHE had good emulsification efficiency and stability. Therefore, the lycopene emulsions could be used in development of soy protein-based food formulations.

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