Stabilizing O/W Emulsions by Soy Protein Concentrate + Maltodextrin and Optimizing the Process by Using Response Surface Methodology

Azadeh Salimi^{1,*}, Yahya Maghsoudlou², Seyyed Mahdi Jafari², Alireza Sadeghi Mahounak², Mahdi Kashani nejad², Aman Mohammad Ziaifar²

¹Veterinary Department, Semanan University, Semann, Iran

²Food Science and Technology Department, Agricultural Sciences and National Resources University of Gorgan, Gorgan, Iran

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Abstract Stability is one of the key quality parameters of emulsion systems, which goes a long way in predicting the shelf life of emulsion products. In this study, the effect of emulsifier (soy protein concentrate (SPC) + maltodextrin (MDX)), dispersed phase (lycopene in oil solution) and homogenizer speed on emulsion stability were investigated and optimized using response surface methodology (RSM). Independent variables were lycopene content (10-20%, w/w), SPC+ MDX as emulsifier and surfactant (30-40%, w/w) and the homogenizer speed (14000-18000 rpm). Responses were droplet size, viscosity and creaming index as stability indicators of the emulsions. According to RSM analysis and models, optimized variables showed a good fit to quadratic equations for droplet size and viscosity with correlation coefficients (R^2) of 0.9571 and 0.9826, respectively. After model simplification with backward stepwise solution, the R^2 values for droplet size and viscosity decreased slightly to 0.9504 and 0.9826, respectively. Creaming values were fitted properly with linear model, and R^2 was 0.8030. Graphical optimization methods were adapted for preparing the best lycopene emulsifying conditions and were predicted to be: homogenizer speed of 18000 rpm; lycopene content of 20% w/w, and SPC+MDX concentration of 36.95% w/w.

Keywords Emulsion, Soy Protein Concentrate, Maltodextrin, Optimization, Response Surface Methodology

of small spherical oil droplets in an immiscible aqueous phase [2]. Generally, emulsions are thermodynamically unstable systems due to an increase in interfacial area following emulsification. Hence, after enough time, they collapse, into parent phases in order to minimize the contact area [3]. It is possible to form a kinetically stable (metastable) emulsion for a reasonable period of time by reducing their destabilization rate to adequately low level [4] by adding emulsifiers and/or thickening agents prior to homogenization [2]. In order to increase the activation energy of the system [5], some other variables, such as Proteins are usually employed in O/W emulsions due to their ability to reduce interfacial tension between oil and water, and to form films that act as a physical barrier, as well as prevent flocculation and coalescence of droplets [6]. In particular, soy protein is a good alternative due to its high nutritional value and low cost than other sources of proteins. Emulsifying and foaming properties are important properties of soy protein concentrates (SPC), and are useful in stabilizing emulsion systems.

Polysaccharides are another group of biopolymers which can be used as stabilizers mainly because of their hydrophobicity, highly-branched structure, high molecular weight, thickening ability and gelling characteristics. They form macromolecular barriers against destabilizing mechanisms by increasing the continuous phase viscosity [7]. Polysaccharides, such as gums and MDX, can be used in combination with other biopolymers, such as whey proteins, to optimize their emulsification and film forming properties [8].

Droplet size is one of the most important parameters that influences emulsion stability, and is affected by homogenization method, quantity of dispersed and continuous phases, as well as viscosity of the two phases.

1. Introduction

Oil-in-water emulsions are systems that are composed of two immiscible liquids such as oil and water [1]. They consist

Lower droplet size can be achieved by using increasing pressure, shear rate and energy density, which leads to more stability in emulsions [9, 10, 11]. Also, it is important to improve the performance of the systems and to increase the yield of the processes without increasing the cost. The method used for this purpose is called optimization. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes, in which a response of interest influenced by several variables is optimized [12, 13].

The objective of this study was to develop an RSM-based optimization technique for improving lycopene emulsion stability by natural biopolymers (SPC+MDX) and to obtain optimum operating conditions for further processing.

2. Materials and Methods

2.1. Materials

Lycopene was purchased from Anhui Minmetals Development Company (Anhuai, China). Maltodextrin (DE20) and SPC 80% were produced from Qinhuangdao Lihua Starch Co. Ltd (Qinhuangdao, China), and Wachsen Company (Shandong, China), respectively. Soy oil was purchased from Salimi Industrials (Tabriz, Iran). Sodium azide and sodium dodecyl sulfate (SDS) were purchased from Sigma Aldrich (USA) and Merck (Germany), respectively.

2.2. Methods

2.2.1. Preparation of Emulsions

SPC powder was rehydrated using deionized water at room temperature, and allowed to stand for 2 h to ensure complete hydration. This solution was adjusted to pH 8.0 using phosphate buffer, and heated in a water bath (WB 14, Memmert, Germany) to 90°C for 30 min in order to denature proteins completely. Being denatured in proteins structure, result to opening their chain and a better contact between non polar and polar bonds which finally can improve emulsifier property and more stability in emulsions. Also denatured SPC is more viscose and can reduce droplet aggregation in emulsions which finally can reduce rate of creaming of them [14]. The denatured SPC solutions were cooled, and held at room temperature for 1 h. On the other hand, MDX powder was dispersed in distilled water, and stirred overnight. Denatured SPC was mixed with MDX (in a ratio of 1:9) to form solutions with different solid concentrations (30, 35 and 40% w/w) [15]. Then, lycopene (5% w/w) was dissolved in soy oil, and was mixed gradually (10, 15 and 20% w/w) with SPC + MDX solutions using a rotor-stator homogenizer (D 91126, Heidolf industries, Germany). To prepare coarse emulsions, 10000 rpm was used for 5 min. Furthermore, to prepare final emulsions, different rotation speeds (14000,

16000 and 18000 rpm) were used for 10 min. Sodium azide (0.01% w/v) was added into emulsions to prevent microbial decay during storage. Emulsions were kept in glass tubes in water bath (30°C) for further analysis.

2.2.2. Microstructure and Droplet Size Determination of Emulsions

The microstructure of emulsions was analyzed using a digital microscope (VIVA-BW 1008, Gaungdong, China) connected to a computer. Microscopic size measurement was carried out by taking photos of emulsions, and processing them by Image J (1.44P) software. Briefly, one droplet of emulsion was diluted in 100 mL 0.1% w/v SDS to prevent further droplet coalescence, and to reduce the droplet concentration down to about 0.01% v/v, because the original emulsions were too concentrated for drop size analysis [16]. One droplet of this solution was placed on a slide glass, and photographed using microscope at 100x [17].

2.2.3. Creaming

Stability test was carried out during storage at 30°C according to the reference method [18, 19]. Emulsion samples were separated into a “cream” layer at the top and a transparent (or turbid) “serum” layer at the bottom after being transferred into glass tubes and stored at the appropriate temperature. The extent of creaming was characterized by the creaming index (CI) as described in Eq.1

$$CI = 100 \times (H_s + H_c) / H_t$$

Hs: height of the serum layer inside the glass vessel

Hc: height of the cream layer inside the glass vessel

Ht: total height of emulsion

2.2.4. Viscosity of Emulsions

Approximately, 15 mL of each emulsion was taken, and its viscosity was measured using a viscometer (D 220, Brookfield, USA) at 30°C.

2.3. Experimental Design and Data Analysis

Design Expert software version 6.0.2 (Minneapolis, MN) was used to design the experiment for stability optimization of emulsions. Face centered ($\alpha=1$) central composite design with four central points was applied to determine the influence of three independent variables, SPC+MDX concentration (30-40w/w%), lycopene content (10-20w/w%) and homogenizer speed (14000-18000rpm) on stability of the emulsions. The un-coded (actual) and coded levels of the independent variables are illustrated in Table 1.

Table 1. Symbols, un-coded (actual) and coded levels of the independent variables

Symbol	Independent variables	Coded levels		
		-1	0	1
X1	Homogenizer speed (rpm)	14000	16000	18000
X2	Lycopene content %	10	15	20
X3	SPC+MD%	30	35	40

Droplets size, creaming index and viscosity were considered as responses. The terms statistically found non-significant ($p>0.05$) were removed from the initial models, and the experimental data were refitted to only the significant ($p<0.05$) factors to produce the final reduced models. Also, the fitting quality of models was expressed by the correlation coefficients (R^2) and adjusted R^2 .

For the graphical analysis of the independent variable interactions, use of 3D surface plots of the regression models has been highly recommended [20]. Thus, these plots were depicted from the fitted equations to explain the interactive effects of the independent variables against the response variables.

3. Results and Discussions

3.1. Fitting the Models

Measured responses (experimented and predicted values) which were considered as dependent variables, were given in Table 2.

Table 2. Predicted and experimental values of droplet size, viscosity and creaming index obtained from central composite experimental design of emulsions

Experiment number	Independent variables					Dependent variables			
	Homogenizer speed (rpm)	Lycopene content% w/w	SPC+MD% w/w	Droplet size (μm) ²		Viscosity (cp)		Creaming index%	
	X1	X2	X3	Experimented	Predicted	Experimented	Predicted	Experimented	Predicted
1	16000	10	35	26.02	24.64	37.10	35.29	45.14	41.20
2	16000	15	40	26.11	26.62	95.40	94.93	43.17	32.53
3	14000	20	40	39.99	38.43	99.80	106.85	54.95	49.88
4	16000	15	35	25.77	25.93	40.20	43.86	50.83	47.61
5	14000	10	40	23.90	20.89	58.80	53.55	40.31	47.07
6	18000	15	35	30.90	30.53	56.30	55.87	45.10	36.67
7	16000	15	30	45.11	36.44	21.00	14.35	56.12	62.68
8	14000	20	30	26.92	25.93	22.80	19.97	79.21	80.03
9	16000	15	35	25.07	25.28	40.50	43.86	50.00	47.61
10	18000	20	30	25.83	25.93	25.70	32.73	68.04	58.15
11	16000	15	35	25.82	27.97	40.50	43.86	50.52	47.61
12	18000	20	40	21.64	22.22	25.70	32.73	10.10	28.00
13	14000	10	30	25.38	37.64	40.50	43.86	57.04	67.22
14	18000	10	40	22.11	22.83	172.80	166.86	7.32	15.19
15	14000	15	35	32.30	35.64	18.80	26.52	54.64	58.55
16	16000	15	35	25.51	25.93	84.50	89.11	50.52	47.61
17	18000	10	30	23.19	24.72	38.40	31.71	46.19	45.34
18	16000	20	35	26.16	27.69	40.00	43.86	47.74	54.01

Table 3. Analysis of variance for the regression coefficients of the fitted models for emulsions

Variables	Droplet size			Viscosity			Creaming index		
	Regression coefficient	F-value	P-value	Regression coefficient	F-value	P-value	Regression coefficient	F-value	P-value
X_0	25.93			43.86			47.61		
Linear									
X_1	-7.29	160.16	<0.0001	12.08	29.17	0.0004	-10.94	17.60	0.0009
X_2	1.53	7.04	0.0226	17.80	63.32	<0.0001	6.40	6.03	0.0277
X_3	-1.95	11.93	0.0060	40.29	324.43	<0.0001	-15.08	33.43	<0.0001
2FI									
X_1X_2	-01.54	5.71	0.0359	6.11	5.97	0.0371	-	-	-
X_1X_3	0.72	1.04	0.3376	11.81	22.31	0.0011	-	-	-
X_2X_3	-0.29	0.17	0.6868	14.96	35.80	0.0002	-	-	-
Quadratic									
X_1^2	2.24	5.04	0.0463	-0.069	0.0002	0.9883	-	-	-
X_2^2	0.23	0.036	0.8543	9.23	5.25	0.0476	-	-	-
X_3^2	2.64	6.95	0.0232	10.78	7.17	0.0253	-	-	-
R^2	0.9571			0.9826			0.8030		
Adj- R^2	0.9089			0.9630			0.7608		

Most of the experimental values agreed well with the predicted ones obtained from RSM design and R^2 was high enough for all the variables to conclude that these models could properly fit the experimental values.

Analysis of variances (ANOVA) was used to evaluate the significance of the coefficients for each model (Table 3).

According to the ANOVA results, optimized variables showed a good fit to quadratic equations for droplet size and viscosity with correlation coefficients (R^2) of 0.9571 and 0.9826, respectively. After model simplification with backward stepwise solution, the R^2 values for droplet size and viscosity decreased slightly to 0.9504 and 0.9826, respectively. Creaming values were fitted properly with linear model and R^2 was 0.8030.

For any of the terms in the models, a large F-value and a small p -value indicate a more significant effect on the respective response variables [21]. Thus, the variable with the largest effect on the emulsion droplet size was the linear term of homogenizer speed, followed by the linear term of SPC+MDX concentration and lycopene content ($p < 0.05$). The quadratic terms of homogenizer speed and SPC+MDX concentration, as well as the interaction between homogenizer speed and lycopene content had significant influences on the droplet size of emulsions ($p < 0.05$).

For the creaming of emulsions, the largest effect was observed in case of linear term of SPC+MDX concentration, followed by those of homogenizer speed and lycopene content respectively ($p < 0.05$).

In case of viscosity, the highest influence was observed in case of linear term of SPC+MDX concentration, followed by those of lycopene content, interactive effect of SPC+MDX concentration and lycopene content, linear

term of homogenizer speed and interactive effect of SPC+MDX concentration and homogenizer speed, respectively ($p < 0.05$). Similarly, viscosity was significantly affected by the quadratic terms of lycopene content and SPC+MDX concentration, as well as interactive effect of homogenizer speed and lycopene content ($p < 0.05$).

3.2. 3D Surface Plots of the Emulsion Droplet Size

The effect of independent variables on responses was visualized using 3D surface plots of the quadratic and linear models generated by two independent variables within the experimental range, while holding the other one constant at the central point.

Figure 1. 3D surface plots of the emulsion droplet size as a function of lycopene content and homogenizer speed at a constant SPC+MD concentration (35%)

Results showed that an increase in homogenizer speed from 14000 to 18000rpm had a remarkable influence on droplet size of final emulsions and the droplet size significantly decreased with an increase in homogenizer speed ($p<0.05$), mainly attributed to the influence of higher energy densities on the collision of droplets accompanied with enforcing them between the rotor and stator of the device. Our results agreed with those reported by Haznedar and Dortunc (2004), Sun and Gunasekaran (2009) and Gardouh et al., (2012) and Gupta et al (2015), [22, 23, 24, 25].

Also, the concentration of lycopene had significant effect on droplet size of emulsions, which slightly increased at higher concentrations ($p<0.05$). It has been previously reported that higher concentrations of dispersed phase, hinder homogenization process, so higher energy density is needed to break down droplets and distribute them in the whole continuous phase (Haznedar and Dortunc, 2004; Mehnert and Mader, 2001 and Ghorab et al., 2004 and Mohammed et al., 2016), [22, 26, 27, 28].

Figure 2. 3D surface plots of the emulsions droplet size as a function of the SPC+MD concentration and homogenizer speed at constant lycopene content (15%)

It was revealed that higher concentrations of SPC+MDX (from 30 to 40 % w/w) resulted in a significant decrease in droplet size ($p<0.05$). In fact, higher viscosity of the continuous phase increases the impact rate between lycopene and the external phase, which results to a decrease in droplet size. Moreover, more concentrated emulsions provide larger surface areas between emulsion droplets and the continuous phase, which allows the formation of smaller droplets. These findings were in agreement with those data obtained by Haznedar and Dortunc (2004), Mehnert and Mader, (2001) and Boom (2008) and Hosseini et al., 2015, [22, 26, 29, 30].

3.3. 3D Surface Plots of the Emulsion Creaming Index

Figure 3. 3D surface plot of the creaming of the emulsions as a function of the lycopene content and homogenizer speed at constant SPC+MD concentration (35%)

As shown in Figure 3, both homogenizer speed and lycopene content had a significant effect on creaming index ($p<0.05$). Higher creaming index was observed at higher concentrations of lycopene, attributed to hindering of homogenization process, which resulted in larger droplets and accelerated phase separation due to gravitational forces.

On the other hand, homogenizer speed exhibited reverse effect on creaming index, which is attributed to larger and heavier droplets at lower homogenizer speeds [17]. These results were agreed with the findings of Haznedar and Dortunc (2004), Mehnert and Mader, (2001) and Ghorab et al. (2004), [22, 26, 27].

Figure 4. 3D surface plot of the creaming of the emulsions as a function of the SPC+MD concentration and homogenizer speed at constant lycopene content (15%)

Higher SPC+MDX concentration resulted in higher stability and lower creaming index. Using surfactants or (bio) polymers, which can adsorb onto the interface and rise electrostatic or steric repulsions between the droplets can postpone un-stabilization of emulsions. Also biopolymers can dissolve in continuous phase and influence the rheology of the continuous phase [28]. Our results were agreed with the findings of Dlużewska et al. (2006), Haznedar and Dortunc (2004) and Jafari et al. (2007b) and Hosseini et al., 2015. [31, 22, 11, 30]

3.4. 3D Surface Plots of Emulsion Viscosity

Effect of lycopene content and homogenizer speed on viscosity of emulsions was illustrated in Figure 5.

Figure 5. 3D surface plot of the viscosity of the emulsions as a function of the lycopene content and homogenizer speed at a constant SPC+MD concentration (35%)

The viscosity was significantly affected by lycopene contents ($p < 0.05$). One of the most important factors, which can influence viscosity, is the disperse phase volume fraction, which is equal to the volume of emulsion droplets divided by the total volume of emulsion [6]. Disperse phase droplets can change hydrodynamic and colloidal interactions and consequently, the viscosity of system by increasing degree of disturbance of normal liquid flow [28, 30, 32, and 33]. Our results agreed with those of Daik et al. (2007), [34].

Additionally, viscosity was significantly affected by homogenizer speed ($p < 0.05$). A reduction in droplet size, resulted in an increase in the number of droplets in the system, and consequently, resulted in enhanced droplet-droplet interactions and an increased resistance to flow. These findings were agreed with those reported by Daik et al. (2007) and Hosseini et al., 2015. [33, 30]

Figure 6. 3D surface plot of the viscosity of the emulsions as a function of the SPC+MD concentration and homogenizer speed at constant lycopene content (15%)

Viscosity was more significantly affected by the concentration of solid material (SPC+MDX) as compared to homogenizer speed ($p < 0.05$). By increasing and adding more soluble materials in to emulsions, they bound with free water, and finally resulted to more viscose systems. This function, in emulsions, can have significant effect on lowering creaming index and producing long-lasting systems.

3.5. Optimization of Conditions for Preparing Lycopene Emulsions

An emulsion can be more stable by decreasing droplet size and creaming index, so optimization was carried out by first superimposing the contour plots for the droplet size, creaming index and viscosity of the emulsion as functions of SPC+MDX concentration and lycopene content at a fixed homogenizer speed of 18000rpm (Figures 7a, 7b and 7c). Economically and technically, an emulsion with higher contents of lycopene and lower emulsifier concentration (SPC+MDX) is suggested. These assumptions, resulted in an optimum zone, in which, every point would represent a combination of SPC+MDX concentration and lycopene content that would give minimum values for the droplet size and creaming index, and maximum viscosity of the emulsions. Finally, from the principles mentioned above, the point at the lycopene content and emulsifier concentrations (SPC+MDX) of 20 and 36.95% w/w, respectively, was chosen as the optimum point, along with the optimized homogenizer speed of 18000rpm.

(a)

(b)

(c)

Figure 7. Contour plots for the droplet size (a), creaming index (b) and viscosity(c) of the emulsion as a function of SPC+MD concentrations and lycopene content to find optimized point at a fixed homogenizer speed of 18000rpm

4. Conclusions

Results showed that, according to RSM analysis and models, quadratic model was adequately fitted to optimize and predict the responses of droplet size and viscosity, but linear model was suggested for creaming index. The linear terms of homogenizer speed, SPC+MDX concentration and lycopene content, had the largest effects on the emulsion droplet size.

Linear terms of SPC+MDX concentration, homogenizer speed and lycopene content, significantly affected creaming index, but none of the quadratic terms and interactive effects significantly affected it.

Finally, emulsion viscosity was highly affected by linear terms of SPC+MDX concentration, lycopene content, as well as interactive effect of SPC+MDX concentration and lycopene content, linear term of homogenizer speed and interactive effect of SPC+MDX concentration and homogenizer speed, respectively. Additionally, quadratic terms of lycopene content and SPC+MDX concentration and interactive effect of homogenizer speed and lycopene content exhibited significant effect on viscosity.

The graphical optimization method was adapted to find the best emulsifying conditions, and it was predicted that the optimum conditions for preparing lycopene emulsions were: homogenizer speed of 18000 rpm; lycopene content of 20% w/w, and SPC+MDX concentration of 36.95% w/w.

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