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Soy Protein Isolate and Gum Arabic Composite Affects Stability of Beverage Emulsion

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Abstract

The aim of the present work was to evaluate the effect of soy protein isolate (SPI)-gum arabic (GA) composite on the stability of beverage emulsion prepared as the orange oil-in-water (O/W) emulsion. Response surface methology (RSM) was used to study the influence of the two independent variables, each at three levels (homogenization speed as x_1 (1500, 15700, and 30000 rpm) and GA:SPI ratio as x_2 (1, 0.6, and 0.2) on the two dependent variables namely, size of the emulsion droplet (EPS) as y_1 and emulsifying activity (EA) as y_2. The results were discussed on the two empirical models obtained. Analysis of variance (ANOVA) showed a high coefficient of determination (R^2) value of 0.937 and 0.979 for EPS and EA, respectively, ensuring a satisfactory adjustment of the second-order regression model with the experimental data. The negative sign for the regression coefficient of x_1, indicated that size of the emulsion droplet increased with the decreased level of factor x_1 from 30000 to 1500 rpm, while EA increased with increased levels of factor x_1. Thus, GA: SPI ratio with the lowest amount of GA along with the homogenization speed at high level, gave the smallest size of emulsion droplet (1μm). This size corresponded to the high emulsifying activity and stability. Sodium chloride and freeze-thaw cycling are two environmental factors affecting emulsion stability. Results of freeze-thaw cycling test showed a large decrease in the EA of the sample prepared with a low level of SPI:GA ratio and no dependence of the EA on the NaCl concentrations in the range of the tested levels was observed (50-150 mM). Long-term storage stability test (storing the emulsion samples at 4, 16, and 30ºC each for 8 days) showed the size of the emulsion droplet increased with the storage time. Results of use of SPI as a readily available plant source of protein for preparation of the encapsulant for orange oil-emulsion were encouraging, especially when one considers the dependence of the beverage industry on the unreliable source of supply of GA. RSM was successfully applied for the modeling of the emulsion preparation.

Keywords: *Central composite design, Gum arabic, Oil-in-water emulsion, Microencapsulation, Response surface methodology, Soy protein isolate*

Introduction

Gum arabic (GA), the dried exudate from certain species of the *Acacia* trees and *Acacia senegal*, grown principally in Sudan, is the main species used in foods [1]. The ability to form stable emulsions (i.e., oil-in-water emulsion, O/W) over a wide range of pH and in the presence of electrolytes, made this biopolymer an appropriate natural emulsifier which has been intensively used in

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food industries, particularly in the beverage sector [2]. Considering the complexity of GA molecular structure and its relation to the emulsifying function has encouraged many researchers to conduct studies in the hope of finding a suitable substitute for GA. GA is shown to be a hetergenous polymer and the presence of the three main structural components have been recognized in it. Proteins are present in all these fractions, while the proteins in the arabino-galacto-protein (AGP) fraction is shown to give GA its emulsifying properties [2,3]. As proposed, the hydrophobic and protein rich backbone at GA adsorbs onto the O/W emulsion interface while the carbohydrate units having sparse amounts of the charged amino acids make up the hydrophilic part of the GA polymer. Its configuration on the interface of the O/W emulsion shows that those units project into the aqueous solution [2].

During the process of making a mixture of two immiscible substances, minimal contact between the oily phase and water can initially be achieved by the formation of small spherical droplets through the input of work and addition of emulsifier, facilitating the formation of these small particles by reducing interfacial tension. In fact, the aim of emulsification such as O/W is to produce a small size of the dispersed phase (oil) and the size of the droplets should not be increased during emulsion storage. This means that emulsions have to be stable for a certain period of time.

Proteins stabilize emulsions through structuring water molecules in the ordered manner, thus the contact of hydrophobic groups with water can be minimized. This state is thermodynamically most favorable [4]. In fact, upon unfolding protein structure (i.e., destabilized proteins) the orientation of the hydrophilic segment of the protein is toward the aqueous phase and the hydrophobic part directs itself toward the organic phase. In this manner surface or interfacial tension is reduced (i.e., facilitation of emulsification).

Among the gains of applying spray-drying microencapsulation process in the food industry, two are considered major ones: improving the chemical stability of volatile flavor compounds and providing controlled release of the flavor from microcapsulated flavorant products [5]. There is relationship between moisture content of the spray-dried product and viscosity of the liquid emulsion prior to spray-drying [6]. The structure and porosity of the spray-dried particles are other factors that affect the water–holding properties of the product during the drying process. Parameters such as drying rate, drier inlet and exit air temperature differentials also influence the properties of the spray dried products [5]. The efficiency of the microencapsulation process in the production of spray-dried O/W microcapsules could be evaluated by testing several properties of the capsules such as moisture content, particle size, total and surface oil. Studies have been directed towards the use of food proteins for the efficient production of the capsules [6, 7].

Optimization of many industrial and research processes could conventionally be performed by use of one-at-a-time variation at treatment variables. This method assumes that various treatment parameters do not interact and that the response variable is only a function of the single varied parameter. However, the response obtained from a treatment of interest could be a function of the interactive relationships which exist between the different variables. Thus when a combination of several independent variables and their interactions affect the desired dependent variable, use of response surface methodology (RSM) has been found to be an effective optimization method, statistically [8, 9]. For fitting a model by performing least squares techniques, RSM uses the design of an experiment such as the central composite design (CCD) [9]. The adequacy of the proposed model is then revealed using the diagnostic checking tests on the basis of applying analysis of variance (ANOVA). The response surface plots can then be employed to study the surfaces and locate the optimum

position.
In the present work the stability of the O/W beverage emulsion prepared with soy protein and GA composite was evaluated statistically and by use of RSM, the process was optimized in terms of the modeling equations for the activity and stability of the emulsion.

Materials and methods

Materials

Commercial SPI was purchased from Gusheen Biological Technology Group, China (product code, GS 5100). Natural orange cold pressed oil was obtained from Givaoudan Company, Switzerland (product code, 012210). GA was a gift from the Zam Zam Beverage Company, Tehran-Iran.

Emulsion preparation

Orange oil-in-water emulsion was prepared in accordance with the procedure described [10]. In order to provide a predetermined ratio of GA to SPI (i.e., l, 0.6, 0.2), varying quantities of GA and SPI, in total 6 g, was added to 74 ml distilled water in a beaker and mixed for 10 min at 20-25ºC with a magnetic stirrer at 900 rpm. The mixture was transferred to a mixer (Janke and Kunkel-Ika-Werk, Re166/D7813, Staufen, Germany) and 20 g cold pressed orange oil was gently added to the mixture, while mixing continued for 2 min. The coarse emulsion was then subjected to homogenization (Edmund Buhler, 7400 Tubingen, HO4) and homogenized for 5 min (Table 1).

Emulsifying activity and stability

Spectrophotometric methods for measuring emulsifying activity and stability were used [7, 11, 12]. The emulsion was diluted to about 0.002 wt% of the orange oil, using acetate buffer solution (0.2 M, pH 4-4.2). The diluted emulsion sample was then transferred into a cuvette and the absorbance was read almost immediately at 600 nm (emulsifying activity). Absorbance reading after storing the emulsion in the cuvette for 24 hrs at room temperature was then recorded as the emulsion stability. Depth of cream layer and centrifugal methods were also used for stability determination of the O/W emulsion [7]. Briefly, 20 ml emulsion was poured in a test tube and the tubes were stored for a pre-determined time at room temperature (20-25ºC). The depths of free oil and cream layers that formed were measured with calipers. Also, a sample of the emulsion was placed in the centrifuge tube and then incubated at 30ºC for 24 hrs. The emulsion sample was centrifuged for 10 min at 3500 rpm. With the use of the formula given, below stability was reported as percentage: $S=(v_0-v)/v_0\times100$ where S is the emulsion stability, v_0 and v are the volume (ml) of the initial and of the discharged phase, respectively [13].

Light microscopy-emulsion droplet size determination

The morphology of droplets in the O/W emulsion was obtained using a light microscope equipped with a camera (Leica, DMR HCS, Germany). Microscopic size measurement was carried out using Windows v.2.41. A drop of the emulsion was placed on a slide glass and observed at 1000x magnification.

Environmental stresses on the orange oil-in-water emulsion

Environmental stresses on the O/W emulsion were performed in two forms as effects of: freeze-thaw cycling and different concentrations of NaCl. The emulsion stability was then determined spectrophotometricaly [12, 14]. The emulsion was diluted to about 0.002 wt% of the orange oil using acetate buffer (0.2M, pH 4-4.2) and 10 ml of the diluted sample was then transferred into a test tube. The sample was frozen in a freezer (-20ºC) and after 24 hrs thawed in a water bath at 30ºC for 2 hrs. This freeze-thaw cycle was repeated five times. The EA was measured each time, by applying the procedure mentioned above.
NaCl stability test was also performed: the

emulsion was diluted to about 0.002 wt % of the orange oil using the acetate buffer while the appropriate concentration of NaCl was provided in each of the test samples as needed (mM):50, 100, 500. The EA was recorded according to the description given above.

Experimental design and data analysis

A CCD in the form of 2^2 full factorial design was used in which two independent variables were converted to dimensionless ones (x_1, x_2) with coded values at 3 levels: -1, 0, +1. The arrangement of CCD as shown in Table l was in such a way that allows the development of the appropriate empirical equations (second-order polynomial multiple regression equations) [8,9]:

$$y=\beta_0+\beta_1x_1+\beta_2x_2+\beta_{11}x_1^2+\beta_{22}x_1^2+\beta_{12}x_1x_2 \qquad (1)$$

The predicted response (y) was therefore correlated to the set of regression coefficients (β's): the intercept (β_0), linear (β_1, β_2) interaction (β_{12}) and quadratic coefficients (β_{11}, β_{22}). The Design Expert (version 5) and Statistica (version 5) softwares were used for regression and graphical analyses of the obtained data.

Results and discussion

Size of orange oil emulsion particles

The ability of the microencapsulants to coat, uniformly, the dispersed phase droplets (i.e., oil phase in an O/W emulsion) during homogenization would prevent coalescence of the emulsion particles after homogenization. Thus, the size of the emulsion droplets affects the physical stability of the dispersed phase. In the present work the quantitative relationship between the two criteria of the O/W emulsion (size of the emulsion particle, y_1 and emulsifying activity, y_2) and two controllable parameters (proportion of GA and SPI used to make emulsion and keeping orange oil inside the particles and speed of homogenization) were studied. A CCD arrangement shown in Table 1 allows the development of mathematical equations where each response variable y is assessed as a function of these two regressors and the equation is calculated as the sum of a constant, two first order effects (terms in x_1 and x_2), one interaction effect (term in x_1x_2) and two second order effects (x_1^2 and x_2^2) (Eq. 1). The results obtained were then analyzed by ANOVA to assess the goodness of fit. Only terms which were found to be statistically significant were included in the model. β_{22} and β_{12} coefficients for the emulsion particle size and β_2 and β_{11} coefficients for the emulsion activity were found to be non-significant. Therefore, these coefficients were dropped from the model and a new ANOVA was then performed for the reduced model. The models for EPS and EA (y_1 and y_2) were significant by the F test at the 5% confidence level (Prob>F<0.05). It was then possible to investigate quantitatively the effects of x_1 and x_2 on the characterization of the O/W emulsion by use of the following fitted regression models (equations in terms of coded values for the regressors):

size of the emulsion particle (EPS):

$$y_1=4 - 4.5x_1+ 1.67x_2+ 3.5x_1^2$$

Emulsifying activity (EA):

$$y_2=0.66 + 0.33x_1+ 0.082x_1x_2 - 0.13x_2^2$$

Table 1. Arrangement of the CCD for the two independent variables along with the experimental and predicted responses

Experiment order	actual and coded value for design variable		Responses*	
	Homogenization speed(rpm,x_1)	GA:SPI ratio (x_2)	y_1 (EPS) (μm)	y_2(EA) abs_{600} nm
1	1500(-1)	0.2(-1)	(10.330)12	(0.282)0.2796
2	30000(1)	0.2(-1)	(1.330)1	(0.778)0.777
3	1500(-1)	1(1)	(13.670)14	(0.118)0.16
4	30000(1)	1(1)	(4.670)5	(0.942)0.9865
5	1500(-1)	0.6(0)	(12.000)10	(0.330)0.311
6	30000(1)	0.6(0)	(3.000)3	(0.990)0.9838
7	15750(0)	0.2(-1)	(2.330)2	(0.530)0.4774
8	15750(0)	1(1)	(5.670)6	(0.530)0.491
9	15750(0)	0.6(0)	(4.000)4	(0.660)0.6505
10	15750(0)	0.6(0)	(4.000)4	(0.660)0.6505

* Theoretically predicted value for each of the two responses is given in parenthesis.

Statistical parameters obtained from the ANOVA for the two fitted models (reduced quadratic models) for the O/W emulsion characterization are given in Table 2. Since R^2 always decreases when a regressor variable is removed from a regression model, in statistical modeling the adjusted R^2 which takes the number of regressor variables into account, is usually selected [15]. The coefficient of determination of R^2 gives the proportion of the total variation in the response variable which is explained or accounted for by the regressors (x's) included in the model. In the present study the adjusted R^2 for the size of the emulsion particle and emulsifying activity were 0.937 and 0.979, respectively. The R^2 coefficient in this study ensured a satisfactory adjustment of the reduced quadratic model to the experimental data. As given in Table 2, the coefficient of variance (CV) for the y_1 and y_2 was found to be (%): 18.12 and 7.03, respectively. The CV as the ratio of the standard error of estimate to the mean value of the observed response (as a percentage) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if its CV is not greater than 10% [16]. The model fitted for the size of the emulsion particles had a relatively high CV which may be related to some of the difficulties in obtaining a representative sample for the microscopic examination (i.e., the emulsion droplet size determination). This model had high R^2 value and showed no lack of fit. By applying diagnostic plots such as normal probability plot of residual, and plot of residuals versus predicted values for the emulsion particle size, the assumptions of normality, independence and randomness of the residuals were satisfied. The fitted model for y_1, thus was accepted. The adequate precision value is a measure of the signal to noise ratio and was found to be 17.64 and 34.05 for the size of the emulsion droplets and emulsifying activity, respectively. A ratio>4 is desirable and indicates an adequate signal [16]. The predicted models for y_1 and y_2 were satisfactory and could be used to navigate the space defined by the CCD.

Table 2. Statistical parameters obtained from the analysis of variance of the fitted model.

Parameter	(μm) $\hat{y}_1$	(abs_{600} nm) $\hat{y}_2$
R^2	0.958	0.986
R^2 adjusted	0.937	0.979
F value	45.7	146.33
Prob>F.0	0.0002	<0.0001
Coefficient of variance	18.12	7.03
Std, Dev.	1.11	0.041
Adequate precision	17.64	34.05

The relative contribution of each of the two regressors to each of the two dependent variables (y_1 and y_2) was directly measured by the respective coefficient in the fitted model. A positive sign for β_2 in the fitted model for the emulsion particle size indicates that the droplet size increased with increased GA: SPI ratio. The largest size of the droplet (~14 μm) corresponded to GA: SPI ratio =1, while the speed of homogenization was lowest (1500 rpm) (Table 1). A negative sign for the regression coefficient of β_1 in the fitted model of y_1 indicates that the particle size of the emulsion decreases with increase in the speed of homogenization (Table 1). However, the contribution of the β_1 coefficient in the y_1 fitted model was found to be more as compared to that in the y_2 model. Interaction between x_1 and x_2 exists in the y_2 model, i.e., effect of x_1 on the emulsifying activity depends on the level of x_2. The highest EA was at x_2=0.6 and x_1=30000 rpm, and the lowest level of EA was at the high level of GA:SPI ratio=1 when x_1 was equal to 1500 rpm (Table 1). These results are in agreement with the findings reported in the relevant literature [7, 11, 12]. The theoretically predicted values for the emulsifying activity and the emulsion droplet size in the present study are also given in Table 1. The same table includes the experimental data for these response variables. The predicted models are presented in Figs. 1 and 2 as the three dimensional response surface plots. The emulsion droplet size and the emulsion activity were affected by the changing levels of the two regressors, namely speed of homogenization (x_1) and the proportion of GA:SPI ratio (x_2). It is apparent from Fig. 1 (3D) that at low levels of homogenization speed when the GA:SPI ratio was also low, large size emulsion droplets were obtained and this emulsion property was evident even when the ratio of GA:SPI was high while the speed of homogenization still was at its low level (see also Table 1, exp. order of 1 and 3). The photomicrograph prepared using a light microscope was in accordance with these results (Fig. 1a). Fig. 1b shows that distribution of the particles would be uniform when GA:SPI ratio was at low level and the speed of homogenization was at its highest level. Figure 2 shows that EA was at its low level when x_1 was kept at the low and GA:SPI ratio was also low (see also Table 1, exp. order of 1). This low level of EA was obtained when x_1 was at its low level while x_2 was highest (see also Table 1, exp. order of 3).

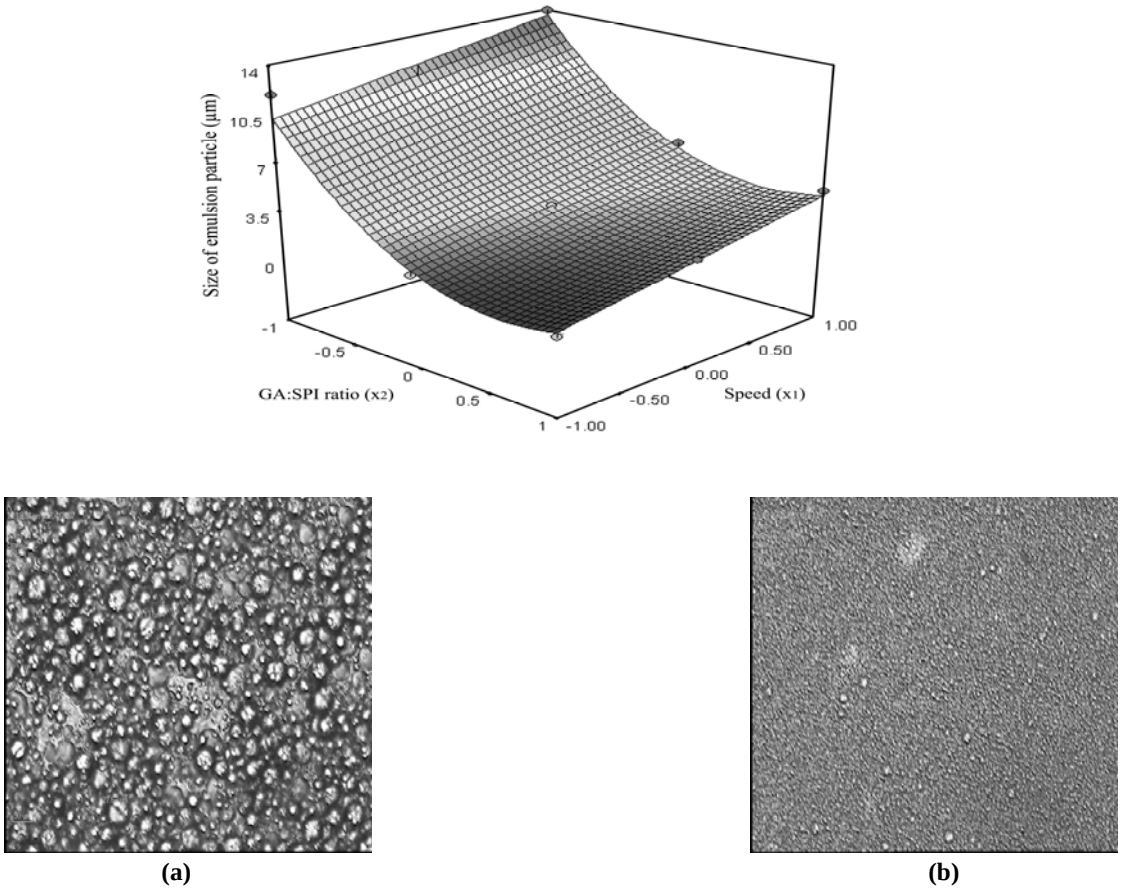

Figure 1. Response surface diagram for the orange oil emulsion droplet size as affected by the changing levels of the two regressors, namely speed of the homogenization (x_1) and the GA:SPI ratio (x_2) (the three dimensional plot, 3D). Photomicrographs of the O/W emulsion were also shown: x_1=1500 rpm and x_2=1 (a); x_1=30000 rpm, x_2=0.2 (b).

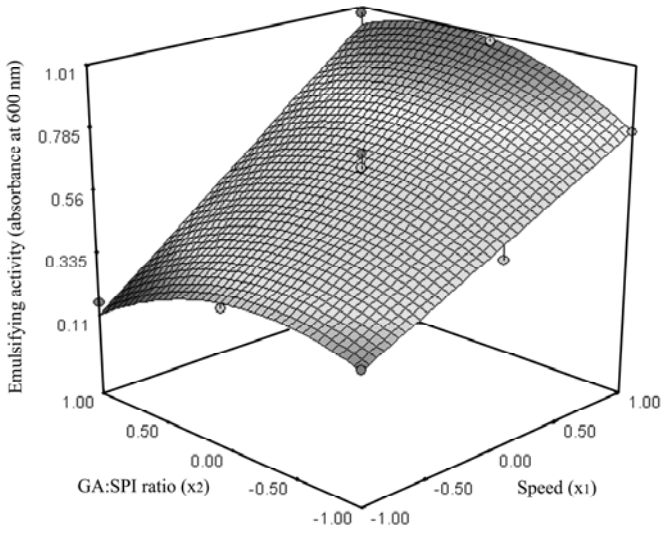

Figure 2. Response surface plot for the emulsifying activity as affected by the changing levels of the two regressors, namely speed of the homogenization and the GA:SPI ratio.

Physical stability of orange oil emulsion particles

By measuring the depth of the cream layer that formed during storage of the emulsion for 15 days at 20-25ºC as described under the materials and methods section, the physical stability of the emulsion was evaluated. Results in Table 3 indicate that the emulsions made of the GA:SPI ratio of 0.2 and with use of high speed homogenization, were the most stable (higher absorbance corresponding to higher emulsifying activity and stability). The emulsion stability is highly related with the small droplet size (Table 1 and 3). Emulsion with a droplet size ranged 1-2 μm showed the highest emulsion stability (100%) according to the centrifugal method (Table 3). Moreover, the results of the centrifugal method and depth of the cream layer were in good accordance with the results of the emulsifying stability based on the turbidimetric technique (Table 3). Although centrifugation is a rapid method of measuring emulsion stability, it does not indicate necessarily that coalescence of the emulsion droplets proceeds with time [13]. As seen, the lowest size of the emulsion droplet (i.e., 1 μm with x_1= 30000 rpm and x_2= GA:SPI ratio =0.2) corresponded to the highest absorbance (0.57 at 0 hr), and the absorbance reading for the emulsion made without SPI and with only GA, decreased to about 90%. Emulsifying activity of GA on the basis of the structural specifications has been studied and using the chemical cleavage approach, researchers showed that glycosyl phos-phatidylinositol (GPI) lipid component had an important role on the EA of GA, although the protein fraction in association with the lipid also had a pronounced effect on this property [3, 11]. Structural basis of the wall prepared from the GA:SPI combination is not clear but changing the proportion of GA to SPI along with the homogenization protocol (i.e., speed) used in the present work certainly affected the emulsion characteristics.

Table 3. Physical stability of the orange oil emulsion

Specification of emulsion preparation	Turbidity measurement (absorbance at 600 nm)	Depth of cream layer (cm)	Centrifugal method (%)
at 30000 rpm:			
GA:SPI=1	0.4477	ND*	50
GA:SPI=0.6	0.1559	ND	85.5
GA:SPI=0.2	0.5716	ND	100
GA	0.0491	0.311	0.16
at 15750 rpm:			
GA:SPI=1	0.313	0.33	40
GA:SPI=0.6	0.1240	ND	57.14
GA:SPI=0.2	0.2316	ND	100
GA	0.0363	0.366	0.28
at 1500 rpm:			
GA:SPI=1	0.0117	0.63	40
GA:SPI=0.6	0.0645	0.31	57.14
GA:SPI=0.2	0.0543	0.26	62.85
GA	0.1296	0.55	≈ 0

* No cream layer was detected.

Further studies were carried out on some selected emulsions with the particle size (μm): 1, 3, and 5, which were prepared using the highest speed of homogenization (30000 rpm) (Table 1). Fig. 3 shows the results obtained from the influence of several environmental factors on the emulsifying activity of the emulsions. Emulsion particle size as a function of sodium chloride concentration showed no dependence of the turbidity on the NaCl concentration (Fig 3a). Behavior of proteins in solution in relation to the ionic strength of the surrounding environment (aqueous media as the solvent) depends on interactions made both internally within the protein molecules and between the protein and its surrounding solvent. The 'salting in' effect is related to the non-specific effect of salt on increasing the ionic strength of the protein solution (decreasing sphere of influence of each charged site on the protein, 'Debye length') while some salts at high concentration compete with protein molecules to interact with solvent (H_2O), resulting in decreased protein solubility (salting out) [17]. Apparently, NaCl at the concentrations used in the present work did not affect either the solubility of the protein (i.e., SPI) or its precipitation. Unavailability of proteins to associate with polysaccharide molecules (such as GA) increases the chance of bridging emulsion particles together (increasing possibility of the particle aggregation thus increasing the droplet size). One may say that there could be no positions on the interface of the emulsion available for connecting the wall material and this emulsion behavior corresponds to decreasing emulsifying activity and stability (Fig. 3a). The results reported elsewhere showed that in spite of obtaining emulsion droplet size in the range of 1 μm by use of sodium dodecyl sulfate (SDS) surfactant during the O/W emulsion, creaming was observed (the destabilized emulsion) [18]. In a separate study, use of SDS at low concentration (< 4 mM) resulted in large emulsion droplets in which the particles were unstable to coalescence [12]. The result of the freeze-thaw cycling test showed a large decrease in the emulsifying activity of the emulsions after five times (Fig. 3b). Crystallization of water molecules during the freezing process and penetration of the ice crystals into the interfacial layer and disruption of this layer are among possible changes which may occur during the freezing process and affect the stability of the emulsions [12]. As the emulsion droplets get closer, low absorbance corresponding to lower emulsion activity and stability with the continuation of the water crystallization process, could account for the events occurring during freeze-thaw cycling test [14] (Fig. 3b). Results of the long-term storage stability test also showed that the size of the emulsion droplets increased upon increasing the time of storage and this corresponded to the decreasing level of the emulsifying activity (turbidimetric assay) (Fig. 3c)

Conclusion

Results of this study showed that soy protein isolate in combination with the lowest amount of gum arabic was able to function well for preparation of the O/W emulsion. These emulsions were prepared through a simple protocol. SPI as a readily available food protein may act as a bridging unit between the orange oil dispersed phase and the water continuous phase, and thus facilitates formation of the molecular film layer formed around each oil droplet and has a role in maintaining the integrity of this film. Disruption of the film causes the oil droplets to come closer and coalesce and form larger droplets in which eventually the oil phase separation occurs (destabilized emulsion). Use of only GA as the encapsulant of the orange oil corresponded to the largest particle size and the resulting emulsion was the least stable. Improvement of the physical properties of soy protein isolate film has been reported by blending soy protein with polsaccharides such as starch, sodium alginate, even other proteins (whey or gelatin) (composite film)[19, 20]. The results of the present study regarding use

of the SPI-GA composite were in agreement with these recent findings. Use of the experimental design approach showed that the mathematical relationships exist between two major dependent variables i.e., emulsion droplet size and emulsifying activity, and two independent variables (proportion of SPI and GA composite and homogenization speed). The emulsion droplet size was significantly affected by the two regressors, x_1 and x_2, and the emulsifying activity was affected by x_1 and the joint effect of x_1x_2.

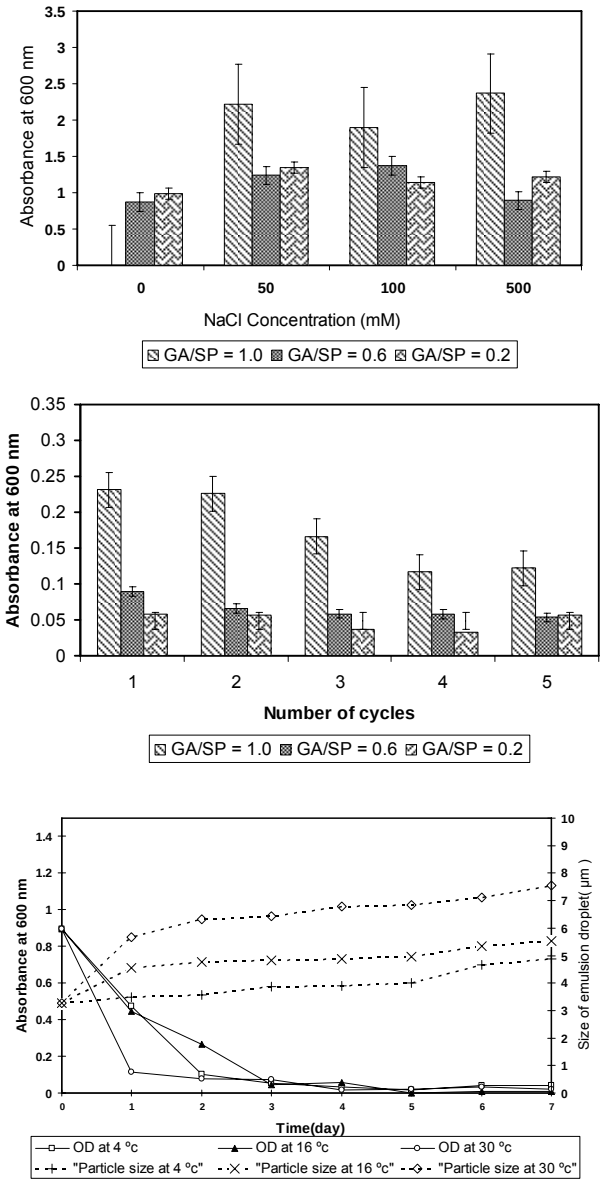

Figure 3. Dependence of the emulsion stability determined as turbidity (λ_{600} nm) on concentration of NaCl (a); on number of freeze-thaw cycles (b). Long term storage stability of the emulsion is also shown (c).

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