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EFFECT OF COMPOSITION AND HOMOGENIZATION PRESSURE OF CHIA OIL EMULSIONS ELABORATED BY MICROFLUIDIZATION

EFECTO DE LA COMPOSICIÓN Y PRESIÓN DE HOMOGENIZACIÓN EN EMULSIONES DE ACEITE DE CHÍA ELABORADAS MEDIANTE MICROFLUIDIZACIÓN

S.C. Pereyra-Castro[1], V. Pérez-Pérez[1], H. Hernández-Sánchez[1], A. Jiménez-Aparicio[2], G.F. Gutiérrez-López[1], L. Alamilla-Beltrán[1]*

[1]*Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Av. Wilfrido Massieu s/n. U. Profesional Adolfo López Mateos. CP 07738. Gustavo A. Madero. Ciudad de México, México.*

[2]*Centro de Desarrollo de Productos Bióticos. Instituto Politécnico Nacional. Carretera Yautepec-Jojutla, Km. 6, calle CEPROBI No. 8. Col. San Isidro. C.P. 62731. Apartado Postal 24. Yautepec, Morelos, México. Tel. (55) 57296000 Ext. 82500*

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Abstract

The effect of continuous phase composition and microfluidization pressure on properties of chia oil emulsions was analyzed using a D-optimal combined design. The results indicated that the suggested models were significantly ($p \leq 0.05$) fitted for all response variables (emulsion droplet size, ξ-potential, Turbiscan stability index and apparent viscosity) and showed high coefficients of determinations (0.897 - 0.933). The soy protein isolate had the major effect on the evaluated parameters due to their emulsifying property and electrical charge. The microfluidization technology allowed the production of emulsions with a droplet size in the range of nanometers (<500 nm) and monomodal particle size distribution. The optimum condition to obtain the minimum emulsion droplet size (300.97 nm), ξ-potential (-40.03 mV), Turbiscan stability index (1.06) and apparent viscosity (18.65 mPa·s) was when a chia oil emulsion was formulated with 4.8% w/w of SPI and 25.2% w/w of MD, applying high microfluidization pressure (104 MPa).

Keywords: soy protein Isolate, maltodextrin, microfluidization, emulsion stability, D-optimal combined design, chia oil.

Resumen

El efecto de la composición de la fase continua y presión de microfluidización sobre las propiedades de las emulsiones de aceite de chía se evaluaron a través de un diseño combinado D-óptimo. En todas las variables respuestas analizadas (tamaño de micela, potencial-ξ, índice de estabilidad Turbiscan y viscosidad aparente) los modelos obtenidos presentaron un ajuste significativo ($p \leq 0.05$) y un coeficiente de determinación alto (0.897 - 0.933). El aislado de proteína de soya mostró la mayor influencia sobre los parámetros evaluados debido a la propiedad emulsificante y carga eléctrica que presenta. La tecnología de microfluidización permitió la producción de emulsiones con tamaño de micela en el rango de los nanómetros (<500 nm) y una distribución unimodal. Las condiciones óptimas para obtener el menor tamaño de gota (300.97 nm), potencial-ξ (-40.03 mV), índice de estabilidad Turbiscan (1.06) y viscosidad aparente (18.65 mPa·s) se obtuvieron al utilizar 4.8% w/w de aislado de proteína de soya y 25.2% w/w de maltodextrina, aplicando una alta presión de microfluidización (104 MPa).

Palabras clave: aislado de proteína de soya, maltodextrina, microfluidización, estabilidad de la emulsión, diseño combinado D-óptimo, aceite de chía.

1 Introduction

Generally, an optimal emulsion for oil encapsulation has small size and narrow distribution of oil droplets, is stable to agglomeration and coalescence, has low viscosity at high solid content for enabling to form a continuous matrix (continuous phase) where oil droplets are uniformly distributed and embedded (dispersed phase) (Bae and Lee, 2008; Turchiuli *et al*., 2014). In the food industry, the main carriers

* *Corresponding author. E-mail*: liliana.alamilla@gmail.com; lalamill@ipn.mx.
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used for oil encapsulation are polysaccharides, starch, celluloses, gums, and proteins; all of them can be used alone or in combination.

However, it has been reported that a mixture of biopolymers can improve the characteristics of the final product. For example, the interfacial properties of a protein can be enhanced by the interactions between an adsorbed protein and a polysaccharide, which may lead to the formation of a secondary macromolecular stabilizing layer (Sheu and Rosenberg, 1995; Taneja *et al*., 2013).

Soy Protein Isolate (SPI) is a mixture of various protein fractions widely used in the food industry to formulate emulsions. These storage vegetal proteins have a globular structure that forms a thick interfacial layer when it is used in emulsification, acting as a physical barrier against the coalescence phenomenon (Roesch and Corredig, 2003; Tang and Liu, 2013). On the other hand, maltodextrin is widely used as wall material for capsule formation, due to its high solubility and low viscosity at high solids contents (Bae and Lee, 2008; Karaca *et al*., 2013; Negrao *et al*., 2017). Maltodextrins with a high dextrose equivalent (as 20 DE) increase the glass transition temperature of microcapsules and promotes the retention of components during spray drying (Fongin, Kawai, Harnkarnsujarit, & Hagura, 2017). Particularly, it has been reported that the combination of proteins (of animal or vegetable origin) and carbohydrate is an effective wall material blend for oils encapsulation (Chronakis, 1998; Sheu and Rosenberg, 1995).

The structural components of an emulsion system are the continuous phase formed by polysaccharides, starch, gums, and proteins; and the dispersed phase that generally is an oily component. Chia (*Salvia hispanica* L.) oil could be used as a dispersed phase; this oil contains linoleic (17.26%) and α-linolenic (50.57%) acids, and it has been reported that omega-3 unsaturated fatty acids play an essential role in physiology, especially during fetal and infant growth. They are also important for the prevention of cardiovascular diseases, because of their antithrombotic, anti-inflammatory, and antiarrhythmic properties, as well as, favoring platelet stabilizing (Ixtaina *et al*., 2011; Rodea-González *et al*., 2012).

During emulsion formation, the homogenization is an important step due to the emulsion droplet size (EDS) reduction improves its stability. Emulsions are produced by low-energy methods (such as phase inversion and spontaneous emulsion) and high-energy methods (such as microfluidization, pressure valve homogenization, and sonication).

Microfluidization creates high-velocity micro-streams as a fluid accelerates into an interaction chamber, generating high shear and impact forces that cause the formation of fine emulsions (Ciron *et al*., 2010; Shen and Tang, 2012).

It has been reported, that homogenization pressure and the number of cycles have significant effects on emulsion properties, like droplet size and physical stability during storage (Ricaurte *et al*., 2016; Tan and Nakajima, 2005). In order to establish an optimal emulsion process, involving relevant variables as homogenization pressure, cycles, droplet size, or components concentration, an optimization tool is important.

Response surface methodology (RSM) represents a collection of statistical and mathematical techniques that are used for modeling and problems analysis in which a response of interest is influenced by several variables and its optimal value must be attained (Sumic *et al*., 2016). It is important to mention, that several studies have used different statistical experimental designs to establish an optimal emulsion condition based on diverse biopolymers blends (Granillo-Guerrero *et al*., 2017; Mirhosseini *et al*., 2008; Monroy-Villagrana *et al*., 2014; Ricaurte *et al*., 2016; Taghi *et al*., 2012).

However, there is an information lack about the application factors of D-optimal combined design as a tool to evaluate the effect of a process variable and the components mix on the emulsion stability. Some advantages of using this kind of design are the analysis in an irregular experimental region and when resources are limited or there are constraints on factor-settings (Montgomery, 2013).

Therefore, the aim of this work was to evaluate the effect of the microfluidization pressure and the composition of the continuous phase (SPI – MD percentage) on the emulsion stability, droplet size, zeta potential and apparent viscosity of the chia oil emulsions. In order to determine the optimal level of process and mixture factors, to minimize the response variable, a D-optimal combined design was applied. The results can be helpful in industries of foods and nutraceuticals, for product design, in aspects as optimization of the minimal components relation improving the encapsulation efficiency, better control of the release of bioactive agents, and improve long-term emulsions stability.

2 Materials and methods

2.1 Materials

Soy Protein Isolate (SPI) was purchased from Fabpsa (Mexico City), Maltodextrin DE-20 (MD) from Grain Processing Corporation (Iowa, USA) and Chia seeds were acquired from Acatic (Jalisco, México). The oil was obtained by pressing of the seeds using a hydraulic press (Model 500, Taiwan) and applying a pressure of 500 Kgf/cm^2 at 80 °C. Matthäus (2012) reported that in oil extraction, process temperatures up to 170 ºC can occur since kinetic energy generated during pressing is changed into heat. The oil was stored at 4 °C until use in darkness using amber glass bottles without head space.

2.2 Preparation of oil-in-water emulsions

The chia oil emulsions were prepared in three stages: the wall materials dispersion, the coarse emulsion and the final emulsion. 1) Preparation of wall materials dispersion: different proportions of soy protein isolate and maltodextrin were dispersed (Table 1) in grade I water, keeping constant the total solids content (30% w/w).

Table 1. D-optimal combined design.

Run	Independent variables		
	Soy protein isolate (%) A	Maltodextrin (%) B	Homogenization pressure (MPa) C
1	2.50	27.50	114
2	2.50	27.50	114
3	7.50	22.50	86
4	6.25	23.75	93
5	7.50	22.50	86
6	3.75	26.25	93
7	5.00	25.00	114
8	6.25	23.75	107
9	7.50	22.50	93
10	2.50	27.50	86
11	7.50	22.50	114
12	5.00	25.00	86
13	7.50	22.50	100
14	3.75	26.25	107
15	2.50	27.50	100
16	7.50	22.50	114
17	5.00	25.00	100
18	2.50	27.50	86
19	5.00	25.00	114

These solutions were stored overnight at 4 °C to ensure a complete hydration of the biopolymers. By preliminary dissolution assessments (data not shown), the maximum SPI concentration was fixed at 7.5% w/w since at the higher protein concentrations it is technically not possible to flow through the channels of the interaction chambers. 2) Elaboration of coarse emulsions: in this stage the coarse emulsions were prepared by mixing the chia oil (ChO) and the wall materials dispersion at a proportion 1:4 (g oil: g biopolymers) using a high-speed blender (Hamilton Beach, Ontario, Canada) for 1 min. The oil-wall material proportion (1: 4) was used, since it is widely used in microencapsulation processes of oils (Beristain, García, & Vernon-Carter, 2001). 3) Final emulsion: the coarse emulsions were homogenized using a Microfluidizer (Model M-110Y, Microfluidics, Newton, MA, USA) in order to analyze the effect of homogenization pressure using two cycles at different homogenization pressures (89 to 114 MPa, this interval was used since technically, they are minimum and maximum applicable operation pressure given by the microfluidizer). The equipment included a "Y" interaction chamber (75 μm in diameter) to reduce the emulsion droplet size, as well as a "Z" interaction chamber (200 μm in diameter) as an auxiliary processing module (APM). For all experimental runs, it was prepared 100g of emulsion.

2.3 Characterization of emulsions

2.3.1 Emulsion droplet size and ζ-potential

The emulsion droplet size (EDS) and ζ-potential in the emulsions were evaluated by Dynamic Light Scattering, and Laser Doppler Micro-electrophoresis, respectively, using a Zetasizer Nano-ZS laser diffractometer (Malvern Instruments Limited, Worcestershire, UK). Another parameter considered was the Polydispersity Index (PDI) as reported by Villalobos-Castillejos *et al*., (2017). The Water dilution of 1:100 v/v was used in order to avoid multiple scattering effects (Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal, 2016). The measurements were carried out immediately after emulsion preparation.

2.4 Emulsion stability

The stability measurements were performed directly after preparation of the emulsions at different times for 6 hours using a vertical scan analyzer Turbiscan Lab

Expert (Formulaction, France) based on the analysis of multiple light scattering. Emulsions were transferred into a flat-bottomed cylindrical glass cell and scanned from the bottom to the top (total height = 40 mm) by a detection head composed of a pulsed near infrared light source (λ = 850 nm) and two synchronous detectors. The transmission detector receives the light which goes through the sample (at 180° from the incident beam), while the backscattering detector receives the light scattered backwards by the sample (at 45° from the incident beam).

All emulsions were compared using Turbiscan Stability Index (TSI). This parameter considers all single measurements during experiments and the TSI value is obtained from their averaging. This coefficient was calculated with the program Turbiscan Easy Soft (V1.1, Formulaction SA, France) applying the following equation:

$$TSI = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{BS}^2}{n-1}} \quad (1)$$

where: x_i- average backscattering for each minute measurement, $x_{BS}-$ average x_1, and $n-1$ number of scans. The TSI values change in the range from 0 to 100. A higher value indicates a more unstable system (Carbone, Musumeci, Lauro, & Puglisi, 2015; Wisniewska *et al*., 2014).

2.4.1 *Apparent viscosity*

The apparent viscosity (μ_{app}) emulsions was measured upon shear rate ramp-up from 0.1 to 1000 s^{-1}, using a rheometer (RST CC, Brookfield Engineering Labs Inc., USA) equipped with a coaxial cylinder geometry. Coaxial cylinders provides greater contact area and therefore greater sensitivity and precision in the readings. The temperature was maintained at 25 ± 0.1 ºC (Kaltsa, Spiliopoulou, Yanniotis, & Mandala, 2016).

2.4.2 *Emulsion storage stability*

Emulsion samples were placed in glass test tubes and then storage at 4 ºC for 28 days. The EDS, ζ-potential, TSI and viscosity measurements were carried out as described earlier.

2.5 *Experimental design and statistical analysis*

Experimental design and formulation optimization were performed using the Design-Expert software (version 7.0.11 Stat-Ease, Inc., Minneapolis, USA). A D-optimal combined design was employed to identify the relationship between the components of mixture (SPI – MD) and homogenization pressure (P) with the response functions. The components of mixture were SPI (2.5-7.5% w/w) and MD (22.5-27.5% w/w); the process variable was the homogenization pressure (89-114 MPa). The levels of these independent variables were based in preliminary trials. The significant terms in the model were found by analysis of variance (ANOVA) of the regression for each response. The criteria used in this selection were the p-value for the model and the lack of fit and the adjusted coefficient of determination ($R^2_{adj} > 0.8$).

The optimal condition was determined through the desirability function, which allows the finding of the factor levels to reach the best possible value for all the evaluated responses in order to fulfill some prior specifications. This is achieved by involving the multiple responses into the desirability function followed by its optimization. In the first step, each response i is transformed over the experimental domain into an individual desirability function, d_i, which ranges between $d_i = 0$, for a completely undesirable response, and $d_i = 1$, for a fully desired response. This transformation makes it possible to combine the results obtained from different responses. In a second step, the overall desirability function, D, is calculated from this individual desirability functions. Thus, the simultaneous optimization process is reduced to find the level of factors that demonstrate the maximum overall desirability (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008). In this study, the optimal emulsion was selected considering the minor EDS, ζ-potential value, apparent viscosity and TSI. To validate the models and verify the optimization result, the experiments were performed at the optimal condition three times.

3 Results and discussion

3.1 *Model fitting and statistical analysis*

Compared with a factorial design, the application of D-optimal combined design reduced the number of runs required and allowed the combination of mixture components (SPI – MD) and process factors (P). Analysis of variance and regression analysis (Table 2) indicated that a quadratic mixture model combined with the linear process model provided the best fit to explain the EDS and apparent viscosity parameters.

Table 2. Analysis of variance and regression coefficients obtained from experimental data.

Variables	Regresion parameter coefficients			
	Emulsion Droplet Size (nm)	Zeta Potential (mV)	Turbiscan Stability Index (ΔBS)	Viscosity (mPa·s)
A	187.89	−29.69	−208.93	54.08
B	30.04	5.29	−6.51	0.545
C	NS	NS	NS	NS
AB	−9.18	NS	9.93	−1.97
AC	0.034	0.561	4.21	−0.062
BC	−0.101	−0.129	0.132	5.66E−003
ABC	NS	NS	0.2004	NS
AC^2	NS	−2.79E−003	−0.0207	NS
BC^2	NS	6.42E−004	−6.46E−004	NS
ABC^2	NS	NS	9.88E−004	NS
R^2_{adj}	0.9333	0.8978	0.9089	0.9104
Regression (p-value)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Lack of fit (p-value)	0.1925	0.3738	0.6937	0.5109

A: soy protein isolate percentage, B: maltodextrin percentage, C: homogenization pressure, NS: non significant $p > 0.05$

A linear mixture model combined with the quadratic process model explained the ζ-potential parameter. A quadratic mixture model combined with the quadratic process model provided the best fit to explain the TSI parameter. The adjusted-R^2 values ranged from 0.8978 to 0.9333 for the response variables, indicating that the proposed regression models are sufficiently accurate for describing the variation of the response. So, these high values of adjusted-R^2 support a high correlation between the experimental and the predicted values (Swamy, Sangamithra, & Chandrasekar, 2014). Also, a higher adjusted-R^2 occurs when non-significant term are not included in the models (Taghi, Mousavi, Hamedi, & Ghasemlou, 2012).

The p-value was used to measure the significance of the coefficients of the model. For the proposed models, these values were too low ($p < 0.0001$), meaning that the model and the associated terms are statistically significant. Moreover, the fitness of the models was investigated through the lack of fit test ($p > 0.05$), which indicated the suitability of models to accurately predict the variation (Myers, Montgomery, & Anderson-Cook, 2009). The experimental observations were compared with predicted values from the regression equations (Fig. 1). These results indicated that observed responses are in acceptable agreement with the suggested models, because the data point showed a distribution relatively close.

The equations generated are presented below:

Emulsion droplet size (EDS)

$$EDS = 187.89A + 30.04B - 9.18AB + 0.03AC - 0.10BC \quad (2)$$

ζ-potential (ζ-p)

$$\zeta - p = -29.69A + 5.29B + 0.561AC - 0.13BC - 2.79E - 0.003AC^2 + 6.42E - 0.004BC^2 \quad (3)$$

Turbiscan stability Index (TSI)

$$TSI = -208.93A - 6.51B + 9.93AB + 4.21AC + 0.132BC + 0.20ABC - 0.02AC2 - 6.46E - 004BC^2 + 9.88E - 004ABC^2 \quad (4)$$

Apparent viscosity (μapp)

$$\mu_{app} = 54.08A + 0.55B - 1.97AB - 0.06AC + 5.66E - 003BC \quad (5)$$

where: A-soy protein isolate percentage, B-maltodextrin percentage and C-homogenization pressure.

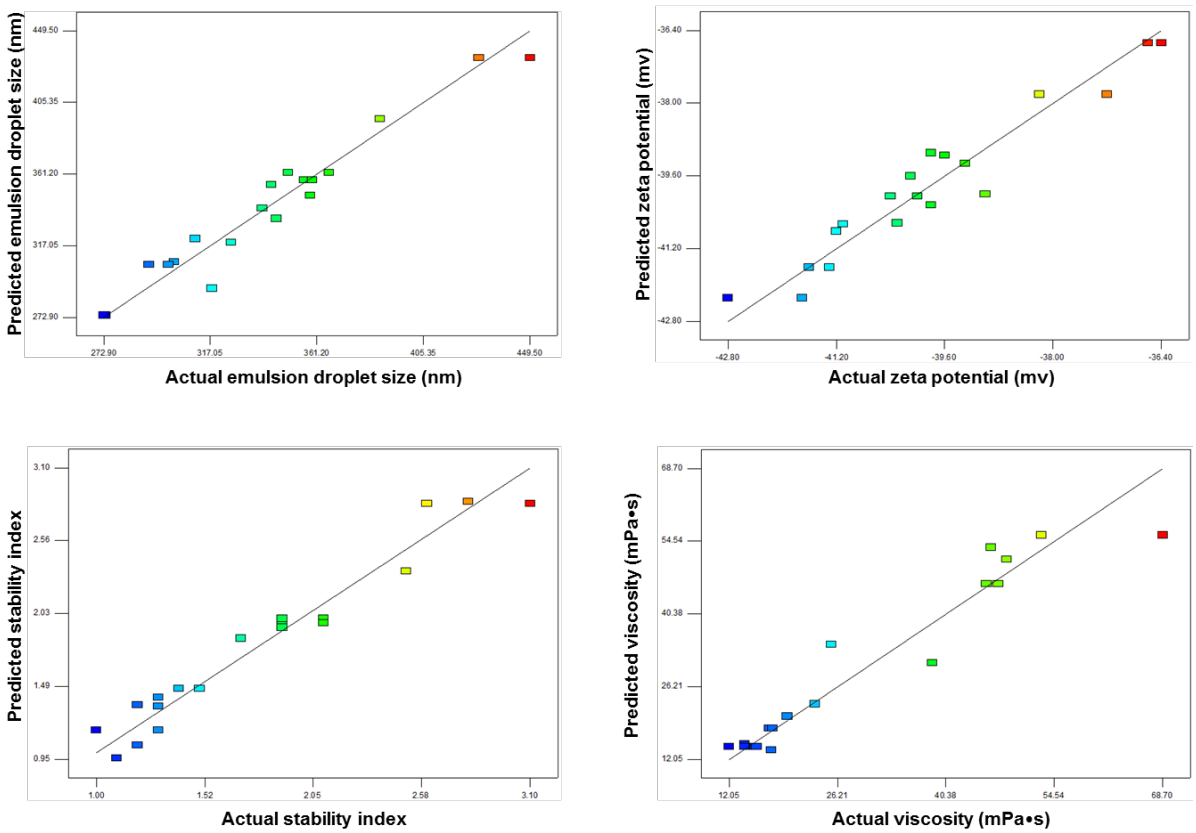

Fig. 1. Comparison between observed and predicted values of response variables.

3.2 Characterization of emulsions

3.2.1 Emulsion droplet size

Table 2 shows that linear and interaction effects of mixture components (A and B) on the emulsion droplet size were significant ($p < 0.0001$). The results obtained pointed out that the SPI-MD concentration presented a quadratic behavior, where the linear coefficients of the mixture components were positive and the interaction between them was negative. Also, the presence of curvatures in the surface plot (Fig. 1a) confirmed this result. As can be seen in Fig. 2a, at constant homogenization pressure, the reduction of droplet size was enhanced by increasing SPI concentration by up 5% (w/w) a further increase in SPI concentration had a negative effect on homogenization process and resulted in an increase in the EDS. Therefore, the increase in the level of SPI not necessary meant a minor EDS. This fact has been reported to be caused by the molecular rearrangement of the proteins into the emulsion, which produce a rise in the internal energy of the system resulting in an increase in the viscosity, which may suppress the formation of eddies responsible for breaking up droplets during the microfluidization process (Julio *et al*., 2015; Qian & McClements, 2011). At constant SPI-MD composition, EDS decreased as pressure increased. This behavior was explained by a linear function.

The ANOVA results showed that the interaction between the homogenization pressure and each mixture component was significant ($p < 0.0001$). In the microfluidization, the reduction in EDS by increment in homogenization pressure has been associated with a rise in the energy input to the system, that produce an increase in the intensity with which one emulsion stream collides with another throughout the interaction chamber (Jafari, He, & Bhandari, 2007).

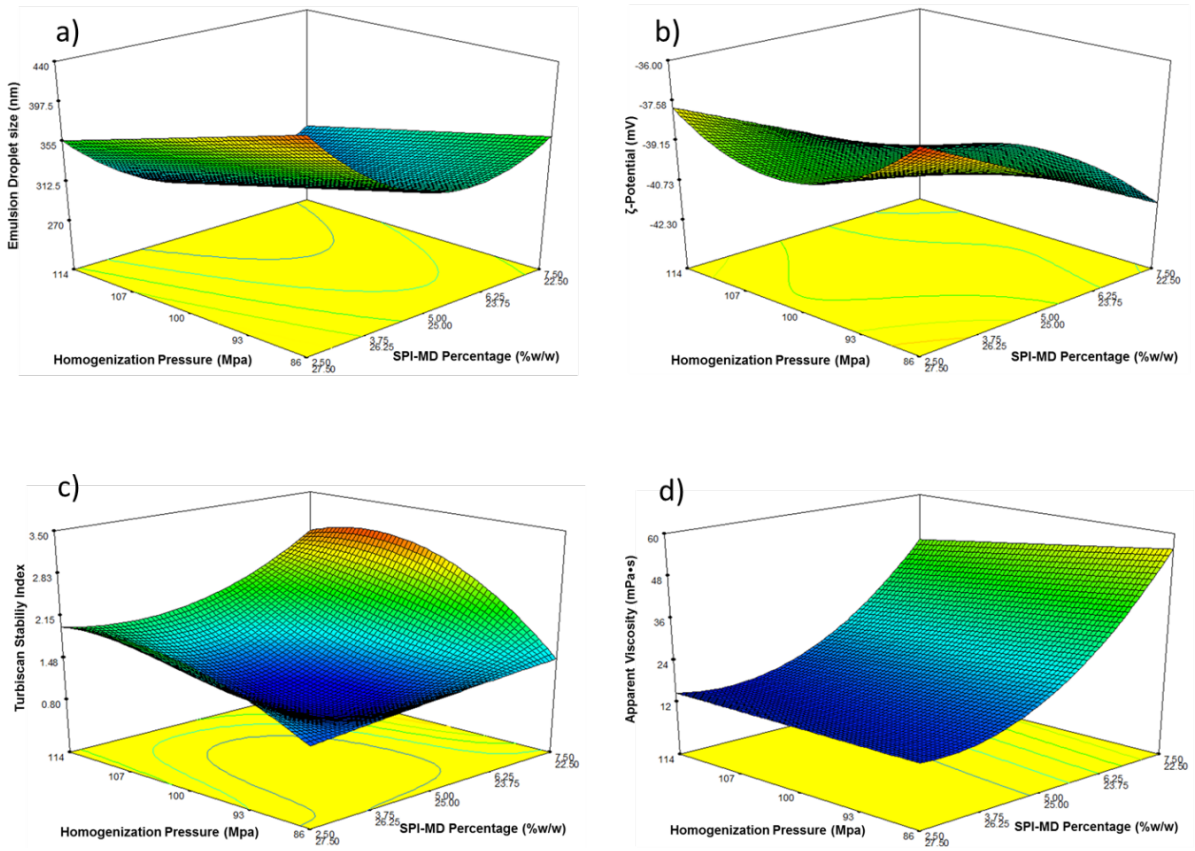

Fig. 2. Response surface plots showing the effects of independent variables on: Emulsion Droplet Size (a), ζ-potential (b), Turbiscan Stability Index (c) and Apparent Viscosity (d).

As shown in Fig. 3, all emulsions exhibited monomodal particle size distribution, independently of their composition. However, the broadness of these curves varies in relation to the composition; this was reflected in the polydispersity index, which varied from 0.213 to 0.322, the highest value corresponded to the sample with the lowest amount of SPI. These results suggest the existence of an optimal concentration of protein that cover the new oil-water interfaces generated during the microfluidization, preventing the immediately coalescence phenomena. It has been reported that protein adsorption depend on their natural properties, such as, molecular weight, chain length, and flexible or rigid packing conformation (Ji *et al*., 2015).

In particular, the soy protein isolated is a rigid protein folding uniquely under native conditions with relatively large molecular weight (Kinsella, 1979). However, microfluidization could change this structure improving their emulsifying properties; more studies are needed to corroborate this assumption.

3.2.2 ζ-potential of emulsions

Table 2 shows that the linear effect of the components of the SPI-MD mixture was significant ($p < 0.0001$) on the ζ-potential value. The interactions between homogenization pressure and each mixture component were also significant ($p \leq 0.05$). Fig. 1b illustrates the effect of the mixture components and the homogenization pressure on ζ-potential parameter. At constant pressure, the ζ-potential decreases as the SPI content increases. Also, the presence of a curvature in the 3D plot indicates that the factor of homogenization pressure has a non-linear effect.

ζ-potential is understood as the difference in the electro-kinetic charge of the drop surface with respect to its dispersing medium (Ricaurte *et al*., 2016).

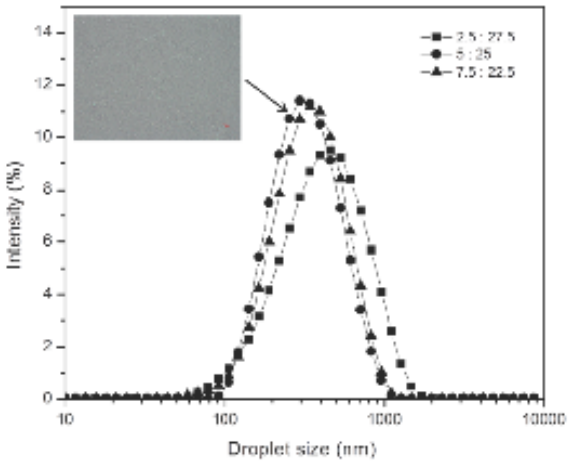

Fig. 3. Droplet size distribution of emulsions with different proportions of soy protein isolated and maltodextrin elaborated at 114 MPa. Structure of emulsion containing 5% w/w SPI and 25 w/w MD.

It has been reported that droplets with ζ-potential more positive than +30 mV or more negative than −30 mV are usually considered to be stable due to electrical charge of droplets is enough strong to assume that repulsive forces between droplets are predominant in the emulsion system (Salvia-Trujillo, Decker, & McClements, 2016). Heurtault, Saulnier, Pech, Proust, & Benoit, (2003) reported that systems with ζ-potentials between 5 and 15 mV showed limited flocculation and systems between 3 and 5 mV presented maximum flocculation. In this study, the ζ-potential of the chia oil emulsions varied from −36.40 to −41.70 mV, these high negative values mean high stability against possible coalescence phenomenon. These values are in agreement with ζ-potential reported for SPI emulsions (−40 mV) with a protein concentration of 6% (w/v) and oil volume fractions (0.2 to 0.6) (Tang and Liu, 2013). It is important to mention that the ζ-potential of the maltodextrin and the soy protein isolated were 21.9 ± 3.4 mV and41.2 ± 2.15 mV, respectively, this values indicate that the maltodextrin has influence on the final ζ-potential of emulsions, due to the emulsion with low ζ-potential correspond to the emulsions with high maltodextrin content. Previous studies have demonstrated that the ζ-potential of the emulsion is strongly dependent on the final pH, emulsifier type and emulsifier concentration (Harnsilawat, Pongsawatmanit, & McClements, 2006; Julio *et al*., 2015; Mirhosseini, Tan, Hamid, & Yusof, 2008). It is important to mention that, the pH values of the emulsions elaborated were within the range from 6.42 to 7.00, giving good stability, because the soy protein isolate were away from their isoelectric point, pI ≈ 4.5 (Kinsella, 1979). Due to the pH value of the ChO emulsions were greater than isoelectric point value of soy protein isolate. The carboxyl groups of the proteins are negatively charged (COO^-) and the amino groups are neutral (NH_2), giving a negative zeta potential. Opposite, the ζ-potential acquires a positive value when the pH value is less than the pI value of proteins, COO^- became neutral (COOH) and NH_2 became positively charged (NH_3^+) (Ji *et al*., 2015).

It is thought that during the adsorption of soybean proteins at the water − chia oil interface, there is a change in the spatial distribution of the amino acids that constitute it. Probably, in the SPI-MD-ChO system the hydrophobic amino acids located inside the globular proteins are exposed to the surface of the chia oil droplets, while the hydrophilic amino acids are directed towards the aqueous phase and the D-glucose units of maltodextrin, acting like a steric barrier against instability phenomena such as flocculation and coalescence.

3.2.3 Emulsion Stability

According to statistical analysis (Table 2), the TSI was significantly ($p < 0.0001$) influenced by the linear effect of mixture components, the interaction between mixture components (AB) and the interaction between soy protein content and homogenization pressure (AC). Also, the BC, AC2 and ABC2 were significant terms ($p \leq 0.05$). As shown in Fig. 2c, there is a zone of a maximum stability which corresponds to emulsion elaborated with protein content less than 5% (w/w) at homogenization pressure less to 107 MPa. This result corroborate that an increase in protein content not necessary means an increase in stability emulsion. In other words, there exists an optimal protein content where all oil droplets presented in the emulsion are stabilized by a monolayer of these molecules that avoid instability phenomena. In relation to the effect of homogenization pressure on the emulsion stability, it was observed (Fig. 2c) that emulsions elaborated with highest homogenization pressures were more unstable, than those elaborated at less homogenization pressure. Probably due to enhanced inter-droplet hydrophobic interactions between these proteins (Shen & Tang, 2012). It has been reported that emulsions stabilized with sodium caseinate - maltodextrin conjugate are more stable than emulsions elaborated with only sodium caseinate.

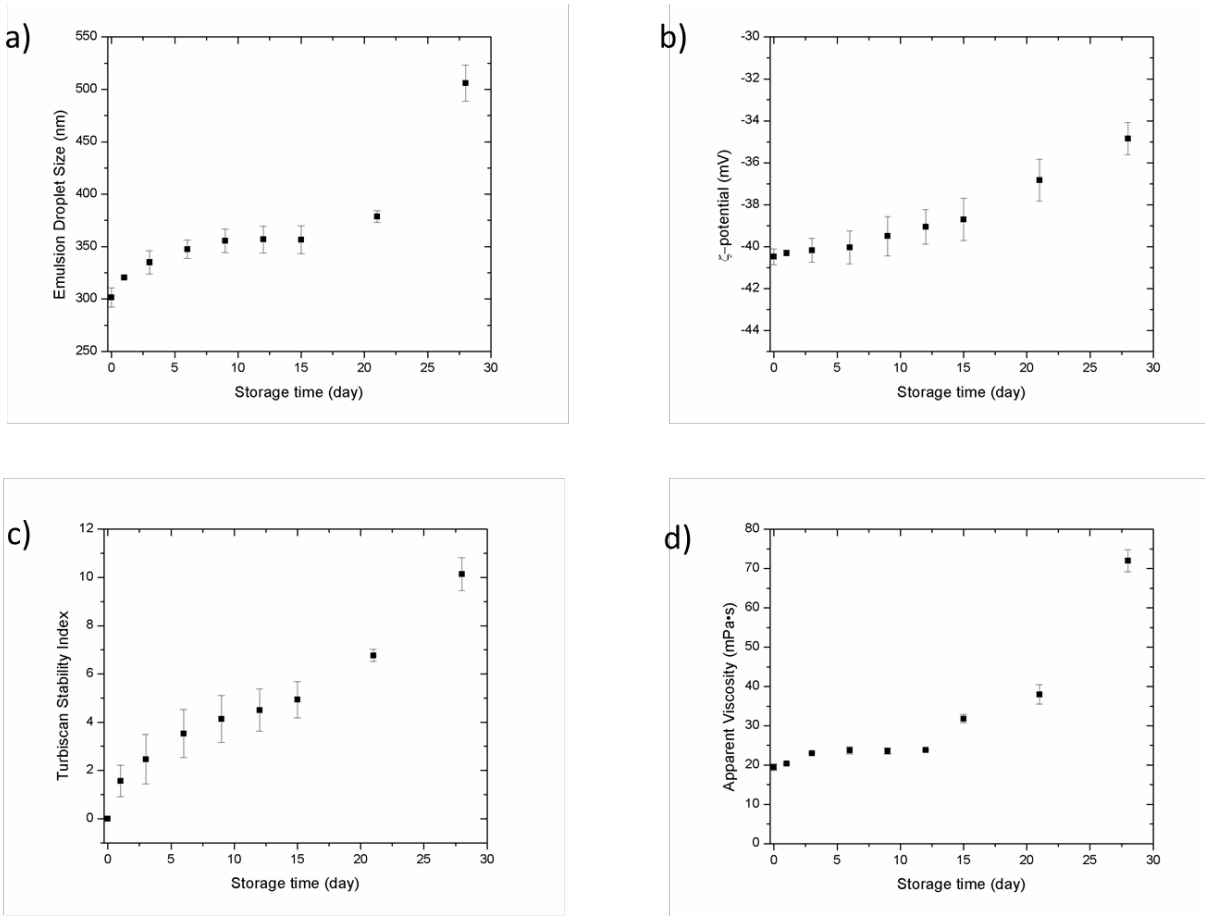

Fig. 4. Emulsion droplet size (a), ζ-potential (b), Turbiscan Stability Index (c) and apparent viscosity (d) of optimal emulsion during storage for 28 days at 4 °C.

Due to the fact that conjugated protein molecules form a more bulky polymeric layer than the non-conjugated protein on the droplet surface, with the MD portion protruding outwards into the continuous phase providing better steric stabilization. Therefore, it is thought that the development of surface active protein − polysaccharide complexes (SPI and MD) could improve the stability emulsions probably by covalent bonding or electrostatic interactions, more studies are needed to verify this.

3.2.4 Apparent viscosity

As shown in Table 2, the apparent viscosity was significantly ($p < 0.0001$) influenced by the linear effect of mixture components. The interaction between mixture components and homogenization pressure was shown to be significant ($p \leq 0.05$). Fig. 2d, shows the effect of mixture components and homogenization pressure on viscosity parameter. It can be seen that at pressure constant, the viscosity increases as the SPI content increases. These results are in agreement with those reported by Julio *et al*. (2015); for emulsions elaborated with sodium caseinate and lactose in the continuous phase. They attributed this increment to the presence of non-adsorbed sodium caseinate in the aqueous phase, which produced high flow resistance.

The increment in the viscosity emulsion is not lineal, this can be visualized as the curvature in the 3D plot, at protein content smaller than 5% w/w the increment in the viscosity parameter was less than the increment in emulsions with higher concentration of protein, and this may be due to the excess of protein in the continuous phase. With respect to the effect of homogenization pressure on the apparent viscosity it was observed that

emulsions with more SPI content (5 to 7.5% w/w) presented a reduction in their viscosity as the homogenization pressure increased (Fig. 2d). Previous study associated the viscosity reduction of lemongrass oil-alginate nanoemulsions with molecular changes on sodium alginate structure as a result of the turbulence generated by the high shear stress of the microfluidization treatment (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2013).

3.3 Optimization and validation of the models

The desirability function was used to optimize multiples responses in order to obtain minimum EDS, viscosity, ζ-potential and TSI, simultaneously.

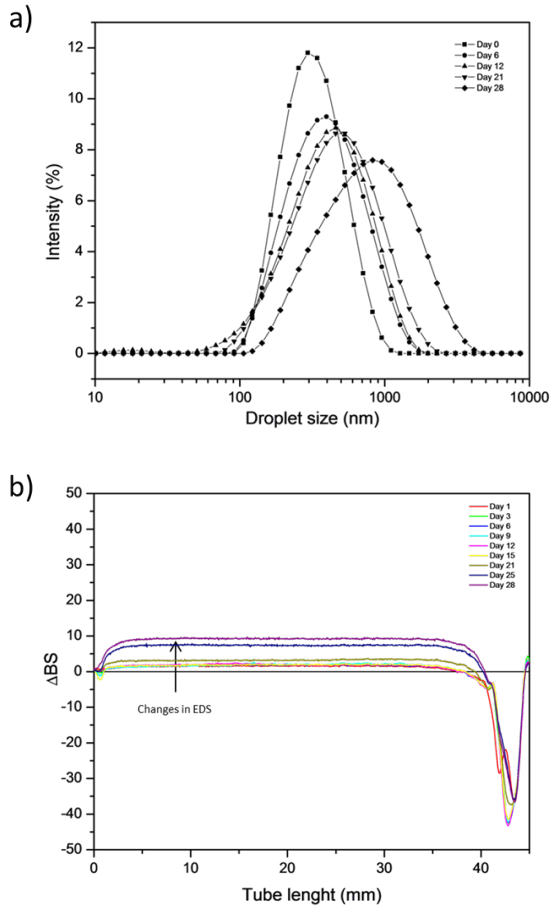

Fig. 5. Changes in (a) emulsion droplet size distribution and (b) backscattering in a reference mode profile of optimal emulsion, during storage for 28 days at 4 °C.

The optimum SPI-MD concentration and homogenization pressure were 4.8 – 25.2% (w/w) and 104 MPa, respectively, with a desirability value of 0.801. Under this optimum condition, the corresponding predicted response values were: emulsion droplet size 300.97 nm, viscosity 18.65 mPa·s, ζ-potential −40.03 mV and turbiscan stability index 1.06. With the aim of validate these optimization results three experiment were carried out, the experimental values for EDS, viscosity, ζ-potential and TSI were 300.56 ± 7.24 nm, 19.42 ± 0.81 mPa·s, −40.46 ± 0.2658 mV and 1.03 ± 0.30, respectively. These results revealed no statistical significant difference ($p > 0.05$) between observed and predicted values, suggesting the adequacy of the fitted models.

3.4 Changes in emulsion properties during storage period

The emulsion properties were monitored during storage for 28 days at 4 ºC. The EDS increased from 301.62 to 505.95 nm (Fig. 4a). The droplet size distribution curve moved to the right on x-axis (Fig. 5a), and the polydispersity index increased from 0.255 to 0.413 (data not shown). It has been reported that polydispersity index greater than 0.7 indicates that the sample has a very broad size distribution (Ricaurte *et al.*, 2016). Therefore, it was considered that optimal emulsion had an intermediate degree of polydispersity. During the storage, the delta backscattering (ΔBS) profiles obtained by the Turbiscan showed an increase of BS in the middle of the tube (Fig. 5b) this was related to the particle size variation characteristic of the physical instability known as flocculation. However, there were not migration phenomena such as sedimentation and creaming. The ζ-potential increased from −40.48 to −34.85 mV (Fig. 4b), this could be correlated with a decrease of sample pH from 7.18 to 6.25 (data not shown). The change in pH is probably due to the modifications in the interaction between protein maltodextrin and oil, the protein aggregation due to an excess of wall material in the continuous phase that generates the liberation of hydrogen or the growth microorganisms (Smith, Mendonca, & Jung, 2009) . In spite of this reduction, the ζ-potential values were in agreement with the reported for stables emulsions (< −30 mV). TSI and viscosity increased from 1.56 to 10.13 and 19.42 to 72.02 mPa·s, (Fig. 4c and 4d), respectively. The viscosity increment may be due to the aggregation of soy protein molecules. Roesch and Corredig (2003) reported that emulsions with 2, 4, 6 and 8 g of soy protein concentrate/100g

emulsion applying a homogenization pressure of 80 MPa showed a gel behavior and stability to creaming upon storage at 4 ºC for 20 days.

During storage of emulsion, the EDS and TSI showed a similar behavior (Fig. 4. a and c respectively), both parameters presented three rates of change (or slope) with respect to the time, indicative about their relationship. If the emulsion droplet size increases, the emulsion stability is also affected by changes in the amount of backscattered light. It is important to mention that the largest changes in emulsion properties were observed from day 15 of storage, probably due to a modification on the kind of interaction presented between SPI-MD-ChO. It has been reported that the emulsion properties would depend on the range and magnitude of the various types of colloidal interactions that exist between emulsion droplets and components of the continuous phase, such as electrostatic, van der Waals, steric, hydrophobic (McClements, 2000). Probably, in the SPI-MD-ChO system, the hydrophobic amino acids of SPI interact with the chia oil droplets and the hydrophilic amino acids of SPI interact with D-glucose units of maltodextrin presents in the continuous phase of the emulsion, forming a barrier against instability phenomenon.

Conclusions

In the emulsions elaborated with potential use for microencapsulation by spray drying, the composition of continuous phase has influence on the emulsion properties, being SPI the component with the highest effect due to its emulsifying properties and electrical charge that gives to the emulsion. The homogenization pressure affected in a minor degree the emulsion properties. However, the microfluidization technique permitted the production of emulsions with a droplet size in the range of nanometers (< 500 nm) and a monomodal particle size distribution, with high effect on emulsion stability. The emulsion stability against creaming may be due to the fact that chia oil droplets were aggregated and entrapped in a structured network of soy protein isolate – maltodextrin. Finally, the desirability function suggested the elaboration of optimal emulsion using 4.8% w/w SPI and 25.2%w/w MD at 104 MPa. The study of the emulsion properties during storage pointed out that after 15 days, the greatest change in the EDS, ζ-potential, TSI and μ_{app} occurred, probably as a result of modifications in the interaction between protein maltodextrin and oil, the protein aggregation due to an excess of wall material in the continuous phase that generates the liberation of hydrogen or the growth microorganisms, more studies are required to corroborate it.

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References

Beristain, C. I., García, H. S., & Vernon-Carter, E. J. (2001). Spray-dried encapsulation of Cardamom essential oil with mesquite gum. *LWT - Food Science and Technology 34*, 398-401.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta 76*, 965-977. http://doi.org/10.1016/j.talanta.2008.05.019

Carbone, C., Musumeci, T., Lauro, M. R., & Puglisi, G. (2015). Eco-friendly aqueous core surface-modified nanocapsules. *Colloids and Surfaces B: Biointerfaces 125*, 190-196.

Fongin, S., Kawai, K., Harnkarnsujarit, N., & Hagura, Y. (2017). Effects of water and maltodextrin on the glass transition temperature of freeze-dried mango pulp and an empirical model to predict plasticizing effect of water on dried fruits. *Journal of Food Engineering 210*, 91-97. http://doi.org/10.1016/j.jfoodeng.2017.04.025

Granillo-Guerrero, V. G., Villalobos-Espinosa, J. C., Alamilla-Beltrán, L., Téllez-Medina, D. I., Hernández-Sánchez, H., Dorantes-Álvarez, L., & Gutiérrez-López, G. F. (2017). Optimization of the formulation simplex centroid and analysis of colocalization of its on. *Revista Mexicana de Ingeniería Química 16*, 861-872.

Harnsilawat, T., Pongsawatmanit, R., & McClements, D. J. (2006). Stabilization of model beverage cloud emulsions using protein- polysaccharide electrostatic complexes formed at the oil-water interface. *Journal of Agricultural and Food Chemistry 54*, 5540-5547. http://doi.org/10.1021/jf052860a

Heurtault, B., Saulnier, P., Pech, B., Proust, J., & Benoit, J. (2003). Physico-chemical stability of colloidal lipid particles. *Biomaterias 24*, 4283-4300. http://doi.org/10.1016/S0142-9612(03)00331-4

Jafari, S. M., He, Y., & Bhandari, B. (2007). Production of sub-micron emulsions by ultrasound and microfluidization techniques. *Journal of Food Engineering 82*, 478-488. http://doi.org/10.1016/j.jfoodeng.2007.03.007

Ji, J., Zhang, J., Chen, J., Wang, Y., Dong, N., Hu, C., ..., Wu, C. (2015). Preparation and stabilization of emulsions stabilized by mixed sodium caseinate and soy protein isolate. *Food Hydrocolloids 51*, 156-165. http://doi.org/10.1016/j.foodhyd.2015.05.013

Julio, L. M., Ixtaina, V. Y., Fernández, M. A., Sánchez, R. M., Wagner, J. R., Nolasco, S. M., & Tomás, M. C. (2015). Chia seed oil-in-water emulsions as potential delivery systems of $\omega - 3$ fatty acids. *Journal of Food Engineering 162*, 48-55. http://doi.org/10.1016/j.jfoodeng.2015.04.005

Kaltsa, O., Spiliopoulou, N., Yanniotis, S., & Mandala, I. (2016). Stability and physical properties of model macro- and nano/submicron emulsions containing fenugreek gum. *Food Hydrocolloids 61*, 625-632. http://doi.org/10.1016/j.foodhyd.2016.06.025

Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of the American Oil Chemist's Society 56*, 242-258.

Mirhosseini, H., Tan, C. P., Hamid, N. S. A., & Yusof, S. (2008). Effect of Arabic gum , xanthan gum and orange oil contents on ζ -potential , conductivity , stability , size index and pH of orange beverage emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects 315*, 47-56. http://doi.org/10.1016/j.colsurfa.2007.07.007

Monroy-Villagrana, A., Cano-Sarmiento, C., Alamilla-Beltrán, L., Hernández-Sánchez, H., & Gutiérrez-López, G. F. (2014). Optimización acoplada Taguchi-RSM de las condiciones de emulsificación por microfluidización de ?-tocoferol en una matriz de goma arábiga y maltodextrina. *Revista Mexicana de Ingeniería Química 13*, 679-688.

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). *Response Surface Methodology, Process and Product Optimization Using Design Experiment*. Wiley, 3rd. Ed. New York.

Qian, C., & McClements, D. J. (2011). Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids 25*, 1000-1008. http://doi.org/10.1016/j.foodhyd.2010.09.017

Ricaurte, L., Perea-Flores, M., Martinez, A., & Quintanilla-Carvajal, M. X. (2016). Production of high-oleic palm oil nanoemulsions by high-shear homogenization (microfluidization). *Innovative Food Science and Emerging Technologies 35*, 75-85. http://doi.org/10.1016/j.ifset.2016.04.004

Roesch, R. R., & Corredig, M. (2003). Texture and microstructure of emulsions prepared with soy protein concentrate by high-pressure homogenization. *LWT - Food Science and Technology 36*, 113-124. http://doi.org/10.1016/S0023-6438(02)00208-6

Salvia-Trujillo, L., Decker, E. A., & McClements, D. J. (2016). Influence of an anionic polysaccharide on the physical and oxidative stability of omega-3 nanoemulsions: Antioxidant effects of alginate. *Food Hydrocolloids 52*, 690-698. http://doi.org/10.1016/j.foodhyd.2015.07.035

Salvia-Trujillo, L., Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2013). Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. *Food Hydrocolloids 30*, 401-407. http://doi.org/10.1016/j.foodhyd.2012.07.004

Shen, L., & Tang, C.-H. (2012). Microfluidization as a potential technique to modify surface properties of soy protein isolate. *Food Research International 48*, 108-118. http://doi.org/10.1016/j.foodres.2012.03.006

Smith, K., Mendonca, A., & Jung, S. (2009). Impact of high-pressure processing on microbial shelf-life and protein stability of refrigerated soymilk. *Food Microbiology 26*, 794-800. http://doi.org/10.1016/j.fm.2009.05.001

Swamy, G. J., Sangamithra, A., & Chandrasekar, V. (2014). Response surface modeling and process optimization of aqueous extraction of natural pigments from Beta vulgaris using Box-Behnken design of experiments. *Dyes and Pigments 111*, 64-74. http://doi.org/10.1016/j.dyepig.2014.05.028

Taghi, G. S. M., Mousavi, S. M., Hamedi, M., & Ghasemlou, M. (2012). Response surface modeling for optimization of formulation variables and physical stability assessment of walnut oil-in-water beverage emulsions. *Food Hydrocolloids 26*, 293-301. http://doi.org/10.1016/j.foodhyd.2011.06.006

Tang, C., & Liu, F. (2013). Cold, gel-like soy protein emulsions by micro fluidization: Emulsion characteristics , rheological and microstructural properties, and gelling mechanism. *Food Hydrocolloids 30*, 61-72. http://doi.org/10.1016/j.foodhyd.2012.05.008

Villalobos-Castillejos, F., Alamilla-Beltrán, L., Leyva-Daniel, D. E., Monroy-Villagrana, A., Jiménez-Guzmán, J., Dorantes-Álvarez, L., & Gutiérrez-López, G. F. (2017). Long term stability of microfluidized emulsions used in microencapsulation by spray drying. *Revista Mexicana de Ingeniería Química 16*, 221-228.

Wisniewska, M., Terpilowski, K., Chibowski, S., Urban, T., Zarko, V. I., & Gun'ko, V. M. (2014). Investigation of stabilization and destabilization possibilities of water alumina suspension in polyelectrolyte presence. *International Journal of Mineral Processing 132*, 34-42. http://doi.org/10.1016/j.minpro.2014.08.007