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COUPLED TAGUCHI-RSM OPTIMIZATION OF THE CONDITIONS TO EMULSIFY α-TOCOPHEROL IN AN ARABIC GUM-MALTODEXTRIN MATRIX BY MICROFLUIDIZATION

OPTIMIZACIÓN ACOPLADA TAGUCHI-RSM DE LAS CONDICIONES DE EMULSIFICACIÓN POR MICROFLUIDIZACIÓN DE α-TOCOFEROL EN UNA MATRIZ DE GOMA ARÁBIGA Y MALTODEXTRINA

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Abstract

The effect of composition (Arabic gum (AG), maltodextrin (MD), surfactants, pH) and processing parameters (pressure and number of cycles of microfluidization) of α-tocopherol (AT) emulsions was studied via a coupled Taguchi-RMS optimization. Creaming index (CI), particle size (PS) and zeta potential (ζ) were selected as response variables. The estimated optimal conditions were found at GA of 40%, MD/surfactant mixture of 60%, 30% AT, pH of 4.9, P of 76 MPa and five cycles of microfluidization for a predicted CI of 0.5 % at 24 h with a PS of 384 nm. Under these conditions the experimental results were 437 nm and 0.5 CI. It was possible to minimize the CI by varying the concentration of GA and modifying processing conditions.

Keywords: emulsion, microfluidization, Taguchi method, response surface methodology.

Resumen

Se evalúo el efecto de la composición (goma Arábiga (GA), maltodextrina (MD), surfactantes, pH) y parámetros de procesamiento (presión y número de ciclos de microfluidización) de emulsiones de α-tocoferol vía una optimización acoplada Taguchi-RMS. El índice de separación (CI), tamaño de partícula (PS) y potencial zeta (ζ) fueron elegidos como las variables de respuesta. Las condiciones óptimas estimadas fueron a 40% de GA, 60% de MD/surfactante, 30% AT, pH 4.9, P de 76 MPa y 5 ciclos de microfludización con una predicción de CI igual a 0.5% a las 24 h con un tamaño de partícula de 384 nm. Bajo estas condiciones fue posible obtener experimentalmente emulsiones con un CI de 0.5% y un tamaño de partícula de 437 nm. Indicando que se puede minimizar la inestabilidad medida como CI, variando las concentraciones de GA y modificando las condiciones de procesamiento.

Palabras clave: emulsion, microfluidización, metodología de Taguchi, metodología de superficie de respuesta.

1 Introduction

Encapsulation of active ingredients, such as flavors, drugs, vitamins and nutraceutics, is an important operation in food, pharmaceutical and cosmetic industry (Wang *et al*., 2009). Encapsulation enhances chemical stability, protecting from moisture, heat and oxidation and provides the capsules, a low susceptibility to degradation during storage by entrapping them into a matrix (Wang *et al*.,2009; Yuan *et al*., 2008; Cao-Hoang *et al*., 2011). For these purpose, the emulsification is a critical step, where smaller particle size (PS) and increased stability are associated with higher encapsulation

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efficiencies (Jafari *et al*., 2007; Quintanilla-Carvajal *et al*., 2011). Microfluidizers are equipments used to prepare emulsions as they are homogenizers operating at high pressures (up to 275 MPa) and are considered superior than other traditional methods of homogenization because the obtained size distributions are more homogeneous, providing an improvement in the bioavailability of different non-polar functional ingredients such as vitamin E (Cano-Sarmiento *et al*., 2014; Jafari *et al*., 2006; Klinkesorn *et al*., 2004). However, minimum size will depend on the interaction chamber used, operating conditions such as applied energy (pressure), number of cycles, residence time of compounds in the interaction chamber and sample composition (type of oil, emulsifier and concentrations) (Brösel & Schubert, 1999; Qian & McClements, 2011).

Recent studies have shown that the use of biopolymers with emulsifying properties, produces more stable emulsions as compared to those where low molecular weight emulsifiers, which are used when the microfluidizer is employed (Jafari *et al*., 2007), but the size of the biopolymers and their interfacial properties are directly involved with the final size of the emulsions, as these take longer to saturate the interfaces and achieve rapid stabilization (Bouyer *et al*., 2013; Chaparro-Mercado *et al*., 2012; Jafari *et al*., 2008). Also, other problems have been found, such as overtreatment and coalescence due to mechanical damage that could undergo such molecules by high applied shear, turbulence and cavitation presented in the interaction chamber (Jafari *et al*., 2008). For all these reasons, a suitable choice of these materials has to be applied, based on the desirable characteristics of the products and the emulsification properties of biopolymers. Polydispersity index (PDI) indicates the degree of heterogeneity of the emulsion, which is related to a wide range of particle sizes and formation of agglomerates as well as sedimentation phenomena so that values of PDI of 1 and close to 1 would indicate non-desirable conditions of emulsification. These criteria have been used in experimental design works for emulsion preparation purposes (Mahfoudhi *et al*., 2014; Perrier-Cornet *et al*., 2005; Ye *et al*., 2012).

According to this, Arabic gum (GA) and Maltodextrin (MD) are the main polysaccharides used in the food industry in emulsification and in spray drying as carriers of bioactive compounds, such as α-tocopherol (AT) (Gomes *et al*., 2010; McClements, 2009). GA is a hybrid polyelectrolyte, which contains protein subunits and polysaccharides in its structure. This biopolymer consists mainly of a mixture of arabino galactan (AG) (80-90% of the total weight of the gum), a glycoprotein (GP) (2-4 % of total weight) and an arabinogalactan protein (AGP) (10-20% of total weight) (Bouyer *et al*., 2011). The AGP fraction is responsible of the emulsification properties of the gum. The protein component is embedded in the oil phase while carbohydrates remain in the aqueous phase (Alftrén *et al*., 2012; Villay *et al*., 2012).On the other hand, MD is one of the most important biopolymers used for encapsulation due to its high solubility, low viscosity and high glass transition temperature (Tg) when dried. This is obtained by enzymatic or acid hydrolysis of starch (Avaltroni *et al*., 2004).

Due to the complexity of the system, a Taguchi's orthogonal array method (TM) followed by a response surface methodology (RSM) were used in this work to determine emulsifying conditions and formulation. The advantages of these methods (TM-RSM) have been demonstrated by different researches (Acherjee *et al*., 2012; León-López *et al*., 2013). The Taguchi's methodology is applied prior to carry out an initial discrimination of variables and then by Response Surface Methodology the final modeling is derived. The objective of this work, was to systematically investigate the influence of pressure, surfactant and GA/MD concentration and pH on stability and particle size, for oil in water emulsions of AT through microfluidization

2 Materials and methods

2.1 Materials

(±) α-tocopherol (AT; HPLC grade, Sigma-Aldrich, Toluca, State of Mexico, Mexico). Maltodextrin DE 20 (MD, Food Supplements, Naucalpan, State of Mexico, Mexico) and Arabic gum from *Acacia Senegal* (GA Alfred L. Wolf, SA de CV, Mexico City, Mexico). Sorbitan monolaurate (Tween 20) (Sigma Aldrich, EUA), sorbitan trioleate (Span 85) (Sigma Aldrich EUA), phosphatidylcholine from soy (lecithin) (Sigma Aldrich, EUA). All solutions were prepared using Type I water.

2.2 Coarse emulsion preparation

The coarse emulsions were prepared according to the combinations indicated in Table 1 for Taguchi experimental design or Table 3 for Box-Behnken design, taking in to account the amount of biopolymer (GA/MD), which were dissolved in Type I water, and

adjusting the pH with 0.1 N hydrochloric acid or sodium hydroxide 0.1 N. Afterwards, the amount of corresponding AT was added. Once the mixture was obtained, it was subsequently homogenized using a knife type blades equipment (Braun, Mexico) for 2 min (González-Rodríguez *et al*., 2007). Percentages of biopolymer and surfactant represent a 100% of surface active material. AT concentration was fixed to 30% of total weight of emulsion for a 20% of total solid content.

2.3 Microfluidization

A microfluidizer (M-110Y, Microfluidics, USA) with a type Y interaction chamber with micro-channels of 75 μm was used, operating at the pressure and cycles indicated by the Taguchi experimental design or Box-Behnken design (See Table 1 and Table 3). The output stream of the equipment was cooled immediately after processing in order to avoid rising of the temperature.

2.4 Zeta potential (ζ)

ζ was measured on a Zeta Plus analyzer (Brokhaven Instrument Corp., USA). An aliquot of the emulsion was evaluated by preparing a 1:200 dilution and adjusting the pH (see Table 1 and Table 3). Each sample was evaluated by triplicate and the mean and standard deviation was reported (Yang *et al*., 2005).

2.5 Particle size (PS)

1 mL of diluted (1:40) emulsion was used to evaluate PS in a Zetasizer Nano S90 (Malvern Instruments, United Kingdom). The PS of the emulsions was defined as the accumulative average of the diameter (z-average) (Yuan *et al*., 2008). PS values and polydispersity index (PDI) were reported. This index was evaluated as the average weight of the particles divided by the average molecular weight and to evaluate of how wide was the range of PS (Mirhosseini *et al*., 2008).

2.6 Creaming index (CI)

A 10 mL aliquot of each emulsion was transferred into test tubes (1 cm diameter x 15 cm high) and stored at room temperature. Serum height (Hs) and the total height of the emulsion (Ht) at 24 hours of emulsion preparation were measured. The emulsion CI was defined as a relative serum height of the stored emulsion to the original emulsion height. The mean and standard deviation of three measurements were calculated. CI (%) was evaluated as follows (Tang & Liu, 2013):

$$CI(\%) = \left(\frac{Hs}{Ht}\right) \times 100 \quad (1)$$

2.7 Experimental design and statistical analysis

A Taguchi experimental design (TM) consisting in a L8-orthogonal array was constructed as shown in Table 1. The purpose was to select the most important factors involved in stability measured as CI and ζ of emulsion and PS. For this purpose two biopolymers were selected: Arabic gum (GA) and maltodextrin (MD); three different surfactants: tween 20, soy lecithin and span 85; pH and pressure, which were taken as variables to be evaluated in two levels according to literature reports (Bouchemal *et al*., 2004; Bouyer *et al*., 2013; Panteloglou *et al*., 2010). An ANOVA was applied to determine the contribution of factors to perform a three level Box-Behnken response surface design for the optimization of stability and PS. Factors and levels are presented in Table 3. Responses from each run were studied by multiple regressions, which were computed by using the Design Expert software 7.0.3 (Stat-Ease, Inc., USA). Quality of fit of the fitted equations was expressed by means of the determination coefficient (R^2). The significance of the equations models and their coefficients were evaluated by applying a one-way ANOVA test.

2.8 Optimization of the conditions to prepare AT emulsions using GA, MD and surfactants for a small PS and low CI

Graphical and numerical optimization procedures were performed to determine the optimum level to form the emulsion with a smaller PS and minor changes to achieved stability measure as CI. Numerical optimization was carried out using the desirability function (León-López *et al*., 2013) to predict the optimal level of independent variables to achieve the objectives of the optimization.

2.9 Optical microscopy

The microstructure of the emulsions was observed for samples obtained at the maximum level of the statistical procedure, using a conventional optical microscope (Nikon H55O5, Japan) with halogen

illumination and maximum aperture. The image capture was performed with a (Nikon Digital Sight DS-2Mv, TV Lens 0.55XDS, Japan) coupled with a digital camera attached to a personal computer (2.67 GHz, 1.0 GB RAM). The images were acquired through the NIS-Elements Software F2.30. Images were captured with an objective of 100X at the resolution of 1280 × 960 pixel^2 and finally stored in jpg format. These images were taken to give a clear picture of the emulsification process.

3 Results and discussion

3.1 Taguchi experimental design

Table 1, summarizes the results for the TM. The influence of GA, MD, surfactant (tween 20, Lecithin or Span 85), pH and pressure (P) was evaluated by ANOVA and results are shown in Table 2. This analysis indicates that P was the factor with the strongest influence on particle size (PS), in a proportional manner; by increasing the P the particle size also increases. PS depends on several factors, as a result of stabilization of new droplets in the emulsions and compared to the re-coalescence (Jafari *et al*., 2008). The contact time between particles should be enough to allow the surfactants to stabilize the new formed interfaces. This results indicated that by using pressures of 103 MPa, the level of turbulence and collision frequency between particles increased thus decreasing the residence time of the emulsion in the microfluidizer and causing a decrease on the contact time between particles resulting in an insufficient time to stabilize the emulsion. As to ζ and CI, the GA and pH were the main factors that influenced them according to the % of contribution (See Table 2). This % was calculated for each factor through the sum of squares of the ANOVA test. Obtained range of ζ values was from -37 to -16 mV (Table 1). In mixtures with GA, ζ values were in the range of -27 to -16 mV, indicating that this factor is highly sensitive to changes in composition and pH. Despite that, ζ is used as an indicator of stability, in this case, the values of ζ did not have a correlation with CI, whose values were from 4.6% to 8.3 %, with the two most stable emulsions containing the GA to a pH of 4.

P, GA and pH were selected as independent variables adding the number of cycles for the RSM. The three levels of the variables were set as follows: P, 69; 76 and 83 MPa. GA, 20; 30 and 40%. pH, 4; 4.5 and 5 and number of cycles 1, 3 and 5. The presence of surfactant was limited to a fixed value of 5% (mixture of wall material: surfactant) for each formulation, being a mixture of Tween20/Span 85 2:1. CI, ζ, and PS were the response variables in a 3^3, Box-Behnken experimental design. Experimental results are shown in Table 3.

3.2 Box-Behnken Design

3.2.1 Creaming index (CI)

The experimental design for this parameter fitted to a second order equation with an R^2 of 0.855. The Equation 2 represents the model for coded factors, and Eq. (3)-(5) are for 1, 3 and 5 cycles of microfluidization respectively.

For coded factors:

$$CI = 11.35 - 3.8GA - 0.91pH + 0.16P - 1.05cycles + 0.33cycles - 8.15GA^2 + 1.67pH^2 + 2.94P^2 \quad (2)$$

Table 1. L8-orthogonal array and response values for Taguchi Experimental Design.

Run	GA (%)	MD (%)	Tween 20 (%)	Lecithin (%)	Span 85 (%)	pH	P (MPa)	CI (%)	PS (nm)	ζ (mV)
1	0.0	90	3.5	0.0	6.5	4	69	7.7	187	-31
2	0.0	90	3.5	6.5	0.0	7	103	6.7	400	-37
3	0.0	90	10	0.0	0.0	4	103	6.1	381	-26
4	0.0	90	3.5	0.0	6.5	7	69	6.9	305	-29
5	90	0.0	3.5	6.5	0.0	7	103	8.3	480	-27
6	90	0.0	10	0.0	0.0	4	103	4.6	367	-17
7	90	0.0	3.5	6.5	0.0	4	69	5.0	340	-16
8	90	0.0	10	0.0	0.0	7	69	7.3	345	-27

Table 2. Analysis of variance of the results of the Taguchi design.

Factor	% Contribution of factors on response variables		
	CI	PS	ζ
Tween 20	14.41	0.00	0.00
GA	1.74	0.00	82.99
pH	29.94	17.67	13.79
Lecithin	0.00	14.19	0.00
P	0.00	68.14	0.00
Tween 20-pH	0.00	0.00	3.22
GA-pH	53.91 0.00	0.00	

For 1 cycle of microfluidization:

$$CI = 446.24 + 4.51GA - 62.03pH - 0.06P - 0.08GA^2 + 6.69pH^2 + 2.94\times10^{-6}P^2 \quad (3)$$

For 3 cycles of microfluidization:

$$CI = 447.62 + 4.51GA - 62.03pH - 0.06P - 0.08GA^2 + 6.69pH^2 + 2.94\times10^{-6}P^2 \quad (4)$$

For 5 cycles of microfluidization:

$$CI = 448.02 + 4.51GA - 62.03pH - 0.06P - 0.08GA^2 + 6.69pH^2 + 2.94\times10^{-6}P^2 \quad (5)$$

According to these results, the variables with the greatest effect on CI were GA, GA^2 and P^2 (p <0.05). The sum of squares obtained for this model (data not shown) showed that the contribution of GA on CI was 76%, considering the most important process variable. This results can be attributed to the emulsifying properties of this gum, which is a highly branched biopolymer with a globular structure (Villay *et al*., 2012) that causes steric repulsion and electrostatic interactions of the particles of the emulsion by forming a viscoelastic layer around the oil droplets, making them thermodynamically and kinetically stable (Bouyer *et al*., 2011). This property is attributed to a sub-unit containing AGP (10-20% of the total weight of the GA) (Bouyer *et al*., 2011; Dickinson, 2003; Panteloglou *et al*., 2010). While the number of cycles of microfluidization was not a variable directly showing significant effects on the stability of emulsions, the obtained results showed that the structure of the GA is affected by the high pressures; therefore, more cycles may induce changes in conformation. However, CI equal to zero was never achieved given a very high turbulence intensity produced by the microfluidizer (Jafari *et al*., 2006).

3.2.2 Particle size (PS)

It was not possible to model PS under the conditions of the experiment. However, as shown in Table 3, the PS for the samples where in an interval of 293-671 nm. PDI indicates that the samples containing 20 and 30% of GA were polydisperse, showing the presence of a wide range of PS on emulsion, while the samples with 40% of GA had the less values of PDI ($\leq$ 0.5) but higher PS (Table 3). Jafari *et al*., (2007) reported that while microfluidization can decrease the particle sizes, PS values lower than the wavelength of visible light, when biopolymers are used as surface active agents, such as modified starch or GA, the particle sizes are bigger than those obtained with small molecule surfactants (eg. Tween 20). High obtained values of PDI (of one and close to one) for emulsions containing 20 and 30% GA helped us to support (together with the CI values reported) and identify the conditions (20 and 30% GA) for which an unstable emulsion turns into a stable one (40% GA).

The GA through its protein portion interacts with the oil droplets creating additional electrostatic interactions to steric barriers, which resulted in an increment of PS and a delay of the onset of instability mechanisms (Bouyer *et al*., 2011; Panteloglou *et al*., 2010). Additionally, the influence of surfactants on emulsion was not clear. Jafari *et al*., (2006) reported an interaction and competition when combining a surface active biopolymer and low molecular weight surfactant. Since surfactants sizes are much smaller than biopolymers, and given that they can reduce the interfacial tension more efficiently and quickly, they tend to control the interface after equilibration (Jafari *et al*., 2006; Kerstens *et al*., 2006) originating a displacement of the biopolymer on interface and generating instability of emulsion.

3.2.3 Zeta potential (ζ)

The ζ of the lipid droplets of the microfluidized emulsion was in between -28 to -20 (mV). In general particles with values of ζ lower than -30 or greater than +30 are considered to be stable, because at these values the electrical charge of droplets generates strong repulsive forces between the particles increasing the stability (Heurtault *et al*., 2003). Nevertheless, in this work, the ζ observed in the emulsions was due to the presence of GA.

Table 3. Box-Behnken response surface design and response results

Run	GA (%)	pH	P (MPa)	Cycles	MD/ Surfactant (%)	PS(nm)	PDI	ζ(mV)	CI (%)
1	40	4.5	83	3	60	463	0.01	-24	0.8
2	40	4.0	76	5	60	492	0.17	-23	0.8
3	20	5.0	76	1	80	382	0.94	-27	3.3
4	30	4.5	76	1	70	437	0.61	-20	8.3
5	40	4.5	83	1	60	525	0.15	-23	1.0
6	30	4.0	69	3	70	386	0.65	-23	16.1
7	20	4.0	76	3	80	339	0.64	-25	6.7
8	30	4.5	76	1	70	452	0.58	-20	7.3
9	30	5.0	89	5	70	335	0.47	-26	16.5
10	30	4.5	76	1	70	457	0.61	-21	7.3
11	40	4.5	69	5	60	437	0.02	-23	0.5
12	30	5.0	83	3	70	360	0.74	-25	14.0
13	30	4.5	76	1	70	454	0.59	-25	7.3
14	30	4.5	76	3	70	375	0.68	-25	10.0
15	30	5.0	83	1	70	424	0.67	-23	14.9
16	40	5.0	76	3	60	429	0.91	-20	3.3
17	20	4.5	83	1	80	385	0.85	-25	10.9
18	20	4.5	83	5	80	301	0.37	-23	10.3
19	30	4.0	83	5	70	356	0.25	-22	16.6
20	30	4.5	76	5	70	332	0.60	-25	11.8
21	30	4.5	76	1	70	437	0.62	-23	10.0
22	30	4.5	76	3	70	375	0.68	-23	16.3
23	20	4.5	69	5	80	305	0.43	-24	11.6
24	20	4.0	76	5	80	314	0.47	-24	10.0
25	30	4.0	69	5	70	362	0.30	-23	16.6
26	30	4.0	69	1	70	451	0.67	-23	16.3
27	30	4.5	76	5	70	349	0.34	-24	16.4
28	20	4.5	83	3	80	326	0.57	-25	9.0
29	40	4.5	69	1	60	391	0.53	-24	2.2
30	40	5.0	76	1	60	295	0.58	-23	2.5
31	40	4.0	76	1	60	632	0.18	-23	1.0
32	30	4.0	83	3	70	381	0.02	-22	19.1
33	30	4.5	76	5	70	341	0.34	-25	12.5
34	20	5.0	76	3	80	318	1.00	-28	8.4
35	40	4.5	83	5	60	308	0.21	-23	1.2
36	20	5.0	76	5	80	293	0.36	-25	6.6
37	20	4.5	69	1	80	395	1.00	-25	12.7
38	30	4.5	76	5	70	352	0.36	-24	11.7
39	30	5.0	69	3	70	365	1.00	-25	14.2
40	30	4.5	76	5	70	349	0.33	-24	14.2
41	40	5.0	76	5	60	348	0.35	-24	3.3
42	40	4.0	76	3	60	472	0.18	-23	1.8
43	20	4.5	69	3	80	331	1.00	-23	11.4
44	30	4.5	76	3	70	373	0.67	-24	11.7
45	20	4.0	76	1	80	403	0.85	-25	10.8
46	30	5.0	69	5	70	340	0.29	-27	16.4
47	30	4.5	76	3	70	363	0.67	-24	14.9
48	30	4.0	83	1	70	445	0.32	-21	20.2
49	30	4.5	76	3	70	378	0.67	-25	10.7
50	30	5.0	69	1	70	429	0.85	-26	10.8
51	40	4.5	69	3	60	671	0.74	-24	2.1

Bouyer *et al.*, (2011) determined ζ in almond oil emulsions using a combination of GA and β-lactoglobulin as wall materials using 2:1 and 1:2 ratios of protein:polysaccharide and pH 4.2. ζ values were -16 and -23 mV for 2:1 and 1:2 respectively. They found that in solutions of GA alone, concentrations of 0.5 and 2.5 % w/v, the ζ evaluated were -25 and -20 mV respectively, while the values of ζ to the β-lactoglobulin at same concentrations were 19 and 10 (both positive), which could indicate that the GA is adhered to the surface of the emulsion particle. This could be due to the larger size of this hydrocolloid as compared to the protein, which was also supported by the net negative charge of the GA at pH's between 4 and 8 (Panteloglou *et al.*, 2010). In this work, a similar phenomenon, may occur: MD, the mixture of surfactants and the AT could be trapped by the GA, given its molecular weight is about 77 times that of the MD (Alftrén *et al.*, 2012; Avaltroni *et al.*, 2004).

3.3 Optimization of the conditions to prepare AT emulsions using GA and MD and surfactants for a small CI and PS.

A multivariable optimization was performed to determine the optimum levels of the independent variables that would result in a stable emulsion with low values of CI in percentage and PS (Design Expert 7.0.3). In this optimization, a graphical interpretation of 3D surface response was constructed (Fig.1) to display the variation of desirability as a function of GA content and pH. Additionally, a numerical optimization was carried out to accurately determine the levels of independent variables resulting GA of 40%, pH of 4.9, P of 76 MPa and five cycles of microfluidization.

In optimized conditions it was obtained a CI of 0.5 % at 24 h after emulsion preparation with a PS of 437 nm, differing very little from the values predicted and achieved by a desirability function which were: CI of 0.5 % at 24 h with a PS of 387 nm and desirability of 0.83. With this optimization it was found that the high residence times associated with low pressures yield response variables with high values of desirability, which may indicate that in this case the kinetic factors were more important than the shear factors. Micrographs of the emulsions on the optimum conditions are shown in Fig. 2. It may be observed that the number of cycles had an important influence on the PS.

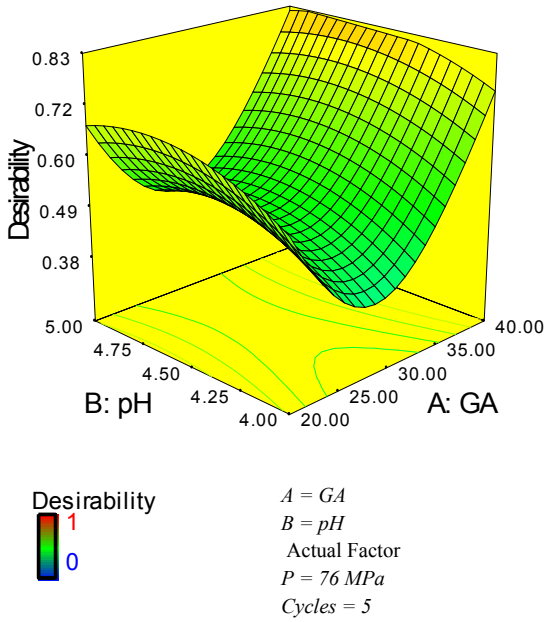

Fig. 1. Surface plot of Desirability of model affected by GA (%) content and pH (P = 76 MPa; Cycles = 5).

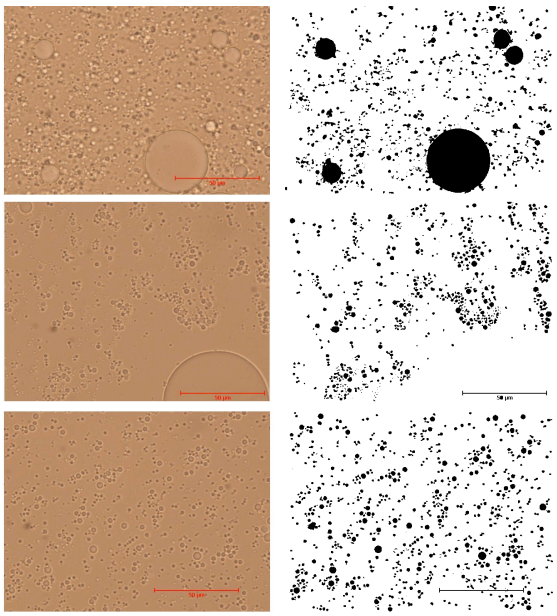

Fig. 2. Images of microfluidized emulsions for 1, 3, y 5 cycles and 76 MPa. Reference bar 50 μm.

It would also be desirable to continue experimenting more cycles until PS is independent of this variable. The number of cycles can be decisive in changing the structure of the GA, also providing enough time so that GA particles adhere to the interfaces, and therefore

had an influence on the stabilization time.

Conclusion

The coupled approach produced good results when applied to the optimization of the conditions to prepare AT emulsions by using GA and MD and low molecular weight surfactants. Taguchi design was firstly used to choose the main factors involved in obtaining the more stable emulsions with the lowest PS. RMS (3^3 Box-Behnken) was applied for optimizing conditions to prepare de emulsions. Obtained optimal formulations were: GA content 40%, MD-surfactant 60%, pH of 4.9, P of 76 MPa. Being the concentration of GA the most influential parameter on CI and PS. At concentrations of 40% of GA, the system had the lowest rates of separation (0.5-3.3%). It is possible to obtain stable emulsions by modifying GA concentrations, pH, P and microfluidizing cycles.

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