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Villalobos-Castillejos, F.; Alamilla-Beltrán, L.; Leyva-Daniel, D.E.; Monroy-Villagrana, A.; Jiménez-Guzmán, J.; Dorantes-Álvarez, L.; Gutiérrez-López, G.F.

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LONG TERM STABILITY OF MICROFLUIDIZED EMULSIONS USED IN MICROENCAPSULATION BY SPRAY DRYING

ESTABILIDAD A LARGO PLAZO DE EMULSIONES MICROFLUIDIZADAS PARA ENCAPSULACIÓN POR SECADO POR ASPERSIÓN

F. Villalobos-Castillejos, L. Alamilla-Beltrán, D.E. Leyva-Daniel, A. Monroy-Villagrana, J. Jiménez-Guzmán, L. Dorantes-Álvarez, G.F. Gutiérrez-López*

Departamento de Graduados e Investigación en Alimentos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Carpio y Plan de Ayala s/n, CP 11340 DF, México.

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Abstract The long-term (60 days) effect of microfluidization on the properties of β-carotene emulsions in a matrix of biopolymers (maltodextrin and gum arabic) for spray drying was evaluated. Microfluidization showed a significant effect ($p \leq 0.05$) in the reduction of Emulsion Droplet Size (EDS) (262-721 nm). Emulsions with higher contents of gum arabic produce the smallest EDS (383-721 nm). EDS and the polydispersity index were directly related while stability and lightness were inversely related to EDS. The majority changes in the stability of emulsions were observed during the first 10 days, promoting the broke up for those emulsions with higher content of maltodextrin. Emulsions with large content of gum arabic remain stable for longer time, despite depletion flocculation due to excess biopolymer.

Keywords: emulsions, microfluidization, long term stability, encapsulation, depletion flocculation.

Resumen

Se evaluó el efecto a largo plazo (60 días) de la microfluidización sobre las propiedades de emulsiones de β-caroteno en una matriz de biopolímeros (maltodextrina y goma arábiga) destinadas a secado por aspersión. La microfluidización mostró un efecto significativo ($p \leq 0.05$) en la reducción del tamaño de micela (EDS) (362-721 nm). Los menores EDS se obtuvieron con mayores contenidos de goma arábiga (383-422 nm). El EDS mostró una relación directamente proporcional con el índice de polidispersidad e inversamente proporcional a la estabilidad y la luminosidad. Se observaron durante los primeros 10 días de análisis los mayores cambios en la estabilidad, presentándose la separación de fases en emulsiones con mayor contenido de maltodextrina. Las emulsiones con mayor contenido de goma arábiga se mantuvieron estables por más tiempo, a pesar de presentar floculación por agotamiento debido el exceso de biopolímero.

Palabras clave: emulsiones, microfluidización, estabilidad a largo plazo, encapsulación, floculación por agotamiento.

1 Introduction

Emulsification is one of the most important steps for the encapsulation of active lipid-soluble agents through spray drying (Chaparro-Mercado *et al*, 2012; Shahidi & Han, 1993; Jafari *et al*, 2007). The Emulsion Droplet Size (EDS) has an important effect over the efficiency of trapping and stability of the active agent in the capsules, and it is dependent on the method of emulsification used (Jafari *et al*, 2007; Cubero *et al*, 2002; Becher, 2001; Zuidam & Shimoni, 2010; Quintanilla-Carvajal *et al*, 2014). The low energy emulsification methods, such as phase inversion temperature (PIT) and the phase inversion composition (PIC) involve modification of factors that affect the Hydrophilic-Lipophilic Balance (HLB) of the system. However, these methods require great amounts of surfactants and are not applicable at industrial levels (Domínguez-Hernández *et al*, 2016; Cano-Sarmiento *et al*, 2014; Jafari *et al*, 2007). The high energy emulsification methods such as microfluidization, have a flexible control of EDS distribution and produce emulsions with smaller EDS (Ochoa *et al*, 2016; Jafari *et al*, 2007; Salvia-Trujillo *et al*, 2013; Qian & McClements, 2011).

During microfluidization, the emulsions are formed by the action of the shear forces (laminar flow), turbulence (inertial flow) and cavitation (implosion of

* *Corresponding author. E-mail*: gusfgl@gmail.com

steam bubbles) produced in the interaction chamber containing micro-channels of different dimensions (microns) and arrangements ("Y" and "Z"), where the flow stream is conducted by a pneumatically powered pump at high pressures between 150 kPa to 150 MPa (Jafari *et al*, 2007; Dalgleish *et al*, 1996; Pinnamaneni *et al*, 2003; Schultz *et al*, 2004).

As with the emulsification method, the wall material selection influences the characteristics and properties of the capsules obtained by spray drying (Tovar-Benítez *et al*, 2016; Shahidi & Han, 1993). The biopolymers most commonly used in spray drying are the gum arabic and maltodextrin. The gum arabic has good solubility, active surface properties, low viscosity at high concentrations and emulsification properties, meanwhile, the maltodextrin, in spite of not having emulsification properties, acts as supporting agent in the formation of microstructure of capsules obtained by spray drying (Quintanilla-Carvajal *et al*, 2014; Monroy-Villagrana *et al*, 2014). The oil/wall material ratio used for encapsulation purposes with gum arabic and maltodextrin has been reported between 0.2 to 0.4, meaning an excess of wall material for emulsification but enough for encapsulation by spray drying (Quintanilla-Carvajal *et al*, 2014; González-Rodríguez *et al*, 2007; Pérez-Alonso *et al*, 2003; McNamee *et al*, 2001). An excess of wall material in the emulsions causes a phenomenon known as depletion flocculation, which could destabilize the emulsion (Jafari *et al*, 2007; Mirhosseini *et al*, 2008).

It has been reported that the emulsification by high pressures modifies the structural composition of biopolymers, improving the stability of the emulsions. The impact of the structural modifications is dependent of the type and intensity of the high pressure used: hydrostatic pressures above 800 MPa affects the viscoelasticity of the gum arabic due to the exposure of proteins (Panteloglou *et al*, 2010), pressures above 150 MPa in a microfluidizer promotes the loss of the tertiary and quaternary structure of the proteins due to the breakup of weak bonds (Jafari *et al*, 2007).

The investigations carried out for emulsions obtained by microfluidization, and used for spray drying, have been focused on the effect of the emulsification method and the biopolymers on the properties of emulsion and the characteristics of the capsules obtained (Jafari *et al*, 2007; Quintanilla-Carvajal *et al*, 2014; Soottintantawat *et al*, 2003; Soottintantawat *et al*, 2005). Due to the fact that spray drying is often carried out on the same day after the emulsification process, the properties of the emulsion have been considered only at the moment in which they are elaborated, which has limited the knowledge of the stability of those emulsions during longer periods. The objective of this study was to evaluate the long-term stability of emulsions elaborated by microfluidization and destined to encapsulation by spray drying.

2 Materials and methods

2.1 Materials

The β-carotene (βC) was supplied by BASF S.A. de C.V. (Mexico City) and dissolved in commercial corn oil (final concentration 0.59 mg/mL), the gum arabic (GA) and maltodextrin DE20 (MD) were acquired from Alfred L. Wolf S.A. de C.V. (Mexico City) and Química LEFE S.A. de C.V. (Mexico City). Distilled water was used for the preparation of all solutions and emulsions.

2.2 Methods

2.2.1 Pre-emulsion preparation

The continuous phase consisted in a solution of 20% (w/w) of dissolved solids composed of maltodextrin and gum arabic. The disperse phase was β-carotene (βC) dissolved in commercial corn oil. The wall materials were hydrated in distilled water during 12 h before emulsification and maintained in agitation at 25 °C to accomplish rehydration. The amounts of each material used were determined according to a D-optimum mixture design (Table 1). The mixture was homogenized with a high speed emulsifier (Rival IB901-MIX, USA) at 3000 rpms for 3 minutes.

Table 1. Matrix of the D-optimal mixture design

Experiment	Active agent (%)	Wall Material (%)	
	βC	MD	GA
1	5	0.00	95.00
2	2	0.00	98.00
3	2	98.00	0.00
4	2	98.00	0.00
5	3.5	48.25	48.25
6	2.75	24.13	73.13
7	5	0.00	95.00
8	5	95.00	0.00
9	3	0.00	97.00
10	4.25	71.62	24.13

Formulations at 20% of total solids (w/w)

2.2.2 Microfluidization process

For each one of the experiments, 200 mL of pre-emulsions were processed in a microfluidizer (M-110Y, Microfluidics, Newton MA, USA) operating at 68.95 MPa and using two cycles at 25 °C. A "Y" interaction chamber (F20Y, ϕ=75 μm) as well as a "Z" auxiliary chamber "Z" (H30Z, ϕ=200 μm) were used (Monroy-Villagrana *et al*, 2014). The properties of the emulsions were evaluated at time zero and at 60 days.

2.2.3 Emulsion Droplet Size (EDS) and polydispersity index (PDI) analysis

The EDS and the PDI in the pre-emulsion and emulsion were evaluated using a particle size analyzer Zetasizer Nano (Malvern Instruments Ltd, England). The EDS and PDI were measured at time zero and at the end of the 60th day. The emulsion (1 mL) was diluted in 40 mL of distilled water and shaken manually.

2.2.4 Turbiscan Lab® stability (TSI) analysis

The stability of the emulsion was evaluated with a Turbiscan Lab® equipment (Expert, Formulation Inc., France) through the Turbiscan Lab® Stability Index (TSI). TSI values close to zero indicated a high stability of the emulsion (Microfluidics, 2008). The measurements were carried out each 10 days until the end of the study.

2.2.5 Colorimetric parameters evaluation

The colorimetric parameters were measured by the CIEL*a*b* system by using a colorimeter (CR-10, Konica Minolta, Japan) with a tungsten light, measuring area of Ø8mm, illumination angle of 8° and diffused vision. Emulsion (5 mL) was placed in plastic Petri dishes of 2.5 cm in diameter. Five measurements for each sample were carried out, reporting the average at the beginning and end of the study.

2.2.6 Spectroscopy RAMAN analysis

β-carotene and biopolymers were analyzed individually before and after microfluidization. At the end of the evaluation, emulsions were analyzed using the Spectrometer RAMAN (LAB-RAMAN Model HR800, Horiba JobinYvon, Japan) with a magnitude of 50X, a laser of 784.29 nm and an aperture of 0.75.

2.2.7 Statistical analysis

The experiments were carried out based on a mixture D-optimum design and the results presented are the average of at least twice. A quadratic model was utilized to analyze the variance (ANOVA) with significance level of 0.05, by using the software Design-Expert 9.0.0.7. (Stat-Ease, Inc., USA).

3 Results and discussion

At day 60, all the experiments presented the formation of 2 phases, identified as a color change in the top of the sample; the upper one was denominated as phase 1, meanwhile the phase at the bottom as phase 2. The properties of the emulsion were determined at day 0 and day 60 for each one of the phases formed, except the Turbiscan Lab® Stability Index which was evaluated each 10 days.

3.1 Emulsion Droplet Size (EDS) and polydispersity index (PDI) analysis

It was observed that EDS and PDI values were directly related. Microfluidization produced emulsions with EDS between 362 to 721 nm, decreasing the EDS of pre-emulsions at 50 % (700 to 1500 nm). According to the EDS and PDI shown in Table 2, it was observed that gum arabic had the greatest influence over EDS, mainly due to its emulsification properties. The emulsification properties of gum arabic are given by the arabinogalactan protein fraction in the gum (10-20% of total weight). The protein fraction was embedded in the oil phase while carbohydrates remained in the aqueous phase (Bouyer *et al*, 2011; Monroy-Villagrana *et al*, 2014).

The presence of gum arabic gives place to small EDS in emulsions elaborated by microfluidization (Soottintantawat *et al*, 2005) which has been reported in emulsions of D-limonene and gum arabic at 82.8 MPa and in emulsions of α-tocopherol/maltodextrin20DE/gum arabic at 68 MPa and two microfluidization cycles (Quintanilla-Carvajal *et al*, 2014).

Although diverse biopolymers are widely applied as wall materials in emulsions destined for spray drying, these require longer times of emulsification to be adsorbed in the interface of the micelles due to their high molecular weight which can be shortened by using microfluidization, due to the simultaneous forces of coalition, deformation and cavitation that produce EDS

Table 2. Emulsions Droplet Size (EDS) and Polydispersity Index (PDI) of the emulsions at day 0 and day 60.

Experiment	Formulation % (p/p)	Day 0		Day 60			
	βC/MD/GA	EDS (nm)	PDI	phase 1		phase 2	
				EDS (nm)	PDI	EDS (nm)	PDI
1	5/0/95	418 ± 1.1	0.11 ± 0.01	548 ± 3.5	1	412 ± 2.7	0.07 ± 0.01
2	2/0/98	405 ± 9.1	0.04 ± 0.05	466 ± 8.6	0.082 ± 0.01	431 ± 10.8	0.13 ± 0.02
3	2/98/0	567 ± 82	0.66 ± 0.50	1044 ± 5	1	452 ± 10.6	0.99 ± 0.01
4	2/98/0	563 ± 16	0.58 ± 0.60	1000 ± 2.1	1	434 ± 15.6	0.39 ± 0.05
5	3.54/48.25/48.25	439 ± 10	0.32 ± 0.40	593 ± 12.2	1	428 ± 5.3	0.54 ± 0.05
6	2.75/24.13/73.13	422 ± 11	0.05 ± 0.01	635 ± 12.4	0.729 ± 0.02	450 ± 21.85	0.01 ± 0.01
7	5/0/95	406 ± 6	0.09 ± 0.04	409 ± 9.2	1	414 ± 2.7	0.01 ± 0.01
8	5/95/0	718 ± 4	0.85 ± 0.21	1052 ± 70.1	1	669 ± 42.3	0.72 ± 0.03
9	3/0/97	383 ± 29	0.19 ± 0.16	659 ± 27.1	0.407 ± 0.01	314 ± 11	0.36 ± 0.01
10	4.25/71.62/24.13	538 ± 25	0.97 ± 0.01	556 ± 9.6	1	513 ± 1.5	0.19 ± 0.02

in the order of microns so increasing their stability (Jafari *et al*, 2007b; Paquin, 1999).

The EDS values were adjusted to the variables depicted in Equation (1) by the following model (R^2=0.995) in which all variables and their interactions were significant (p<0.05).

$$EDS = 2.59\times10^{7}(\beta C) + 28248.03(MD) + 28169.24(GA) - 2.78\times10^{7}(\beta C\cdot MD) - 2.77\times10^{7}(\beta C\cdot GA) + 26460.3(MD\cdot GA) \quad (1)$$

As shown in Table 2, after 60 days, the phase 1, presented EDS between 466 to 1050 nm, which were higher to those in phase 2 (314 - 669). The high EDS values in phase 1 were due to coalescence, particularly in the experiments with a higher content of maltodextrin. However, in the experiments with large contents of gum arabic and low contents of βC, no difference in EDS was observed in both phases so indicating that the formation of the phases was not induced by coalescence.

3.2 Turbiscan Lab® stability (TSI) analysis

The measurements of TSI were carried out during 4 h after preparation of emulsions. The greatest changes in TSI, were observed during the first 2 h, obtaining values between 0.2 and 3 ΔBS/h. TSI was related to the EDS and PDI mentioned in the previous section. It was found that EDS and TSI were directly related.

The emulsions with large contents of gum arabic as wall material (experiments 2, 6, 7 and 9), had the highest stability (0.27-0.49 ΔBS/h). The protein component in gum arabic is adsorbed in the surface of the oil droplets while the carbohydrates fraction inhibits the flocculation and coalescence through electric and stearic repulsions (Monroy-Villagrana *et al*, 2014; Williams & Phillips, 2009). Emulsions containing only maltodextrin (experiments 3, 4 and 8) showed the lowest stability (1.75-3.28 ΔBS/h). The presence of maltodextrin in the preparation of emulsions was due to its solubility, low viscosity and its capacity for the formation of capsules during spray drying (Avaltroni, *et al*, 2004; Monroy-Villagrana *et al*, 2014).

An important reduction in TSI was observed at day 10 (Figure 1), followed by a gradual loss of stability. Experiments with large contents of βC (> 5 % w/w) maintained the highest stability during the first 40 days so increasing the TSI until the end of the evaluation period. Experiments in which maltodextrin concentration were high, showed the largest TSI as from the 10th day.

It has been reported the exposure to media and breakup of weak bonds, such as hydrogen bonds, in proteins structures during microfluidization (Jafari *et al*, 2007; Panteloglou *et al*, 2010), therefore, an increase of the availability and exposure to media of protein groups of gum arabic could explain the increase in the stability of the emulsions with high contents of gum arabic.

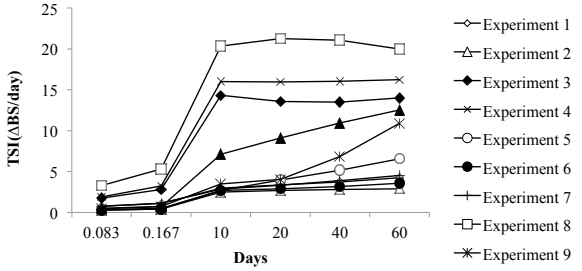

Fig. 1. Turbiscan Lab® Stability Index (TSI) of emulsions obtained by microfluidization at different times.

Table 3. Colorimetric parameters for emulsions recently elaborated

Experiment	Colorimetric parameters		
	a*	b*	L
1	12 ± 0.1^{a}	$46 \pm 1.1^{a,b,c,d}$	64 ± 1.2^{a}
2	6 ± 1.1^{b}	33 ± 2.4^{d}	$57 \pm 0.2^{a,b}$
3	6 ± 1.9^{b}	$35 \pm 7.2^{c,d}$	$53 \pm 4.8^{b,c}$
4	6 ± 2^{b}	$39 \pm 7.2^{b,c,d}$	48 ± 6^{c}
5	12 ± 1.1^{a}	$48 \pm 1.1^{a,b}$	64 ± 1.4^{a}
6	8 ± 1.7^{b}	$39 \pm 0.4^{b,c,d}$	$60 \pm 1.2^{a,b}$
7	12 ± 0.1^{a}	$45 \pm 1.5^{a,b,c}$	64 ± 1.8^{a}
8	9 ± 0.1^{b}	$39 \pm 1.1^{b,c,d}$	$53 \pm 5.4^{b,c}$
9	12 ± 0.6^{a}	$43 \pm 7.3^{a,b}$	63 ± 3.6^{a}
10	13 ± 2^{a}	51 ± 1.9^{a}	65 ± 1.4^{a}

Figures in the same row, followed by different letters are significantly different ($p \leq 0.05$).

3.3 Colorimetric parameters evaluation

Color and opacity are the main optical properties in emulsions and are dependent of its composition and structure (Chung & McClements, 2014). In Table 3, the colorimetric parameters of the emulsions at day 0 are showed. At the end of the evaluation, the phases of the emulsion did not allow obtaining conclusive data in relation to these parameters. In the present, values of luminosity (L*) were determined since it has been inversely related to EDS (Chung & McClements, 2014).

EDS was inversely related to L*. An increase in L* was observed with large content of βC due to the increase of light dispersed by the oil drops (Chung & McClements, 2014). Besides, the presence of chromophores such as β-carotene influences the color of the emulsion. The βC gave a yellow-orange coloration to emulsions. It has been reported the use of L* and a* parameters to determine the degradation of β-carotene (Flores-Miranda *et al*, 2015; Desobry *et al*, 1997),

3.4 Spectroscopy RAMAN analysis

The RAMAN spectroscopy was a quick and useful tool to observe possible changes in the chemical structure of components of the emulsion due to high pressures in the microfluidization, as well as to know the composition of each one of the phases presented at the end of the study.

For β-carotene, three main bands were identified (a, b, c), located approximately at 1005, 1157 and 1524 cm^{-1} respectively. These bands were attributed to the flexion in the plane of -CH (1008 cm^{-1}), symmetric stretching of C-C (1157 cm^{-1}) and symmetric stretching of C=C (1524 cm^{-1}) (Tschrimer, 2011). The maltodextrin and gum arabic have in their RAMAN spectrum, regions 1 (300-700 cm^{-1}) and α (~850 cm^{-1}), in contrast to spectra of various polysaccharides (Vandenabeele *et al*, 2000).

No differences were found between RAMAN spectra of experimental materials (data not shown), before and after microfluidization which means that high pressures did not cause ruptures in the chemical structure of the biopolymers as claimed by Salvia-Trujillo *et al* (2009) for sodium alginate, however an exposure of the protein fraction in gum arabic could improve the stability in emulsions (Jafari *et al*, 2014 & Panteloglou *et al*, 2010).

In Figure 2, the RAMAN spectrum of experiment 5 is shown. This experiment was selected because both polymers are used in the same concentration (βC:MD:GA, 3.5:48.25:48.25 w/w). In phase 1, the presence of β-carotene was observed with greater intensity (bands a, b, c), due to the increase in the concentration of micelles. Also the β-carotene, phase 1 showed the presence of maltodextrin, in agreement to results by Jafari *et al* (2007) and Mirhosseini *et al* (2008), who pointed out that not absorbed biopolymers, can be found in the surface of the emulsion.

The presence of gum arabic (region α) was notorious in both phases (1 and 2). There was a sufficient amount of emulsifier to achieve stability in the emulsion, nonetheless, when in excess, the emulsifier promotes a phenomenon known as depletion flocculation, which caused the formation of phase 1 in some experiments and not the coalescence, explaining the minimum differences between the EDS

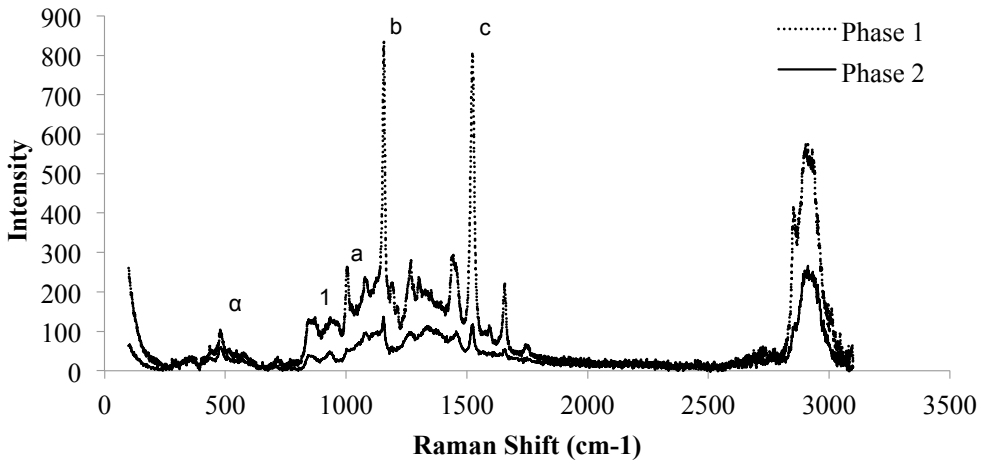

Fig. 2. RAMAN spectra of phase 1 and 2 from experiment 5 (β-carotene/maltodextrin/gumarabic 3.5/48.25/48.25 (w/w)) at day 60.

in both phases (Jafari *et al*, 2007; Mirhosseini *et al*, 2008) (see Section 3.1). It is important to point out that in spite of the presence of the depletion flocculation, stability and EDS were not affected.

Conclusions

The combination of the emulsification by microfluidization and the emulsifying properties of gum arabic produce small EDS and maintain the long term stability of the emulsions elaborated for the purpose of encapsulation given the small variation of EDS values. It was found that EDS, PDI and TSI were directly related while EDS and L*parameter were inversely related. In spite of the presence of the depletion flocculation, due to the excess of wall material, the stability was maintained and this phenomenon was identified by means of RAMAN spectroscopy.

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