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Stability of a colloidal system based on avocado (*Persea americana* Mill. cv. Hass) and others: Effect of process and composition

Estabilidad de un sistema coloidal a base de aguacate (*Persea americana* Mill. cv. Hass) y otros: Efecto del proceso y composición

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Abstract

Avocado (*Persea americana* Mill. cv. Hass), is a perishable fruit, which compositionally presents nutritional benefits, which has led to its productive chain to look for new alternatives of agroindustrialization to improve its competitiveness in the market, being guacamole a potential option. The aim of this research was to evaluate the influence of composition and homogenization process on the colloidal system stability based on avocado and other ingredients for a potential use in the aspersion drying process. We used the surface response methodology and a central composite experimental design for experimental optimization of the process, considering three independent variables as follows: avocado dry solids ($DS_{avocado}$), homogenization time and tert-butylhydroquinone concentration (TBHQ), and six dependent variables: zeta potential (-ζ), color (L *: brightness, a *: green-red chromaticity, b *: yellow-blue chromaticity), viscosity (μ), spectral absorption stability index, peroxide index (PI) and particle size (D_{10}, D_{50} and D_{90}). The best emulsion and process formulation was achieved with 47.1% of $DS_{avocado,}$ 5 min of homogenization and 100 $mg.kg^{-1}$ of TBHQ, obtaining an emulsion with $-\zeta = -27.67 \pm 0.29$, $L^* = 51.3 \pm 1.0$, $a^* = -5.8 \pm 0.8$ and $b^* = 30.0 \pm 1.9$, $\mu = 1034.56 \pm 95.91$ cP , $R = 0.78 \pm 0.03$, $IP = 0.73 \pm 0.30$ meq $H_2O_2.Kg^{-1}$ emulsion, $D_{10} = 8.1 \pm 0.7$ μm, $D_{50} = 56.2 \pm 11.5$ μm and $D_{90} = 346.6 \pm 94.6$ μm. The emulsion based on avocado and other ingredients, presents a physico-chemical, physical stability adequate stability, which guarantees a potential use for aspersion-drying process, since it meets with the criteria design for viscosity and total solids, of the pilot unit PSALAB, Vibrasec S.A.

Key words: Colloids, fruits, functional foods, guacamole.

Resumen

El aguacate (*Persea americana* Mill. cv. Hass) es un fruto perecedero que composicionalmente presenta bondades nutricionales, lo que ha llevado a su cadena productiva a buscar nuevas alternativas de agroindustrialización para mejorar su competitividad en el mercado, siendo el guacamole en polvo una potencial opción. El objetivo de la investigación fue evaluar la influencia de la composición y el proceso de homogenización sobre la estabilidad de un sistema coloidal a base de aguacate y otros ingredientes, para un potencial uso en el proceso de secado por aspersión. Se utilizó la metodología de superficie de respuesta y un diseño experimental central compuesto para la optimización experimental del proceso, considerando tres variables independientes: sólidos secos aportados por el aguacate ($SS_{Aguacate}$), tiempo de homogenización y concentración de ter-butilhidroquinona (TBHQ), y seis variables dependientes: potencial zeta (-ζ), color (L*: luminosidad, a*: cromaticidad verde-rojo, b*: cromaticidad amarillo – azul), viscosidad (μ), índice de estabilidad por absorción espectral (R), índice de peróxido (IP) y tamaño de partícula (D_{10}, D_{50} y D_{90}). La mejor formulación de la emulsión y proceso se alcanzó con 47.1% $SS_{Aguacate}$, 5 min de homogenización y 100 mg/kg TBHQ, obteniendo una emulsión con $-\zeta = -27.67 \pm 0.29$, $L^* = 51.3 \pm 1.0$, $a^* = -5.8 \pm 0.8$ y $b^* = 30.0 \pm 1.9$, $\mu = 1034.56 \pm 95.91$ cP, $R = 0.78 \pm 0.03$, $IP = 0.73 \pm 0.30$ $meqH_2O_2/kg$ emulsión, $D_{10} = 8.1 \pm 0.7$ μm, $D_{50} = 56.2 \pm 11.5$ μm y $D_{90} = 346.6 \pm 94.6$ μm. La emulsión a base de aguacate y otros ingredientes presenta una estabilidad adecuada en términos fisicoquímicos, físicos que garantiza un uso potencial para el proceso de secado por aspersión, ya que cumple con los criterios de diseño en cuanto a viscosidad y sólidos totales, de la unidad piloto PSALAB, Vibrasec S.A.

Palabras clave: Alimentos funcionales, frutas, guacamole, métodos de optimización.

Introduction

Avocado (*Persea americana* Mill. cv. Hass) is a climacteric fruit which is characterized by its high nutritive value, having an important content of unsaturated fatty acids, fat soluble and water soluble vitamins, especially A and C. In addition, it has been pointed out that this fruit may be a functional food due to beneficial effects tested for human health (Jacobo-Velázquez & Hernández-Brenes, 2012).

This have allowed an increase in avocado consumption worldwide, mainly in countries such as the United States, France, Germany and Spain, which has resulted in an increase in planted areas, and in turn a variety of possibilities of uses as industrialized products (Duque, Londoño-Londoño, Álvarez, Paz, & Salazar, 2012).

Currently, the main industrialized avocado products, which are marketed as follows: pulp, as a base for spreadable products, both fresh and chilled or frozen, and oil. Avocado puree is the product, which has a greater volume of production when used as a base for spreadable products, mainly guacamole, a product composed of other ingredients such as tomato, onion, garlic, cilantro, jalapeño chili pepper, salt, acids and preservatives. Guacamole, is a popular seasoning in Mexico, and currently in the United States and Europe, as the basis of food called "Tex-Mex".

Guacamole, constitutes an oil-in-water colloidal system type emulsion, which is thermodynamically unstable, with its stability representing a critical and essential variable in the shelf-life of this food.

The physical destabilization of colloidal systems mechanisms or colloidal disruption, can be presented without variations in particle size, for example, when sedimentation or cremation and flocculation occur, or with particle size variation, including coagulation phenomena, coalescence, Ostwald maturation or phases inversion (Mirhosseini, Tan, Hamid, & Yusof, 2008w/w, x1).

Some authors have evaluated the effect of different compounds on the stability of oil-in-water emulsions as follows: globular protein sources (soybeans, peas and whey proteins) and the casein ratio in emulsions with soybean oil (Liang, Wong, Pham, & Tan, 2016), sodium caseinate use in emulsions based on peanut oil (Long *et al.*, 2015) with stability enhanced by increased concentrations of Na-CN. The lower equilibrium interfacial tension along with greater negative \ u03b6-potential of PO revealed that Na-CN was preferentially adsorbed to the PO interface. Adding 0.05mol/L NaCl to the PO emulsions minimized depletion flocculation caused by the unadsorbed Na-CN, but further NaCl addition increased oil droplet size and concomitant coalescence. For the PO-DAG emulsions, adding 0.2mol/L NaCl did not significantly (p>0.05, texturized whey protein incorporation into emulsions containing corn oil and butter oil (Ruttarattanamongkol, Nor Afizah, & Rizvi, 2015), sucrose esters and Tween 20.60 & 80, among others. In addition, the use of homogenization high speed and ultrasound in the preparation of stabilized emulsions with fenugreek gum (Kaltsa, Yanniotis, & Mandala, 2016), oil content, starch (starch octenyl sodium succinate) and pressure homogenization on the o/w emulsion stability (Domian, Brynda-Kopytowska, & Oleksza, 2015).

The aim of this research was to evaluate the influence of composition and homogenization process on a colloidal system stability based on avocado (*Persea americana* Mill. cv. Hass) and other ingredients, for a potential use in aspersion drying process.

Materials and methods

Raw materials

We used fresh avocados (*Persea americana* Mill cv. Hass), from the municipality of Abejorral, Antioquia-Colombia, which were processed between 11 and 15 days of ripening, gum arabic (Master Gum FT), maltodextrin (between 18 and 20 dextrose equivalent), TBHQ (terbutylhydroquinone), sodium chloride, food grade, onion (*Allium cepa* L.), garlic (*Allium sativum* L.), bell pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), cilantro (*Coriandrum sativum* L.), lemon (*Citrus aurantiifolia* (Christm.) Swingle) and water.

Formulation and emulsion preparation

Emulsion formulations were standardized according to the avocado (*Persea americana* Mill cv. Hass), dry solids content ($DS_{avocado}$), with a total acidity of 0.1%, provided by lemon juice adjusted to 7% as citric acid. The other ingredients used were fixed in emulsions. Batches of 2000 g of emulsion were prepared. Initially, avocado was homogenized with lemon juice and partial water content, the total solids being adjusted if required. Subsequently, a homogenized maltodextrin, gum arabic, TBHQ, NaCl, vegetable ingredients premixture and the rest of water was added. An Ultraturrax IKA homogenizer model TK50, USA, was used at a speed of 10.000 rpm.

Emulsions stability characterization

Z potential (ζ)

Emulsions zeta potential was measured using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcester, UK) (Rezvani, Schleining, & Taherian, 2012). Nano zetasizer calculates zeta potential by determining the electrophoretic mobility and then applying the Henry equation. To avoid multiple dispersion effects and air bubbles presence, emulsions were diluted with deionized water in an emulsion: water ratio (1: 100).

Stability index by spectral absorption (R)

It was determined from the absorbances ratio at two wavelengths (800 and 400 nm) (A800/A400) (Mirhosseini *et al.*, 2008)w/w, x1, using a UV-Visible Spectrophotometer (Thermo Scientific Evolution 60). Emulsion samples were diluted in water (1: 100) and triplicates were performed by emulsion. The R stability index is based on the light scattering properties, which is related to the average droplet size and wavelength.

Viscosity (μ)

It was determined using the rheometer (Brookfield DV-III Ultra, Brookfield Engineering Laboratories, Inc., USA), coupled with a Brookfield thermostated bath model TC-502, controlled temperature of 25° C, equipped with the spindle RV4 at a speed of 0.01 to 100 rpm, the viscosity being reported in cP at a speed of 100 rpm. A fixed emulsion volume was measured in a 600 mL beaker, and spindle depth was constant throughout the measurements (Mirhosseini, Tan, Hamid, Yusof, & Chern, 2009).

Peroxide index (PI)

It was determined by a spectrophotometric method, which relies on the peroxides ability to oxidize ferrous ions to ferric ions which react with various reagents that produce colored complexes. It was expressed as H_2O_2 milliequivalents per kg sample, using equation 1. Where Am and Ab, correspond to the sample and blank absorbance respectively, at a wavelength of 500 nm; M = calibration curve slope, W = sample weight (g); 2 = conversion factor to express as H_2O_2 meq, 55.84 = iron molecular weight and Vt = final volume (mL) of the reaction mixture (Equation 1).

$$\frac{\mathrm{H_2O_{2\ meq}}}{\mathrm{kg\ sample}} = \frac{(\mathrm{A_m} - \mathrm{A_b}) * 1/\mathrm{m}}{\mathrm{w} * 2 * 55{,}84} * \mathrm{v_t}$$

Color

It was determined using an X-Rite spectrophotometer, model SP62 with D_{65} illuminant and 10° observer as reference. From the spectra reflection, we obtained the color coordinates for the ICD-La * b *, where L * is a luminosity indicator, a * (green chromaticity (-) to red (+)) and b * (blue chromaticity (-) to yellow (+)).

Particle size

Particle size distribution of the prepared emulsions, was determined using laser light diffraction in a Mastersizer 3000 (Malvern Instrument Ltd., Worcestershire, UK). Sample was added in 500mL of distilled water until a darkening value of 10 ± 1%, was obtained. The size distribution was calculated using the Mie theory by the refractive index of 1.52 (Millqvist-Fureby & Smith, 2007). Particle size was reported as D_{10}, D_{50} y D_{90} percentiles.

Experimental design and statistical analysis

Response surface methodology was used with a composite central design to establish the main and combined effects of the independent variables as follows: (A) $DS_{avocado}$ (33.07% - 50.32%), (B) homogenization time (3 - 7 min) and (C) TBHQ concentration [TBHQ] (50 - 100 $mg.kg^{-1}$) on dependent variables: ζ, L*, a*, b*, μ, R, IP, D_{10}, D_{50} and $D_{90,}$ obtaining 20 randomized experiments with six replicates at the central point. Table 1, describes the experimental design used. In addition, independent variables to optimize the emulsion proportion components in terms of dependent variables, were determined. We used a quadratic model (Equation 2), to analyze each dependent variable (Y) as a function of independent variables, where β_0, is a constant, β_A, β_B and β_C are the linear coefficients for each factor; β_{A2}, β_{B2} and β_{C2}, are the quadratic coefficient of each factor; β_{AB}, β_{AC} and β_{BC} are the product coefficient of factor interactions.

$$Y = \beta_0 + \beta A\beta_B B + \beta_C C + \beta_{A2} A^2 + \beta_{B2} B^2 + \beta_C{}^2 C^2 + \beta_{AB} AB + \beta_{AC} AC + \beta_{BC} BC$$

The models adequacy was determined using the non-fit test and the coefficient of determination (R^2). In addition, variance analysis (ANOVA), was performed with a significance level of 5%. The matrix of the experimental design, the results analysis and the procedure optimization was carried out using the software Statgraphics Centurion XVI.I®.

Table 1. Experimental design for formulation of emulsion optimization based on avocado (*Persea americana* Mill. cv. Hass) and other ingredients

Experiments	$DS_{Avocado}$ (%)	Homogenization time (min)	[TBHQ] ($mg.kg^{-1}$)
1	50.32	3	50
2	44.15	5	75
3	44.15	5	75
4	33.07	3	50
5	50.32	5	75
6	44.15	7	75
7	33.07	5	75
8	44.15	3	75
9	44.15	5	75
10	44.15	5	75
11	50.32	7	50
12	33.07	7	100
13	44.15	5	75
14	44.15	5	50
15	50.32	3	100
16	33.07	7	50
17	33.07	3	100
18	50.32	7	100
19	44.15	5	75
20	44.15	5	100

Results and discussion

Tables 2 present the mean values and the standard deviations the dependent variables. Table 3 shows the p-values of the effects of the the full quadratic model for each of the response variables. Figure 1, shows the response surface graphs of dependent variables, which were statistically significant ($p<0.05$).

Table 2. Quality attributes of avocado (*Persea americana* Mill. cv. Hass) based emulsions and other ingredients

Experiment	Variables dependientes									
	ζ (mV)	R	μ (cP)	IP ($H_2O_2meq.k\text{-}g^{-1}$)	L*	a*	b*	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
1	-28.8±0.6	0.833±0.006	1395.3±132.0	1.14±0.11	50.9±0.1	-5.7±0.0	31.1±0.1	8.3±0.5	75.4±10.0	556.3±293.9
2	-27.8±0.4	0.758±0.007	691.3±57.4	1.44±0.10	53.1±0.4	-6.3±0.1	28.5±0.5	6.4±1.2	27.1±4.7	90.2±39.3
3	-30.9±1.0	0.817±0.011	834.6±18.5	0.66±0.12	50.1±0.3	-6.1±0.1	32.1±0.2	9.6±0.5	72.5±6.1	673.0±70.3
4	-27.8±0.6	0.759±0.042	476.6±17.2	0.93±0.42	56.0±0.5	-5.9±0.1	24.7±0.3	3.3±0.3	59.4±21.4	348.0±147.7
5	-27.8±1.2	0.896±0.003	1102.6±9.0	1.22±0.07	56.5±0.2	-5.7±0.1	29.6±0.6	7.7±1.2	99.4±82.8	969.0±605.6
6	-29.2±1.0	0.831±0.005	755.0±57.9	1.01±0.28	48.5±1.8	-2.8±0.0	29.3±1.1	4.3±1.1	34.7±27.6	348.1±269.6
7	-29.3±0.5	0.817±0.004	376.0±25.5	0.62±0.09	57.6±0.5	-3.0±0.1	27.5±0.1	2.3±0.2	25.0±24.3	354.0±124.0
8	-30.3±0.5	0.852±0.004	926.0±40.4	0.86±0.12	55.7±0.4	-4.5±0.0	28.9±0.7	7.3±0.8	72.8±24.8	509.4±209.2
9	-28.4±0.5	0.850±0.011	922.0±20.7	0.82±0.18	56.3±0.2	-4.0±0.0	27.0±0.5	7.7±0.7	153.5±88.5	1007.9±461.9
10	-27.8±1.1	0.756±0.008	724.0±31.1	0.95±0.07	53.8±1.1	-4.3±0.2	27.2±0.3	4.5±0.5	27.8±4.7	201.7±61.6
11	-28.3±2.4	0.803±0.006	1263.3±39.3	1.28±0.24	49.6±0.7	-3.9±0.1	27.3±0.5	8.1±0.4	40.0±5.4	421.3±41.4
12	-29.4±0.5	0.761±0.020	381.3±28.9	0.33±0.13	50.0±0.8	-2.6±0.0	23.2±0.5	3.38±0.18	36.9±10.7	243.7±82.2
13	-28.2±0.7	0.828±0.010	712.6±29.0	0.83±0.21	48.0±0.4	-4.8±0.0	23.9±0.4	5.1±1.33	82.4±79.5	470.6±356.1
14	-27.4±0.7	0.808±0.023	778.0±54.1	0.44±0.17	51.5±0.3	-5.6±0.1	24.9±0.6	5.8±1.2	123.6±163.0	795.9±698.0
15	-26.9±1.0	0.858±0.004	954.0±5.2	1.04±0.51	54.7±0.6	-4.5±0.1	30.3±0.3	7.30±0.28	32.5±10.0	538.7±99.5
16	-28.2±0.8	0.777±0.004	424.0±12.4	0.83±0.09	52.2±0.5	-3.0±0.3	24.4±0.5	22.3±29.1	509.6±685.6	1110.8±1090.7
17	-29.3±0.4	0.772±0.000	338.6±47.3	0.20±0.08	52.6±0.3	-4.8±0.0	27.3±0.5	5.2±1.1	105.5±109.6	674.7±484.3
18	-30.0±1.0	0.836±0.012	1146.0±50.4	0.68±0.12	47.9±0.3	-4.9±0.1	33.4±0.9	8.7±0.7	58.8±13.4	508.9±214.7
19	-27.9±2.7	0.824±0.012	762.6±8.0	0.50±0.23	50.8±0.3	-5.9±0.0	31.8±0.1	8.7±0.5	64.6±6.7	687.7±160.8
20	-29.1±0.8	0.848±0.005	752.6±11.0	0.37±0.01	50.6±0.2	-4.5±0.0	30.9±0.5	6.5±0.9	54.1±14.7	377.3±259.9

Table 3. *p-values* for response surface models of avocado (*Persea americana* Mill. cv. Hass) based emulsions and other ingredients

Variables	Principal effects			Quadratic effects			Interaction effects		
	A	B	C	AA	BB	CC	AB	AC	BC
ζ	0.581	0.633	0.236	0.622	0.216	0.411	0.628	0.430	0.307
R	0.005*	0.265	0.139	0.296	0.213	0.052	0.229	0.198	0.638
μ	0.000*	0.678	0.045*	0.220	0.266	0.876	0.915	0.232	0.153
IP	0.011*	0.954	0.021*	0.077	0.129	0.036*	0.71	0.272	0.598
L^*	0.301	0.022*	0.540	0.064	0.339	0.119	0.730	0.309	0.568
a^*	0.150	0.045*	0.421	0.911	0.205	0.462	0.246	0.723	0.348
b^*	0.018*	0.574	0.190	0.777	0.859	0.608	0.625	0.548	0.695
D_{10}	0.767	0.177	0.149	0.991	0.740	0.616	0.116	0.116	0.090
D_{50}	0.146	0.213	0.073	0.967	0.977	0.497	0.118	0.125	0.091
D_{90}	0.802	0.967	0.387	0.566	0.542	0.863	0.569	0.570	0.262

*: Significant (p<= 0.05).

Z Potential (ζ)

As can be seen in Table 3, the potential-ζ did not show significant differences ($p > 0.05$) with respect to the considered independent variables. However, it was observed in all the experiments, absolute values of zeta potential greater than 25 mV (26.94 - 30.85mV) were performed, which indicates a good repulsive interaction or repulsive forces among colloidal particles, contributing to a good thermodynamic stability of the system (Mirhosseini *et al.*, 2008; Rezvani *et al.*, 2012) w/w, x1.

Avocado (*Persea americana* Mill cv. Hass), is rich in mineral salts such as potassium, sodium, phosphorus, iron, copper and chlorine, which when are dissociated, produce an important ionic strength in continuous phase, whose ions are strongly adsorbed at the particles interface, which also have ionizable charge groups, producing or resulting in a strongly adsorbed negative charge at the particles interface. This situation also causes an electric potential to occur on the outskirts of this adsorbed layer (stern layer) associated with the zeta potential found. Furthermore, it contributes to the formation of a diffuse co-ions second layer distributed in a solution close to the interface. Both layers, form the known double-layer ion or Debye length (κ^{-1}).

Stability index by spectral absorption (R)

Stability index by spectral absorption (R) was significantly ($p < 0.05$) influenced by $DS_{avocado}$ (A), observing the avocado increased content (increase in the system oil phase or dispersed phase), had a direct effect on R (Figure 1a), i.e., particles of larger diameter are produced in the emulsion. The prepared emulsions were stable, without phase separation, which was favored by the high viscosities of the evaluated formulations, which diminished from the difference between oil and water specific gravity (Dłuzewska, Stobiecka, & Maszewska, 2006). Similar results were reported by Gharibzahedi *et al.* (2012)including turbidity loss rate, density, size index, particle size and stability, was investigated using response surface methodology (RSM), where oil content had a direct effect on the R index in walnut emulsions. In addition, Mirhosseini *et al.* (2008)w/w, x1, also reported the oil content had an inverse influence on R.

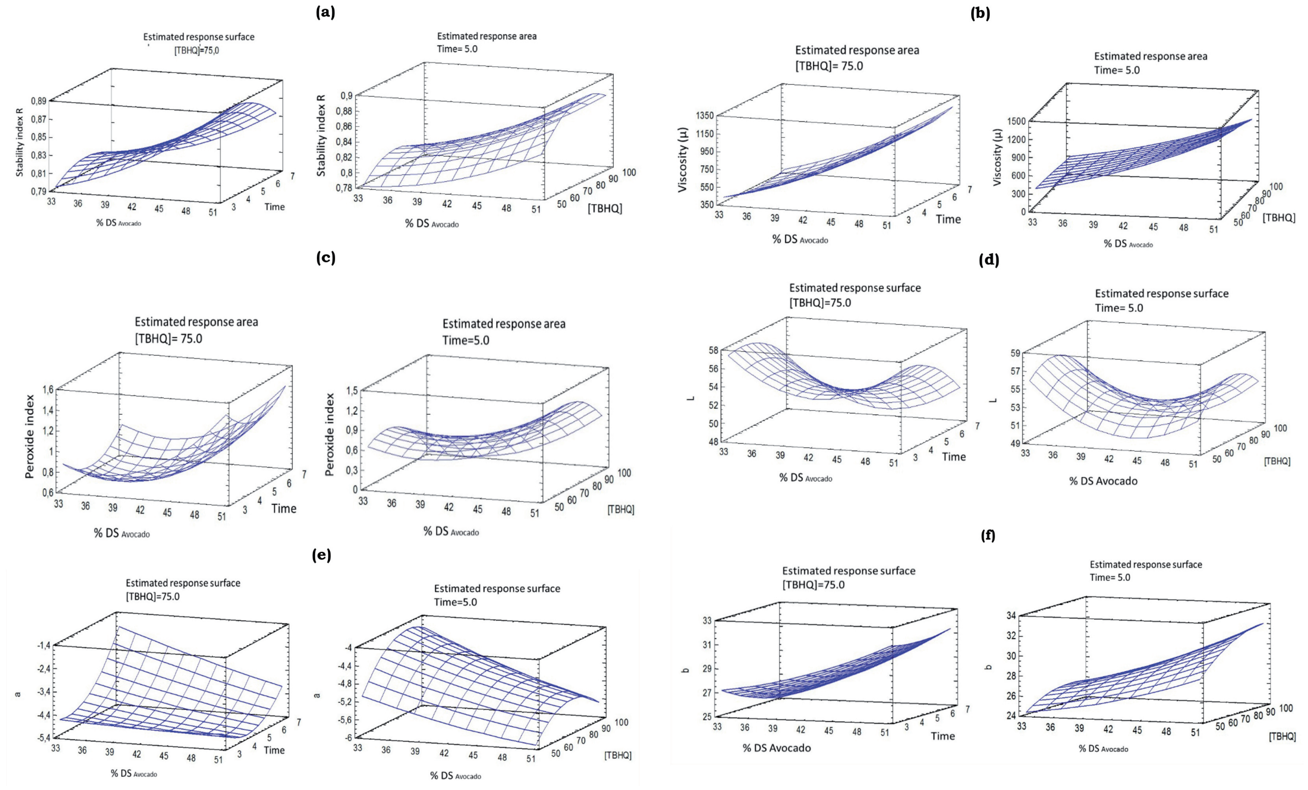

Figure 1. Response surface graphs of the quality attributes for emulsions based on avocado (*Persea americana* Mill. cv. Hass) and other ingredients depending on the evaluated independent variables

Viscosity (μ)

The $DS_{Avocado}$ (A) and [TBHQ] (C) lineal effects was significantly ($p < 0.05$) in viscosity (μ), which fluctuated between 338.66 and 1395.33cP. In Figure1b, the increase of $DS_{Avocado}$ in formulation, increases the emulsion viscosity, due to the higher ratio $DS_{Avocado}$/water. However, to higher $DS_{Avocado}$, is where the effect of [TBHQ], becomes more important tending to reduce viscosity (μ) with the [TBHQ] increased. The emulsion viscosity decreased with increasing spindle speed, probably with shear rate increased. The drop-droplet interaction is modified and eventually interrupted, which reduces floc size and decreases viscosity. Given these concerns, the prepared emulsions behave as a non-Newtonian pseudoplastic fluid (Mirhosseini *et al.*, 2009). This behavior is usual in food emulsions, for example, in seasoning salad emulsions (Chatsisvili, Amvrosiadis, & Kiosseoglou, 2012)in orange juice emulsions (Mirhosseini *et al.*, 2009), in gum arabic drinks (Dłuzewska *et al.*, 2006), among others.

Peroxide index (PI)

As can be seen in Table 3, the linear effects of $DS_{Avocado}$ (A) and [TBHQ] (C), was significantly ($p<0.05$) in PI, as well as the quadratic effect of [TBHQ] (C^2) ($p<0.05$). Figure 1c, shows that the increase in $DS_{Avocado}$, contributes to increasing PI, attributable to the higher content of mono and polyunsaturated avocado fatty acids, which are susceptible to oxidative reactions mainly on the particles surface (Kishk & Elsheshetawy, 2013) This situation is similar to that reported by Topuz et al. (2014), in a sauce to marinate fish containing olive oil and pomegranate juice, for Kishk & Elsheshetawy (2013), in mayonnaise and by Paraskevopoulou et al. (2007), in seasonings for greek salads.

The C^2 quadratic effect influence is evident in the curvature of Figure 1c, where the lower values of PI or PI increase delay are reached when the [TBHQ] is of the order of 100 $mg.kg^{-1}$, throughout variation range of the $DS_{Avocado}$. TBHQ addition in concentrations of 100 $mg.kg^{-1}$, reached its minimum value of 0.206 H_2O_2meq. Kg^{-1} emulsion when the $DS_{Avocado}$ were minimal (33%) and the hydrophobic antioxidant action at the fat-water interface becomes more effective by reducing the oily phase oxidation (Kishk & Elsheshetawy, 2013).

Color

ANOVA issued significant differences ($p<0.05$) in L* and a* with respect to homogenization time (B), varying between (47.9 & 57.6) and (-2.8 & -6.3), respectively. Whereas, the b* chromaticity was significantly influenced by the $DS_{Avocado}$ (A), varying between 23.2 and 33.3. Homogenization time (B), had an inverse effect on L *, as this decreases with increasing homogenization time (Figure 1d), which could be attributable to a slight browning experienced by the avocado (*Persea americana* Mill. cv. Hass) based emulsions and other ingredients, which is enhanced with homogenization time, by increasing its temperature from 25 °C to 32° C. On the other hand, it is attributed the observed browning of enzymatic type, due to the polyphenolic content and the present polyphenol-oxidase, which is potentiated with the air occluded during the homogenization process, which also favors lipids oxidation with the hydroperoxides and free radicals production (Kuhn & Cunha, 2012), which induces the found color changes. This phenomenon, intensifies to a longer homogenization period.

The a* chromaticity performed a decrease with the homogenization time (Figure 1e), which could be attributable to the greenish chromaticity decrease as a consequence of chlorophyll pigments and carotenoids degradation present in avocado (Aguiló-Aguayo, Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2014), which is also potentiated at higher temperatures and greater air occluded in the emulsion. The b*chromaticity (yellow-blue) was significantly ($p <0.05$) influenced by the $DS_{Avocado}$ linear effect, increasing with the increase of $DS_{Avocado}$ (Figure 1f), indicating an increase in yellow chromaticity.

Particle size

Particle size reported as D_{10}, D_{50} and D_{90} percentiles was not influenced ($p> 0.05$) by any of the considered independent variables. In general, particle sizes were large and fluctuating D_{10} (2.337- 22.324 µm), D_{50} (25.007 - 509.622 µm) and D_{90} (90.244 - 1110.778 µm), which was not considered critical, because it was sought when the reconstituted powder could be perceived similar characteristics to traditional guacamole, with pieces of vegetables of various sizes. Figure 2, presents particle size distribution of the emulsions at the optimum point, observing a trimodal behavior denoting their variability.

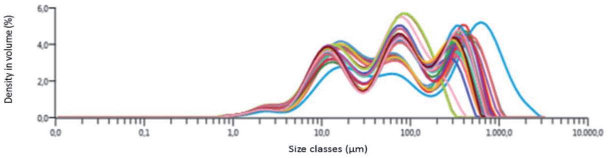

Figure 2. Particle sizes distribution of the emulsion under optimum conditions of composition and homogenization process

At this point, the mean values from 3 replicates correspond to D_{10}= 8.1 µm, D_{50}= 56.2 µm and D_{90}= 346.6 µm This trimodal or bimodal behavior, has been frequent in emulsions of oil-in-water type, for example, in seasoning salad emulsions, a proportion of relatively large particles is present, probably due to the aggregates formation during course of preparation, Which does not appear to diminish with homogenization (*Chatsisvili et al.*, 2012). A bimodal behavior was presented in a sauce (milk emulsion) (Marco-Molés, Llorca, H. & Pérez-Munuera, 2012) and emulsions with linseed oil (Kuhn & Cunha, 2012).

Adjustment of response surface models

Table 4, presents the estimated regression coefficients of the polynomial response surface models with the corresponding R^2 values and the non-fit test. Although, R^2 values for the response variables were not high enough, the lack of fit, which measures the model fitness, resulted in no significant p-value ($p> 0.05$) in terms of the evaluated response variables, which indicates models which were adequate to describe the data behavior.

Table 4. Regression coefficients, R^2 and probability values of the non-fit models for statistically significant variables

Regression coefficients	R	µ (cP)	PI (H_2O_2. meq. Kg^{-1})	L^*	a^*	b^*
β_0	0.616	1118.910	5.423	118.332	-12.464	37.495
β_A	-0.010	-33.401	-0.301	-4.246	0.030	-0.661
β_B	0.053	-259.261	-0.394	4.281	-0.115	-2.769
β_C	0.006	2.017	0.059	0.471	0.190	0.104
β_{AB}	-0.0004	0.201	-0.001	-0.018	-0.027	0.028
$\beta_{AC} \times 10^5$	3.8208	-19491.7	36.217	439.55	-61.62	278. 20
$\beta_{BC} \times 10^5$	-5.250	10483	-70.75	-1050	-739. 25	783.5
$\beta_A^2 \times 10^4$	1.6159	11011.1	37. 07	468.45	10.54	71.41
β_B^2	-0.003	16.610	0.050	-0.381	0.222	0.075
$\beta_C^2 \times 10^5$	-4.3410	-1396.36	-52.446	-414.4	-77.6	-140.27
R^2	87.709	95.5677	84.1065	63.4803	65.4802	53.7763
Unadjusted (*p-value*)	0.2864	0.5373	0.5229	0.8025	0.6261	0.7334

Optimization

In order to obtain experimental optimization using statistical tools for more suitable values of the independent variables, which have allowed to obtain a final product with desired quality attributes. For this case, the following considerations were considered as criteria for optimization: an approximate viscosity of 1000 cP (suitable operating condition of the pilot dryer for avocado (*Persea americana* Mill. cv. Hass) based emulsions) with the highest percentage of $DS_{Avocado}$, an emulsion which retains its luminosity (> L *) and greenish chromaticity (low values of a *), minimizing enzymatic browning and lipids oxidation (low values of IP), which were significantly influenced by homogenization time. Finally, a concentration of TBHQ capable of delaying or reducing the PI, which represents a critical variable in the process. The optimal conditions for the composition homogenization and process, which were defined as 47.1% of $DS_{Avocado}$, since it corresponds to the highest percentage of $DS_{Avocado}$, with a permissible viscosity to perform the aspersion-drying processes, 5 min for the homogenization time as the average level scanned where the color attributes are preserved and 100 $mg.kg^{-1}$ for the TBHQ concentration, where the minimum values are reached for the Peroxide index. At this point, the experimental values obtained from three replicates to the optimal experimental conditions were potential-ζ -27.67 ± 0.29, the color parameters L * 51.3 ± 1.0, at * -5.8 ± 0.8 and b * 30.0 ± 1.9, viscosity 1034.56 ± 95.91 cP, spectral absorption stability index (R) 0.78 ± 0.03, peroxide index 0.73 ± 0.30 $H_2O_2meq.kg^{-1}$ emulsion and particle size D_{10} 8.1 ± 0.7 μm, D_{50} 56.2 ± 11.5 μm and D_{90} 346.6 ± 94.6 μm.

Conclusion

The results indicate this methodology with a central composite design is an effective one to model the dependent variables as a function of the independent variables. In addition, it has allowed to define the most appropriate levels of the independent variables to obtain a stable emulsion from a physical and chemical point of view. The obtained results from the emulsion based on avocado (*Persea americana* Mill. cv. Hass) and other ingredients indicates an influenced stability for the formulation and homogenization operation conditions. The increase in $DS_{Avocado}$ increased stability in terms of viscosity (μ) and PI, although had an inverse influence on R. On the other hand, the increase of homogenization time affects the browning of the colloidal system. Whereas, the increase in [TBHQ] had a PI control, which could be associated with the lipid peroxidation inhibition.

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