Evaluation of *Passiflora tripartita* var. *mollisima* seed oil as potential nanoemulsion excipient.

Jenny Alexandra Hernández-Rivera, Jorge Ariel Martínez-Ramírez, Maritza Rojas-Cardozo, Diana Marcela Aragón Novoa*

Universidad Nacional de Colombia, Departamento de Farmacia, Facultad de Ciencias, Bogotá D.C, Colombia

Received: February 9, 2018*; Accepted:* March 14, 2018

Original Article

ABSTRACT

Passiflora tripartita var. *mollissima* is a fruit that is typically used as food, especially for juices and desserts, discarding the seeds. The seeds can be a source of vegetable oil used i the cosmetics, pharmaceutical and food industries. Nanoemulsions are kinetically stable liquid-in-liquid dispersions with droplets in the range of 50-500 nm. The aim of this work was to evaluate the viability of the seed oil of *P. tripartita* var. *mollisima* as oil phase in oil in water (o/w) nanoemulsion. *P. tripartita* var. *mollissima* seed oil was obtained by solvent extraction and characterized chemically and physicochemically. For nanoemulsion formation, the required hydrophilic-lipophilic balance was determined, and stable nanoemulsions were obtained using a pseudo-ternary phase diagram. Based on these results, it was concluded that seed oil of *P. tripartita* var. *mollissima* could potentially be used as an excipient for the development of o/w nanoemulsions.

KEYWORDS: Nanoemulsion, *Passiflora tripartita* var. *mollisima*, hydrophilic-lipophilic balance, seed oil

INTRODUCTION

Nanoemulsions are kinetically stable liquid-liquid dispersions with small droplet sizes ranging from 50 to 200 nm (transparent or translucent) at 500 nm (milky appearance) and a low polydispersity index (1). These systems consist of an oil and aqueous phase, as well as, an appropriate surfactant, and are mainly divided into the oil-in-water (o/w) type and the water-in-oil (w/o) type (2). In the pharmaceutical field, nanoemulsions are used for oral (3), topical (4) and parenteral (5) drug delivery systems, as they present several advantages over conventional emulsions because their small droplet size provides improved physical stability against gravitational separation, as well as, improving the bioavailability of active pharmaceutical ingredients (APIs) (6). It is well understood that the type and concentration of the surfactant determines the properties of the nanoemulsion. However, given that several types of lipids with different physicochemical properties can be used for the formulation of nanoemulsions, previous investigations have shown that lipid phase composition can also have an important influence on the physicochemical properties of the emulsion, as well as, the bioavailabilty of the active compound (7).

Vegetable fixed oils, most of them obtained from seeds, have been successfully used for the oil phase in the development of nanoemulsions because they are safe and biocompatible (8), and help improve the stability,

*Corresponding adress: Universidad Nacional de Colombia, Departamento de Farmacia, Facultad de Ciencias, Bogotá D.C., Colombia, Tel. +57(1)3165000 Ext. 14630, E-mail: dmaragonn@unal.edu.co

efficacy and solubility, as well as, the controlled release of different kinds of synthetic and herbal drugs (9, 10). The most common seed oils that have been used for the development of nanoemulsions are soybean oil (11-13), sesame oil (13), cottonseed oil (14), safflower oil (15), grape seed oil (16) and evening primrose oil (17).

Passiflora tripartita var. *mollissima*, also known as the banana passion fruit, belongs to the subgenus Tacsonia of the genus *Passiflora (Passifloraceae)* (18). Fruits of *P. tripartita* var. *mollissima*, as for other *Passifloras* fruits, are generally consumed as juices and desserts, or processed to obtain other edible products such as jams, nectars and compotes. The main byproducts of the industrial processing of the fruits are the seeds and the peel. Previous studies have reported that banana passion fruits are rich in pectin (19,) as well as, phenolic and carotenoid compounds (20) responsible for the antioxidant activity of the pulp and seeds, evaluated by FRAP (ferric reducing anti-oxidant power), DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid).

The aim of this work was to evaluate the potential of using the seed oil from *P. tripartita* var. *mollissima* for the oil phase in and oil-in-water (o/w) nanoemulsion that could be used for formulating APIs.

MATERIALS AND METHODS

Plant material

Mature fruits of *P. tripartita* var. *mollissima* (10 kg) were obtained at a market place in Bogota D.C. (Colombia). The seeds of the *P. tripartita* var. *mollissima* fruits were separated manually from the pulp. The seeds were then washed and dried in an air circulating oven at 48°C for 3 days, and finally ground in a knife mill. The milled material was collected in sieve No. 20 (850 μm) (U.S.A. Standard Testing Sieve) and was subjected to further analysis.

Extraction of *Passiflora tripartita* var. *mollissima* seed oil (PTO)

The oil was extracted from 1020 g of dry seeds by the percolation method (rate 1 mL/min) using n-hexane at room temperature for 72 hours, changing the solvent every 24 hours. The plant material:solvent ratio was 1:5 w/v. Subsequently, the solvent was eliminated by reduced pressure and the oil obtained was stored in a desiccator until further analysis. The yield obtained was 10.2%.

The chemical characterization of *Passiflora tripartita* var. *mollissima* seed oil

Preparation of fatty acid methyl esters

Cold esterification of fatty acids was prepared in a 5 mL screw-top test tube by mixing PTO (100 mg), hexane (1 mL) and 0.5 mL of 2 N methanolic potassium hydroxide solution and shaking vigorously for 30 seconds, according to Regulation EC/796/2002 of the European Union Commission (21). After 45 minutes the mixture became clear and the upper organic phase were transferred to a clean autosampler vial, and 1 μL was analyzed using GC-MS/EI.

GC-MS/EI analysis

All GC-MS/EI analyses were performed on a Thermo Scientific™ TRACE™ 1300 gas chromatograph connected to an ISQ QD single quadrupole mass spectrophotometer and an AL1310 autosampler (Thermo Fischer, Massachusetts, USA) in liquid injection mode. The GC conditions were adapted to Standard ISO-5508 (22) as follows: injection volume, 1 μL; split injection 10:1; capillary column, Thermo Scientific™ TRACE™ TR1 30 m×0.25 mm, 0.25 μm film thickness supplied by Thermo (Massachusetts, USA); injection port temperature, 220°C; carrier gas, helium (≥ 99.99% purity); flow rate, 1.0 mL/min; The oven conditions were 180°C initial (15 min) increasing to 193°C at 0.5°C/min; final time 43 minutes; The MS conditions were as follows: full-scan mode (m/z 50–600); dwell time 0.2 sec, electron ionization (EI) mode; ionization energy, 70 eV; ion source temperature, 230°C; interface temperature, 280°C. Data evaluation was performed using a Chromeleon® 7 Chromatography Data System version 7.2.2.6394 software with a NIST 2007 target library.

Physicochemical characterization *Passiflora tripartita* var. *mollissima* seed oil

The density and acid, iodine, peroxide, saponification, esterification and refraction values were determined according to United States Pharmacopoeia (23) official methods, with slight modifications. All determinations were made in triplicate.

Saponification value

PTO (2 g) was taken in a flask and 25 mL of 0.5N potassium hydroxide was added. The flask was heated in a steam bath under an appropriate condenser and kept at reflux for 60 minutes, 1 mL of phenolphthalein was added and the excess potassium hydroxide was titrated with 0.5 N hydrochloric acid. The same procedure was performed for a blank.

Acid value

PTO (5 g) was dissolved in 25 mL of a mixture of ethanol:ethyl ether (1:1) and titrated in triplicate with 0.1N potassium hydroxide. Phenolphthalein was used as an indicator and a blank was measured.

Iodine value

PTO (0.2 g) was dissolved in 15 mL of a cyclohexane: glacial acetic acid (1: 1) mixture. 25 mL of Wijs reagent was added and the mixture was stored in a dark place for 2 hours. Subsequently, 20 mL of a solution of potassium iodide soda and 150 mL of distilled water was added. It was titrated with 0.1 N sodium thiosulfate under continuous stirring until the yellow color disappeared. Finally, starch was added until the blue color disappeared.

Refractive index

The measurement was performed using a refractometer Carl Zeiss Abbe Jena 292210 (Carl Zeiss, Jena, Germany) at 20ºC.

Density

An empty pycnometer was weighed, filled with distilled water (avoiding the formation of bubbles), and weighed. Finally, the pycnometer was filled with the oil obtained and weighed

Determination of the o/w required HLB ($_r$HLB) of PTO

Before to nanoemulsions elaboration, the $_r$HLB of PTO was determined, based on the methodology described by O. Orafidiya *et. al.* (24) evaluating the following parameters: droplet size and uniformity analysis, stability after centrifugation and turbidity of the coarse emulsions prepared.

Preparation of emulsions

To $_r$HLB determination of PTO, coarse oil-in-water emulsions were prepared, using Tween®20 (Sigma-Aldrich, St Louis, USA Lot 125K01034) and Span® 85 (ICI Specialty Chemical, London UK Lot V-5420) as surfactants (5%), PTO as the oil phase (10%) and distilled water as the aqueous phase (85%). The oil phase was added to the aqueous phase and homogenized on a vortex 3 agitator (IKA®, Staufen, Germany) at 2000 RPM for 2 minutes.

Several emulsions with different mixtures of Span®85 and Tween®20 were prepared, to modify the surfactant HLB values for the PTO rHLB determination. Tween®20: Span®85 ratios were mixed as follows: 0: 100 (HLB: 1.8), 16:84 (HLB: 4.2), 32:68 (HLB: 6.6), 50:50 (HLB : 9.3), 68:32 (HLB: 11.9), 76:24 (HLB: 13.1), 84:16 (HLB: 14.3), 100: 0 (HLB: 16.7).

Droplet size and uniformity analysis

The droplet size and the uniformity value of the emulsions were measured by dynamic light scattering (DLS) using a Mastersizer 3000E (Worcestershire, UK) particle size analyzer. Values were obtained from 5 measurements for each emulsion, and droplet size was expressed as surface volume diameter (Dvs).

The effect of centrifugation on droplet size distribution of emulsions

Samples of 3 mL were withdrawn and placed into

glass tubes with closely fitting caps and subjected to centrifugation at 10000 RPM for 10 minutes using a Hermle® (Wehingen, Germany) Model Z383K centrifuge. Emulsions that showed no phase separation were allowed to stand for 3 days, after which they were gently stirred for 60 seconds to ensure the redispersibility of the droplets (24). Droplet size and uniformity were then determined as previously described.

Turbidimetric method

Samples of 5 ml of each emulsion were removed and placed in test tubes and capped properly. The tubes were stored undisturbed at room temperature (19°C) for 7 days. A 1 mL sample was then gently removed from the base of the tube using a syringe, so that the tip of the needle touched the base of the tube. The sample was diluted in 10 ml of distilled water and the absorption was measured at 600 nm with a spectrophotometer (Shimadzu® uv - 1800; Kyoto Japan) (24).

The percentage of transmission (%T) was determined using the following ratio:

$$Absorbancia = 2 - \log\%T$$

The turbidity of each emulsion was calculated as:

$$Turbidity = 100 - \%T$$

Construction of pseudo-ternary phase diagram

Once $_r$HLB of PTO was determined by using the coarse emulsions, twenty-six emulsions of 10 mL were prepared by varying the percentage of water, oil and surfactant mixture using a Q500-SONICATOR® ultrasonicator (Newton, USA) with 80% amplitude, for 1 minute to obtained nanoemulsions. The nanoemulsion region was determined by constructing a pseudo-ternary phase diagram, in which each corner represented 100% water, surfactant blend and PTO.

All formulations were characterized after 1 and 7 days of preparation. The mean droplet size and polydispersity index were determined by dynamic light scattering (DLS) using a Zetasizer nano-ZS (scattering angle 90º, 25ºC) (Worcestershire, UK). 5 µL of each sample was diluted in 1 mL of deionized water and the measurement was performed at 25°C. The effect of dilution on the mean particle size or the size distribution was not taken into consideration. The values were obtained from the mean and standard deviation of 3 measurements for each emulsion.

Statistical analysis

GraphPad Prism® 7 software was used for statistical analysis of the droplet size in the emulsions using a t-Student paired test.

RESULTS AND DISCUSSION

Chemical and physicochemical characterization of PTO

Figure 1 shows a chromatographic profile of fatty acids of PTO describing the similar compounds reported for other seed oils (25, 26). Table 1 summarizes the fatty acid composition of seed oils of PTO and other *Passiflora* species obtained by extraction with organic solvents. As in other *Passiflora* seed oils, the major compound in PTO is linoleic acid followed by oleic acid, two essential fatty acids. However, it is important to note that the linoleic acid content of PTO is higher than that reported for other *Passiflora* species. Likewise, the palmitic acid content of PTO is the lowest of the described seed oils. This finding is reflected in the total content of unsaturated fatty acids (UFA) and polyunsaturated fatty acids (PUFA) of PTO which is higher than reported in other studies. This fact suggests that PTO could be more susceptible to oxidation due to the presence of double bonds, but it is also widely known that the consumption of PUFA has important health benefits (27). Regarding other seed oils employed for pharmaceutical purposes, although the content of UFA is similar among them (77-90%), the PTO content showed more linoleic acid and PUFA than most of them i.e., cottonseed oil (2.12%) (28),

Table 1 Fatty acid composition of *P. tripartita* var. *mollisima* seed oil and other *Passiflora* seed oils previously reported

	P. tripartita var. *mollisima*	*P. alata* (26)	*P. edulis* (26)	*P. tenuiflia* (26)	*P. setacea* (26)	*P. alata* (30)	*P. edulis* var. *flavicarpa* (31)
LINOLEIC ACID C18:2 MW 256.4 g/mol	77±0.05	71.54 ± 0.34	70.52 ± 0.03	70.76 ± 0.07	69.93 ± 0.05	72.04 ± 0.15	73.14 ± 0.05
PALMITIC ACID C16:0 MW 256.4 g/mol	7±0.04	10.38 ± 0.03	11.08 ± 0.02	10.89 ± 0.02	9.55 ± 0.02	11.46 ± 0.03	9.73 ± 0.01
OLEIC ACID C18:1 MW 282.5 g/mol	13±0.05	13.58 ± 0.05	14.37 ± 0.01	14.43 ± 0.03	15.92 ± 0.01	12.93 ± 0.07	13.83 ± 0.04
STEARIC ACID C18:0 MW 284.5 g/mol	3±0.01	2.72 ± 0.02	2.87 ± 0.02	2.72 ± 0.04	3.16 ± 0.01	2.51 ± 0.02	2.58 ± 0.00
Others	ND	1.98	1.16	1.2	1.44	1.06	0.72
SFA	10	13.10 ± 0.05	13.95 ± 0.03	13.60 ± 0.02	12.71 ± 0.02	13.97	12.41
UFA	90	86.8	86.03	86.40	87.29	86.04	87.59
MUSFA	13	14.83 ± 0.08	14.99 ± 0.02	15.10 ± 0.02	16.46 ± 0.001	14.00 –	14.04
PUSFA	77	72.07 ± 0.13	71.04 ± 0.02	71.30 ± 0.03	70.83 ± 0.02	72.04	73.55
AMWFA	279.2	273.4	275.0	274.9	274.6	275.1	276.5

Data are expressed as the mean ± standard deviation (n=3). SFA: saturated fatty acids. UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; AMWFA: Average molecular weight of identified fatty acids in the oil (calculated from available data).

(25), sesame oil (42%) (29), and castor oil (6%) (25).

The physicochemical properties of PTO are similar to those of other *Passiflora* seed oils as shown in Table 2, particularly with regard to density and the refractive index. The differences of the other parameters are related to chemical composition, mainly the high content of linoleic acid and low content of palmitic acid in PTO. The saponification value (SV) is inversely proportional to the average molecular weight and hydrocarbon chain length of all fatty acids in the oil (32). Thus, the saponification value of PTO is slightly lower than that of other *Passiflora* oils due to the fact that average molecular weight of identified fatty acids in the oil (AMWFA), mainly linoleic acid, is slightly higher. The PTO saponification value showed greater differences with other vegetable oils, such as cottonseed oil (SV 189.338) (28), corn oil (187-193), castor oil (SV 176-182, and olive oil (190-195), which has a lower linoleic acid content (33).

The iodine value was related to the content of unsaturated fatty acids (UFA) in the oil. The higher iodine value of PTO is related to its higher UFA, mainly due to the linoleic acid content, as discussed previously.

Determination of $_r$HLB of PTO

The smallest droplet size value was 10.3 ± 0.04 μm and 10.0 ± 0.1 μm for the non-centrifuged and centrifuged coarse emulsions, respectively, and was observed in HLB 11.9. However, the highest uniformity values were recorded in HLB 13.1 (Figure 2). A minimal change in the droplet size values, after

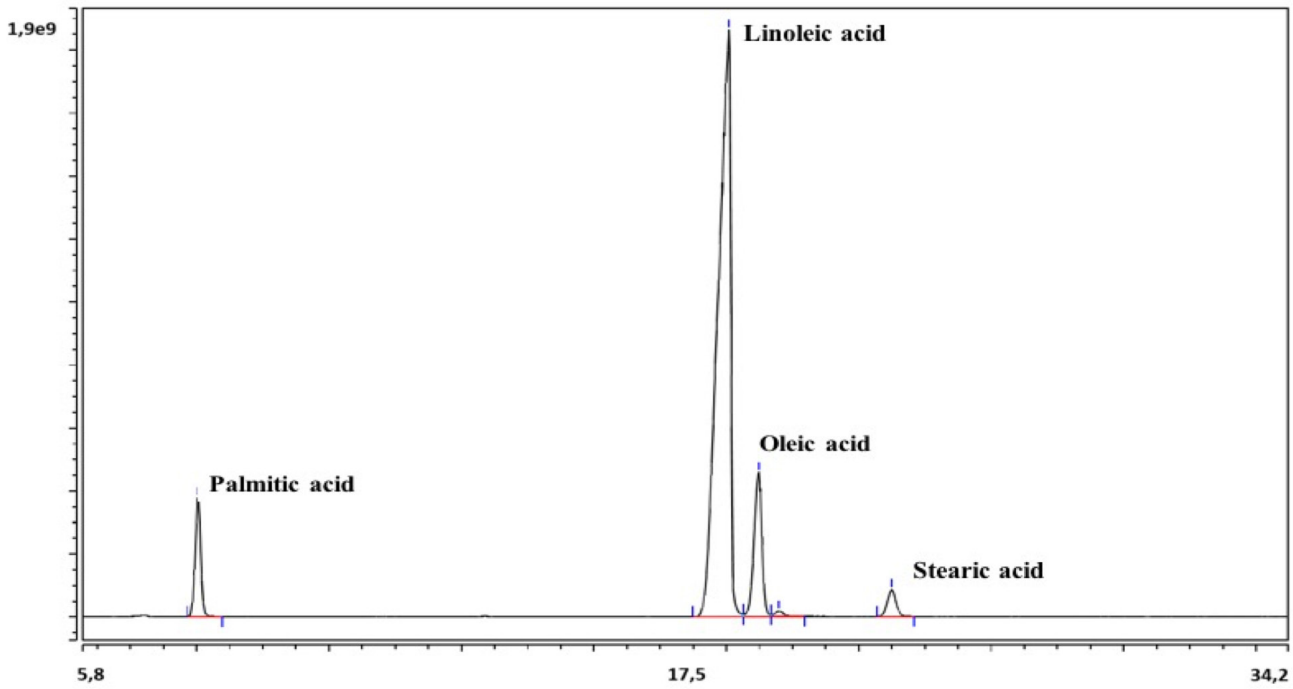

Figure 1 TIC profile EI mass chromatogram of a palmitic acid, linoleic acid, oleic acid, and stearic acid obtained after solid B liquid extraction and derivatization of a seed oil of *Passiflora tripartita* var. *mollisima*.

centrifugation and subsequent storage, indicate optimum stability, which in turn indicates the HLB of the system. In this case, the emulsion that presented the least variation was the one corresponding to HLB 13.1. Based on the statistical analysis it was possible to establish that there is no statistically significant difference between droplet size and uniformity of centrifuged and non-centrifuged emulsions at HLB 13.1, which means that centrifugation and storage did not induce droplet coalescence or change in size distribution. In spite of this minimum change, for the effect of the determination of the required HLB of the oil, the point with less variation was visible. The droplet size values obtained for the emulsions of HLB 11.9 and 13.1 (both centrifuged and non-centrifuged), did not show any statistically

Table 2 Physicochemical properties of *P. tripartita* var. *mollisima* seed oil and other Passiflora seed oils previously reported

PROPERTIES	*P. tripartita* var. *mollisima*	*P. alata* (26)	*P. edulis* (26)	*P. tenuifila* (26)	*P. setacea* (26)	*P. alata* (30)	*P. edulis* var. *flavicawrpa* (31)
Refractive index (21,5°)	1.476 ± 0.002	1.469 ± 0.001	1.468 ± 0.001	1.469 ± 0.001	1.469 ± 0.001	14.698	1.4682 ± 0.0001
Density (g/mL)	0.918 ± 0.001	NR	NR	NR	NR	0.9041	NR
Saponification value (mg KOH/g)	175.6 ± 4.2	179.86 ± 6.52	177.95 ± 0.07	181.77 ± 7.55	178.99 ± 4.89	NR	190.7 ± 2.76
Acid value (mg KOH/g)	1.46 ± 0.07	NR	NR	NR	NR	1.99 ± 0.18	2.35 ± 0.06
Iodine value (g I_2/100g)	137.3 ± 2.1	133.08 ± 3.35	132.17 ± 5.63	134.73 ± 1.19	135.64 ± 0.23	NR	128.0 ± 0.75

NR: Not reported. Data are expressed as the mean ± standard deviation (n=3).

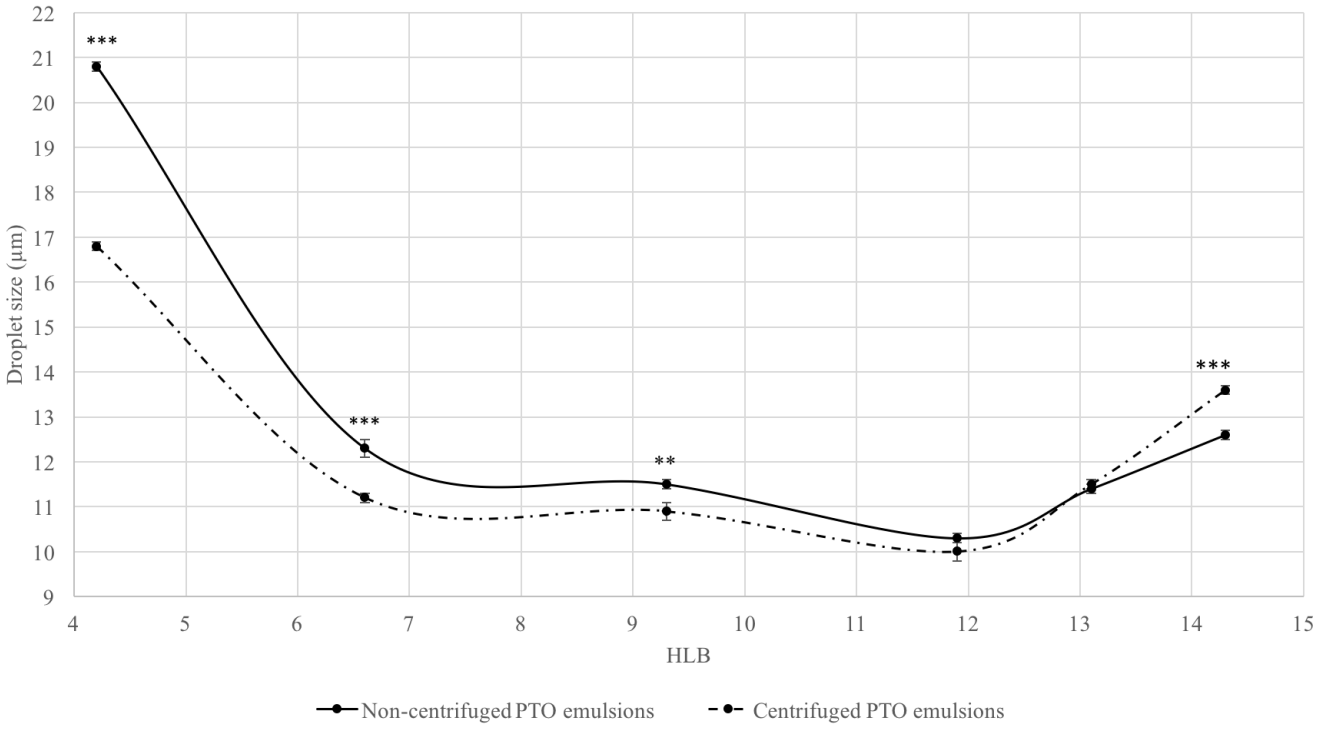

Figure 2 Droplet diameter (µm) of *P. tripartita* var. *mollisim*a seeds oil emulsions with different surfactant HLB. Data are expressed as the mean ± standard deviation (n=5). t-Student paired test; **p< 0.01 and ***p < 0.001 respect to non-centrifuged emulsions.

significant difference, therefore the turbidimetric method was used to determine the required HLB of the PTO, which was defined as 13.1, according to the maximum turbidity (Figure 3).

Construction of the pseudo-ternary phase diagram

The pseudo-ternary diagram is a valuable tool for investigating the influence of a composition on nanoemulsion formation. It is usually constructed by varying the components of the formulation (oil-surfactants-water) (34).

Twenty-six formulations were prepared, with percentages of oil from the oil phase ranging from 5% to 30% v/v based on a previous report (35). The droplet size ranged from 134.2 ± 0.4 nm (5% oil, 30% surfactants and 65% water) to 616.6 ± 32.5 nm (25% oil, 5% surfactants and 70% water) (Table 3). The prepared emulsions had a milky-white appearance, with no apparent phase separation, except for formulations 22 and 25, which had oil on the surface of the tube; this can be attributed to their having a very low surfactant-oil ratio.

In all the formulations, droplet size increased after seven days (in 13 of them this increase was statistically significant). This change was related to the coalescence phenomenon, which also explains the appearance of new populations of droplet size and the increase of the polydispersity index.

In order to establish the nanoemulsion region in the pseudo-ternary diagram, nanoemulsions with a droplet size of less than 500 nm were used after 1 and 7 days of preparation, taking into account the definition of nanoemulsion proposed by Gupta A. *et al.* (36), and a polydispersity index of less than 0.500 after 1 and 7 days of preparation. This ensures the stable behavior of the system, given the possibility of coalescence (a major problem for the stability of nanoemulsions) occurring (34) (Figure 4).

The diagram obtained in the present study coincides

Table 3 Droplet size of emulsions containing *P. tripartita* var. *mollisima* seed oil

FORMULATION	O%	S%	W%	S: PTO	Day 1		Day 7	
					DROPLET SIZE (dvs nm)	POLYDISPERSITY INDEX	DROPLET SIZE (dvs nm)	POLYDISPERSITY INDEX
1	5	10	85	2	209.3±8.4	0.529±0.084	218.4±0.7	0.451±0.026
2	5	15	80	3	306.9±8.8	0.599±0.062	310.5±1.0	0.422±0.012
3	5	20	75	4	252.3±3.8	0.422±0.010	261.2±6.8	0.302±0.039
4	5	25	70	5	186.3±1.0	0.226±0.004	191.9±1.7	0.242±0.003
5	5	30	65	6	134.2±0.4	0.106±0.021	151.9±0.6	0.120±0.016
6	10	5	85	0.5	212.1±0.9	0.390±0.027	418.4±19.3***	0.554±0.096
7	10	10	80	1	266.1±2.1	0.463±0.015	380.6±18.7***	0.518±0.098
8	10	20	70	2	189.4±0.6	0.261±0.013	340.6±4.2***	0.431±0.016
9	15	5	80	0.3	277.7±10.1	0.471±0.018	417.2±17.9***	0.579±0.118
10	15	10	75	0.7	341.7±29.5	0.516±0.016	378.3±11.2**	0.622±0.119
11	10	15	75	1.5	222.2±1.2	0.407±0.005	341.2±11.6***	0.497±0.030
12	10	25	65	2.5	175.1±0.8	0.224±0.009	176.1±1.9	0.187±0.016
13	10	30	60	3	194.9±1.1	0.273±0.005	195.8±0.8	0.144±0.007
14	15	15	70	1	282.8±5.1	0.436±0.029	362.7±7.6***	0.495±0.064
15	15	20	65	1.3	241.6±3.1	0.399±0.015	245.0±0.9	0.264±0.005
16	15	25	60	1.7	177.9±1.2	0.186±0.008	236.3±2.4***	0.342±0.008
17	15	30	55	2	147.4±1.1	0.133±0.006	158.4±1.0	0.141±0.023
18	20	5	75	0.3	365.8±19.4	0.677±0.137	541.6±43.8***	0.765±0.198
19	20	10	70	0.5	348.8±12.4	0.582±0.108	423.2±25.9***	0.626±0.157
20	20	15	65	0.8	356.9±1.7	0.488±0.002	388.0±2.3	0.512±0.007
21	20	20	60	1	255.7±3.7	0.375±0.003	302.6±2.9***	0.406±0.008
22	25	5	70	0.2	616.6±32.5	0.964±0.062	620.2±7.6	0.789±0.051
23	25	10	65	0.4	394.5±7.1	0.520±0.028	493.9±11.9***	0.542±0.029
24	25	15	60	0.6	370.8±2.8	0.479±0.008	401.2±13.2	0.483±0.050
25	30	5	65	0.2	487.4±11.0	0.516±0.015	540.3±14.9***	0.489±0.012
26	30	10	60	0.3	309.7±2.9	0.410±0.015	327.4±2.5	0.424±0.021

O: oil; S: surfactant; W: water; S:PTO: surfactant *P. tripartita* var. *mollisima* seed oil ratio. In all cases, S corresponds to Tween®20: Span®85 76:24. Data are expressed as the mean ± standard deviation (n=5). t-Student paired test; **p< 0.01 and ***p < 0.001 respect to day 1.

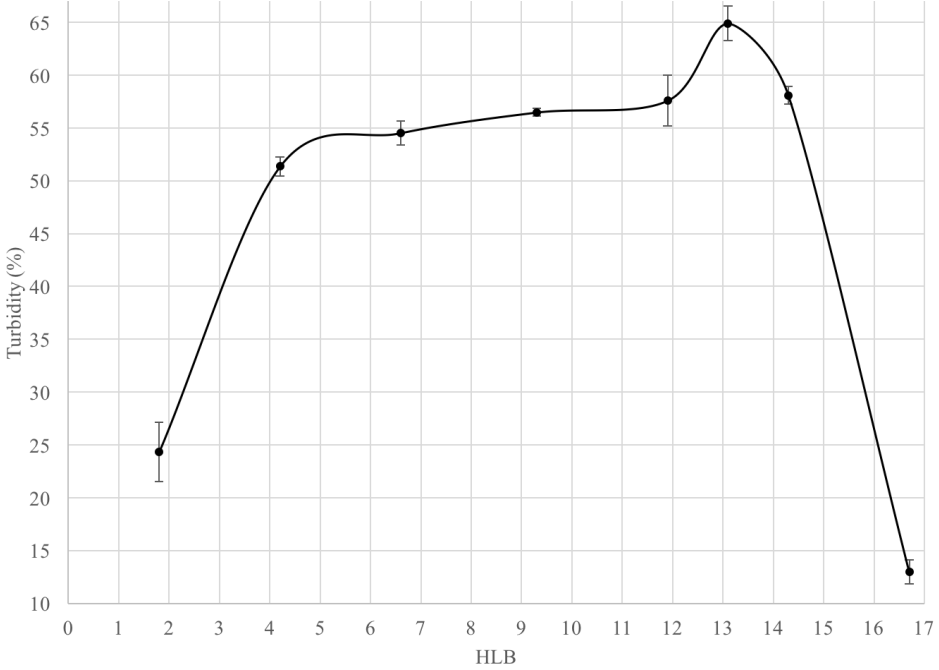

Figure 3 Turbidity (%) of the *P. tripartita* var. *mollisima* seed oil emulsions with different surfactant HLB. Data are expressed as the mean ± standard deviation (n=3)

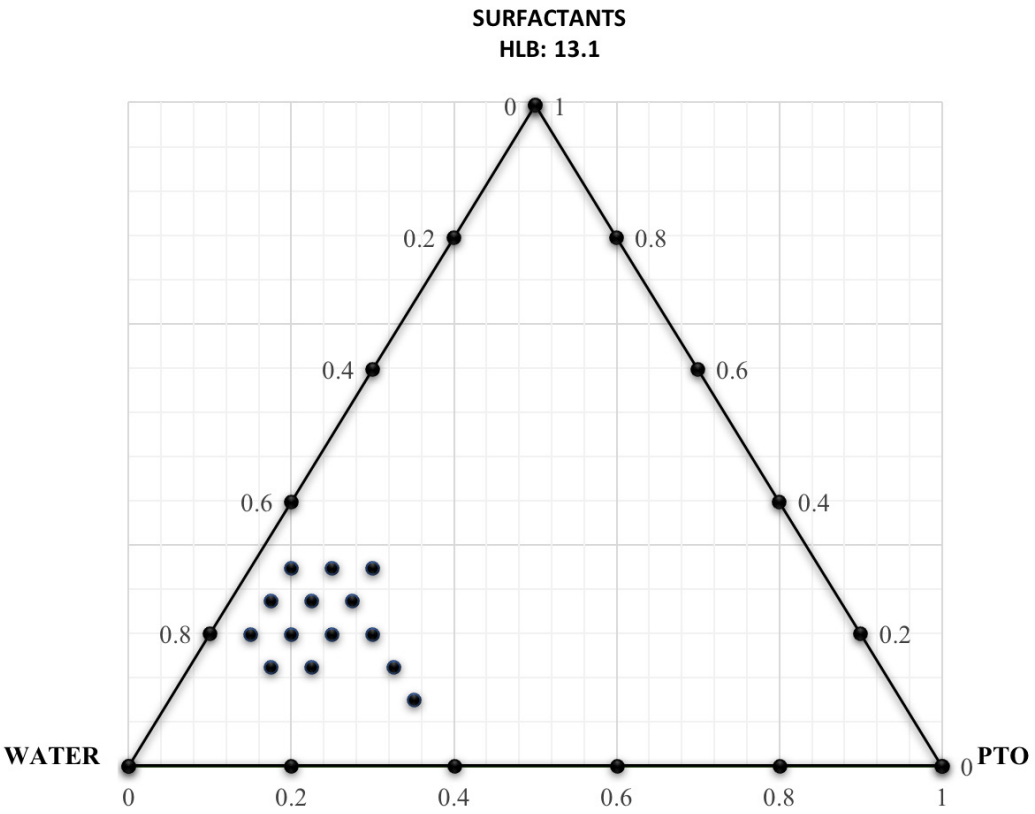

Figure 4 Pseudo-ternary diagram for the determination of the *P. tripartita* var. *mollisima* seeds oil nanoemulsion region

with the typical zone of nanoemulsion formation o/w in the diagram designed by B. Yang *et. al.* (37). In this region, formulations with oil proportions between 5% to 30% and high proportions of surfactants (greater than 10%) tend to form nanoemulsions. In the work carried out by S. Baboota *et. al.*, in which celecoxib nanoemulsions were developed for transdermal administration, the authors constructed a series of pseudo-ternary diagrams, from which they concluded that as the concentration of surfactants increases, the area of nanoemulsion formation becomes larger. This may be due to a more effective interface reduction. Despite obtaining smaller drops with high proportions of surfactants, it is important to clarify that this is not recommended in the case of a topical formulation, because it could cause skin irritation (35).

On the other hand, the type of oil also influences the formation of the nanoemulsions, due to the differences in the chemical composition of the oil. Thus, the area of the PTO nanoemulsion region in the pseudo-ternary diagram is greater than that determined for evening primrose seed oil (17).

Fourteen stable nanoemulsions were obtained after storage for 7 days at 18°C, with a droplet size of less than 500 nm and a polydispersity index of less than 0.500. Specifically, nanoemulsion 5 (5% oil, 30% surfactants and 65% water) had the smallest droplet size and polydispersity index during the 7 days of storage (Figure 5), suggesting the potential of PTO as a new ingredient for nanoemulsion formulation.

CONCLUSIONS

A *P. tripartita* var *mollisima* (PTO) seed oil with chemical profile was obtained by solvent extraction, characterized by a high content of linoleic acid, similar to other *Passiflora* seed oils, and its physicochemical properties were elucidated.

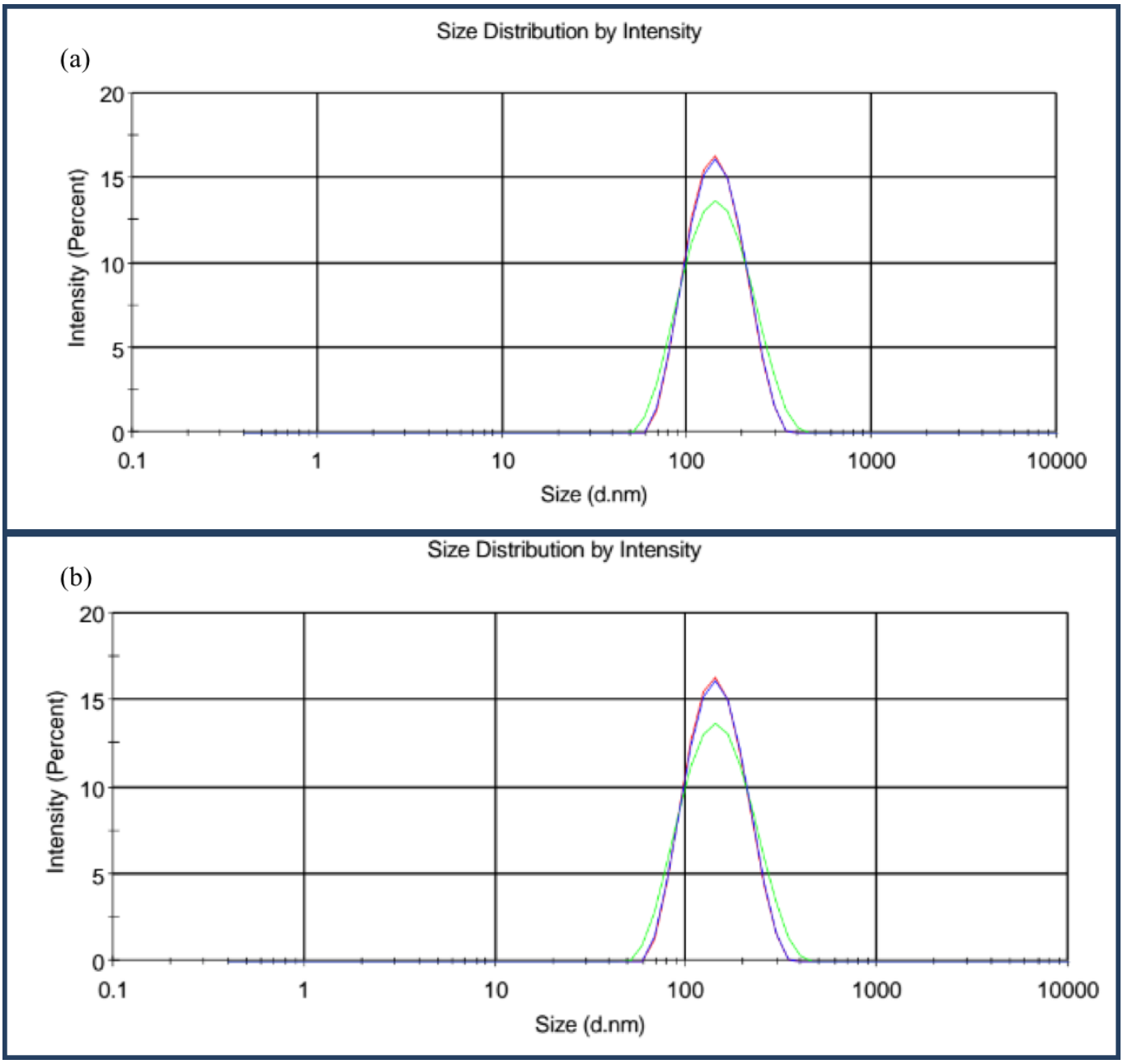

Figure 5 Droplet size distribution of nanoemulsions prepared with 5% (w/w) of PTO, 30% (w/w) of surfactants and 65% (w/w) of water. (a) Day 1: droplet size: 134.2±0.4 nm, polidispersity index: 0.106±0.021. (b) Day 7: droplet size: 151.9±0.6 nm, polidispersity index: 0.120±0.016.

The o/w required HLB of the PTO was determined as 13.1 and from a pseudo-ternary phase diagram, the area of o/w nanoemulsion was established. It was also established that the most stable nanoemulsion (over 7 days of storage) was composed of a 5% oil and 30% surfactant mixture (Span® 85 and Tween® 20) with 65% water. Finally, the feasibility of PTO as a potential ingredient in nanoemulsions for pharmaceutical, sunflower seed oil (62.2%) (25), almond oil (22.8%) food or cosmetics purposes was demonstrated.

ACKNOWLEDGEMENTS

The authors wish to thank the DIB of the Universidad Nacional de Colombia (UNC) for its financial support, as well as, the Department of Pharmacy of UNC for providing equipment and for the use of its laboratories.

REFERENCES

1 Yukuyama M, Ghisleni D, Pinto T, Chacra B. Nanoemulsion: process selection and application in cosmetics – a review. Int Journal Cosmet Sci, 38: 13-24, 2016.

2 Lovelyn C. Attama, A.A. Current state of nanoemulsions in drug delivery. J Biomater Nanobiotechnol, 2: 626-639, 2011.

3 Mou D, Chen H, Du D, Mao C, Wan J, Xu H, Yang X. Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs. Int J Pharm, 353: 270–276, 2008.

4 Khani S, Keyhanfar F, Amani A. Design and evaluation of oral nanoemulsion drug delivery system of mebudipine. Drug Deliv, 23: 2035–2043, 2016.

5 Pawar VK, Panchal SB, Singh Y, Meher JG, Sharma K, Singh P, Bora HK, Singh A, Datta D, Chourasia MK. Immunotherapeutic vitamin E nanoemulsion synergies the antiproliferative activity of paclitaxel in breast cancer cells via modulating Th1 and Th2 immune response. J Control Release, 196: 295–306, 2014

6 Montes de Oca-Ávalos JM, Cándal RJ, Herrera ML. Nanoemulsions: stability and physical properties. Curr Opin Food Sci, 15: 1-6, 2017

7 McClements DJ, Xiao H. Potential biological fate of ingested nanoemulsions: influence of particle characteristics. Food Funct, 3: 202–220, 2012.

8 Bajerski L, Michels L, Colomé L, Bender E, Freddo R, Bruxel F, Haas S. The use of Brazilian vegetable oils in nanoemulsions: an update on preparation and biological applications. Braz J Pharm Sci, 52: 347-363, 2016.

9 Singh Y, Meher JG, Raval K, Khan F, Chaurasia M, Jain Nitin K, Manish K. Chourasi Nanoemulsion: Concepts, development and applications in drug delivery. J Control Release, 252: 28–49, 2017.

10 Gohel M, Soni T, Hingorani L, Patel A, Patel N. Development and Optimization of Plant Extract Loaded Nanoemulsion Mixtures for the Treatment of Inflammatory Disorder. Current Research in Drug Discovery, 1: 29-38, 2014.

11 Anuchapreeda S, Fukumori Y, Okonogi S, Ichikawa H. Preparation of lipid nanoemulsions incorporating curcumin for cancer therapy. J Nanotechnol, 11 pages, 2012.

12 Hwang YY, Ramalingam K, Bienek DR, Lee V, You T, Alvarez R. Antimicrobial activity of nanoemulsion in combination with cetylpyridinium chloride in multidrug- resistant *Acinetobacter baumannii*. *Antimicrob.* Agents Chemother, 57: 3568–3575, 2013.

13 Ghosh V, Mukherjee A, Chandrasekaran N. Eugenol-loaded antimicrobial nanoemulsion preserves fruit juice against, microbial spoilage. Colloids Surf. B Bio interfaces, 114: 392–397, 2014.

14 Abd-Elsalam KA, Khokhlov AR. Eugenol oil nanoemulsion: antifungal activity against *Fusarium oxysporum* f. sp. vasinfectum and phytotoxicity on cottonseeds. Appl. Nanosci, 5: 255–265, 2015.

15 Vyas TK, Shahiwala A, Amiji MM. Improved oral bioavailability and brain transport of saquinavir upon administration in novel nanoemulsion formulations. Int J Pharm, 347: 93–101, 2008.

16 Almeida J, Jezur L, Fontana M, Paese K, Silva C, Pohlmann A, Guterres S, Beck R. Oil-based nanoparticles containing alternative vegetable oils (grape seed oil and almond kernel oil): preparation and characterization. Lat Am J Pharm, 28: 165-172, 2009.

17 Rodríguez R, Costa I, Almeida F, Cruz R, Ferreira A, Vilhena J, Florentino A, Carvalho J, Fernández C. Development and characterization of evening primrose (*Oenothera biennis*) oil Nanoemulsions. Braz J Pharmacogn, 25: 422-425, 2015.

18 Primot S, D'eeckenbrugge G, Rioux V, Ocampo J, Garcin F. Variación morfológica de tres especies de curubas (*Passiflora tripartita* var. *Mollissima, P. tarminiana* y *P. mixta*) y sus híbridos en el valle del cauca (Colombia). Rev Bras Frutic, 27: 467-471, 2005.

19 Espinal-Ruiz M, Restrepo-Sánchez LP, Narvaez-Cuenca C, McClements DJ. Impact of pectin properties on lipid digestion under simulated gastrointestinal conditions: Comparison of citrus and banana passion fruit (*Passiflora tripartita* var. *Mollissima*) pectins. Food Hydrocoll, 52: 329-342, 2016.

20 Contreras-Calderón J, Calderón-Jaimes L, Guerra-Hernández E, García-Villanova B. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. Food Res Int, 44: 2047–2053, 2011.

21 The Commission of the European Communities. Commission Regulation (EC) No 796/2002 of 6 May 2002. Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis. Official Journal of the European Communities 15.5.2002.

22 International Organization for Standardization. Reference number ISO 5508: 199O(E). Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids.

23 U.S. Pharmacopoeia-National Formulary (USP 40 NF 35). Volume 1. Rockville, Md: United States Pharmacopeial Convention, Inc; 2017. Chemical Test (401). Fats and Fixed oils. Pp. 332-336.

24 Orafidiya O, Oladimeji, A. Determination of the required HLB values of some essential oils. Int J Pharma, 237: 241-249, 2002.

25 Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int J Mol Sci, 16: 12871-12890, 2015.

26 Carvalho de Santana F, Branco Shinagawa F, da Silva Araujo E, Costa AM, Mancini-Filho J. Chemical composition and antioxidant capacity of Brazilian *Passiflora* seed oils. J Food Sci, 80: C2647- C2654, 2015.

27 Koba K, Yanagita T. Health benefits of conjugated linoleic acid (CLA). Obes Res Clin Pract, 8: e525-532, 2014.

28 Ibanga OI, Ekpa OD. Fatty acid composition of cottonseed oil and its application in production an evaluation of biopolymers. American Journal of Polymer Science, 3: 13-22, 2013.

29 Gharby S, Harhar H, Bouzoubaa Z, Asdadi A, El Yadini A, Charrouf Z. Chemical characterization and oxidative stability of seeds and oil of sesame grown in Morocco. Journal of the Saudi Society of Agricultural Sciences, 16: 105–111, 2017.

30 Pereira MG, Hamerski F, Andrade EF, Scheer AP, Corazza ML, Assessment of subcritical propane, ultrasound-assisted and Soxhlet extraction of oil from sweet passion fruit (*Passiflora alata* Curtis) seeds. J. Supercritic Fluids, 128: 338–348. 2017.

31 Malacrida CR, Braz NJ. Yellow Passion Fruit Seed Oil (*Passiflora edulis* f. *flavicarpa*): Physical and Chemical Characteristics. Arch. Biol. Technol, 55: 127-134, 2012.

32 Marina AM, Che Man YB, Nazimah SAH, Amin I. Chemical Properties of Virgin Coconut Oil. J Am Oil Chem Soc, 86: 301–307, 2009.

33 Rowe R, Sheskey P, Quinn M. Handbook of Pharmaceutical Excipients. 6th edition. Royal Pharmaceutical Society of Great Britain. London. 2009. Pg 108,119,126 and 470

34 Bernardi D, Pereira T, Maciel N, Bortoloto J, Viera G, Oliveira G. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J. Nanobiotechnol, 9: 1-9, 2011.

35 Baboota S, Shakeel F, Ahuja A, Ali J, Shafiq S. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. Acta Pharm, 57: 315-332, 2002.

36 Gupta A, Burak H, Alan T, Doyle P. Nanoemulsions: formation, properties and applications. Soft Matter, 12: 2826-2841, 2016.

37 Yang, B., Xu, J. Thermophysical properties and SANS studies of nanoemulsion heat transfer fluids in Monteiro WA (Ed.), Neutron Scattering. InTech pp 82-105, 2016.