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Development of W/O emulsion for encapsulation of "Pitanga" (*Eugenia uniflora* L.) leaf hydroethanolic extract: droplet size, physical stability and rheology

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Abstract

The low chemical stability under environmental conditions of "Pitanga" leaf hydroethanolic extract (PLHE) can limit its application in industrial scale. It is known that this extract has high antioxidant and antimicrobial activities and that it must be stored under special conditions or encapsulated into W/O emulsions. The objective of this research was to encapsulate PLHE in a W/O emulsion, analyzing the effect of the concentration of the emulsifier and phase ratios on its droplet size, physical stability and viscosity. In general, the droplet size and stability of the W/O emulsions were affected by the concentrations of emulsifiers and phase ratios. The emulsion with a 20/80 W/O ratio and 3 g PGPR/100 g oil was chosen as the most stable formulation because they presented well distributed droplet sizes (unimodal distribution), the lowest $D_{3,2}$ (0.25 ± 0.02 µm), the highest physical stability at 60 °C and presented Newtonian behavior. In conclusion, the W/O emulsion is able to encapsulate PHLE and can be applied to thermal processed foods.

Keywords: plant extract; hydroethanolic solvent; microencapsulation; colloidal system; viscosity.

Practical Application: A very stable W/O emulsion containing antioxidant extract was developed.

1 Introduction

The "Pitangueira" tree (*Eugenia uniflora* L.) can be found in South America, Southern Asia and Africa (Arai et al., 1999). Its fruits, called "Pitanga" or the Brazilian cherry, are edible and eaten fresh or in marmalade (Rattmann et al., 2012). The leaves of these trees are also well known and used in folk medicine to assist in the treatment of several diseases (Arai et al., 1999; Consolini & Sarubbio, 2002; Rattmann et al., 2012).

Recently, several researchers have demonstrated the high antioxidant activity of the "Pitanga" leaf's extracts (Garmus et al., 2014; Lorenzo et al., 2018; Schumacher et al., 2015; Vargas et al., 2016, 2019). These bioactivities are due to the presence of some phenolic acids and flavonoids (e.g. gallic acid, ellagic acid and myricitrin) (Bezerra et al., 2018; de Oliveira et al., 2018) and terpenoids (i.e. germacrene D and trans-caryophyllene) (Garmus et al., 2014). Nevertheless, these active compounds are very sensitive to environmental conditions, such as light and oxygen, and their storage can be an important challenge.

The encapsulation of PLHE in a W/O emulsion by adding this product in the water phase can be an approach to guarantee its stability (Ali & Akhtar, 2014, Kaimainen et al., 2015, Tepsongkroh et al., 2015, Liu et al., 2018, Rahpeyma & Sekhavatizadeh, 2020). A predominantly hydrophobic emulsifier, such as Polyglycerol polyricinoleate (PGPR), is necessary to produce a W/O emulsion (Fraj et al., 2017). The PGPR has been widely used in researches on W/O emulsions because it is an effective hydrophobic emulsifier, and has been used in several similar studies (Márquez et al., 2010, Ushikubo & Cunha, 2014, Tepsongkroh et al., 2015, Dridi et al., 2016, Matos et al., 2018, Velderrain-Rodríguez et al., 2019). It has been also widely used in food industries to stabilize W/O emulsions such as margarines, butter, salad dressing and chocolat (Okuro et al., 2019).

W/O emulsions containing plant extracts in the W inner phase can be applied in different industries, such as foods, pharmaceuticals and cosmetics. In the food industry, for example, W/O emulsions containing plant extracts are widely used to produce functional oily foods with a lower total fat content and desirable texture (i.e. dairy products) (Rahpeyma & Sekhavatizadeh, 2020), to increase the oxidative stability of the dispersing oil phase (Liu et al., 2018), and to protect and control the release of active aqueous compounds (Mohammadi et al., 2016; Rabelo et al., 2018). In the pharmaceutical and cosmetic industries, W/O emulsions containing plant extracts can be used to produce topical products for the treatment of diseases (Rasul et al., 2011) or pro-age (Huma et al., 2020), respectively.

The aim of this study was to encapsulate PLHE in a W/O emulsion and analyze the effects of emulsifier concentrations and phase ratios on its droplet size and physical stability. Some physical properties, including viscosity, of W/O emulsion with the more stable formulation, were also studied. To the best of our knowledge, no works on PHLE encapsulation in an W/O emulsion has been previously published.

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2 Materials and methods

2.1 Material

"Pitanga" (*Eugenia uniflora* L) leaves were collected in Pirassununga (SP), Brazil. Grinsted Polyglycerol Polyricinoleate (PGPR®) was donated by DuPont (São Paulo, Brazil).

2.2 Production of "pitanga" (Eugenia uniflora L.) leaf hydroethanolic extract (PLHE)

"Pitanga" leaves were washed and dried in an air circulating oven (MA035/5, Marconi, Brazil) at 42 °C/72 h, then they were ground and sifted in a 48-mesh sieve. The leaf powder was dispersed (1g/10 mL) in a 60% ethanol solution and treated by ultrasound at 40 kHz (Odontobras, 1440 DA, Brazil) at 25 °C/40 min. Subsequently, this dispersion was treated in a magnetic heater (Gehaka, AA-2050, Brazil) at 80 °C for 30 min and then filtered using filter paper (Whatman n°1). This extract was evaporated under vacuum (Tecnal, TE-211, Brazil) at 40 °C/4 h and then water was removed by freeze-drying (Heto, model FD 1.0-60, Germany) for 5 days and stored in the dark at 4 °C (Vargas et al., 2019). The freeze-dried powder was re-suspended in distilled water (1 g/10 mL water) prior to the production of W/O emulsions.

2.3 Production of water-in-oil (W/O) emulsions

The W/O emulsion was prepared using PLHE in the aqueous phase (10, 20 and 30% w/w) and soybean oil containing hydrophobic emulsifier PGPR (3 and 5 g/100 g oil) as the oil phase (90, 80 and 70% w/w). Briefly, the W/O emulsion was prepared by dropwise addition of PLHE in the oil phase under homogenization at 15,000 rpm for 5 minutes (Labotechnik, Ultraturrax® IKA T25, Germany). Then, this coarse W/O emulsion was homogenized through power ultrasound (Branson Ultrasonics, Sonifier® SFX550, USA) operating at 20 kHz with an amplitude of 30% during 3 cycles/30 s (Leong et al., 2018), both preparations were in ice bath. These emulsions were produced in triplicate and characterized just after their production.

2.4 Characterization of the emulsions

All emulsions characterizations were done in triplicate, at least.

Droplet size distributions and zeta-potential measurements

Prior to these analyses, the W/O emulsion samples were diluted 100x in chloroform, according to Souilem et al. (2014). Droplet size distributions and Zeta-potential of the W/O emulsions were determined by dynamic light scattering (Malvern Instruments, Zeta Sizer Nano ZS, UK) at 25 °C.

The droplet size distributions and the surface-based average droplet size of the W/O emulsions were calculated using ZetaSizer Nano software (Dammak & Sobral, 2018; Matos et al., 2014). The Sauter size ($D_{3,2}$) and span were calculated using the Equations 1 and 2, respectively, where d_i is the droplet size and n_i the surface frequency of droplets with size d_i.

$$D_{3,2} = \frac{\sum n_i d_1^3}{\sum n_i d_1^2} \quad (1)$$

$$Span = \frac{D_{v,90} - D_{v,10}}{D_{v,50}} \quad (2)$$

When bimodal droplet size distribution occurred, $D_{3,2}$ and span values were calculated for each peak using Equations 1 and 2, respectively, where $D_{v,10}$, $D_{v,50}$ and $D_{v,90}$ corresponded to the surface-based size at 10, 50 and 90% of cumulative surface for each peak (Dammak & Sobral, 2017, 2018).

2.5 Analysis of the physical stability

The physical stability of W/O emulsions was studied using a multi-sample analytical photocentrifuge (L.U.M. GmbH, LUMiSizer, Germany) with the following parameters: 1.8 mL of sample, 2,32 5x g, time 3,600 s, time interval 10 s, at 20, 40 and 60 °C (Dammak & Sobral, 2017, 2018). The instability Index (iI) was calculated as the ratio of the height of the creamy layer in relation of the height of the initial emulsion.

2.6 Confocal laser scanning microscopy (CLSM)

The morphology of the droplets of the W/O emulsion with the more stable formulation was qualitatively analyzed using a confocal laser scanning microscope (Leica Microsystems GmbH, SP5, Germany), with objective of 63x (1.4 aperture and oil immersion). The W/O emulsion was prepared with rhodamine B solution (0.1% m/v) added in the PLHE. Then, the W/O was diluted 200x in chloroform, and the rhodamine B was excited with HeNe laser at 543 nm and the emitted light was recorded between 570 to 640 nm. These analyses were made in the Multi-User Laboratory for Confocal Microscopy – LMMC of FCMRP-USP.

2.7 Physical properties

The W/O emulsion that had the more stable formulation was analyzed for determination of its density, pH, refractive index and rheological behavior. The density was assessed with the help of a digital densimeter (Anton Paar GmbH, DMA 4500, Austria); the pH was measured using an electronic pHmeter (Ind. e Com. Eletrônica Gehaka Ltda., PG1400, Brazil), and the refractive index was measured with an Abbe type refractometer, always at 25 °C. For these three characterizations, the W/O emulsions were analyzed without any prior preparation.

The viscosity of the W/O emulsions was also studied. Steady-state flow measurements were carried out with shear rates ranging from 0 to 125 s^{-1} at 25 °C, after the sample remained unperturbed for 5 min (Dammak & Sobral, 2017). These analyses were performed using a rheometer (TA Instruments, AR-2000, UK), using a cone and plate (cone diameter = 60 mm, angle = 2° and gap = 2 mm) geometry.

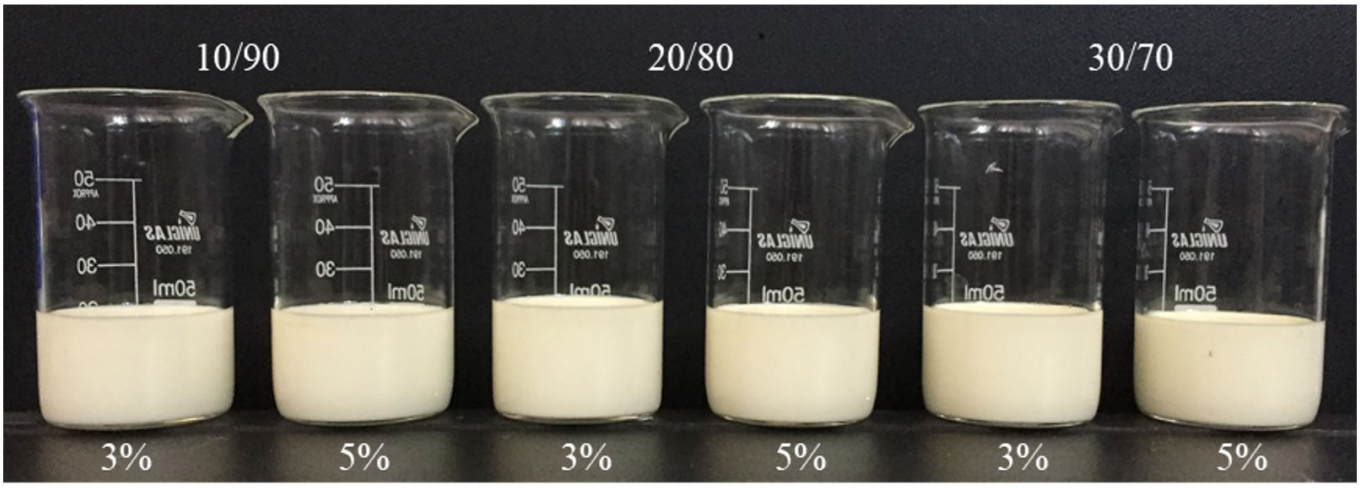

Figure 1. Visual aspects of produced W/O emulsions. PHLE concentration and W/O ratios are indicated in the figure. Source: own authorship.

2.8 Statistical analysis

All the experiments were carried out in triplicate, with three measurements for each replicate. All the experiments were submitted to variance analysis for determining significant differences between the averages using one-way or two-way (ANOVA) analyzes and Tukey test comparison ($p < 0.05$) with Statistica 7 software.

3 Results and discussion

3.1 Characteristics of water-in-oil (W/O) emulsions

All W/O emulsions were opaque and presented a white-yellowish color resembling milk due to the presence of PLHE (Figure 1). This appearance is usual due to the effect of droplet on light diffraction.

Droplet size distribution and average droplet size

The droplet size distributions of the W/O emulsions were mono- or bimodal (Figure 2), according to the concentration of the emulsifier and the dispersed phase. The emulsions containing both 10/90 and 20/80 W/O ratios and 5 g PGPR/100 g oil presented monomodal droplet size distributions, and emulsions with 10/90 W/O ratio and 3 g PGPR/100 g oil and both emulsions with 30/70 W/O ratio presented bimodal droplet size distributions (Figure 2).

Bimodal distribution can occur, for example, when there is insufficient emulsifier present in an emulsion to stabilize all of the droplets formed during homogenization (McClements, 2004). Thus, formed droplets were well stabilized only in emulsions having the higher amount of PGPR and low amount of water (10 and 20%). Nevertheless, these emulsions were produced under the same processing conditions than those described by Tepsongkroh et al. (2015), which observed only monomodal distributions, probably because they added biopolymers into the W inner phase, which contributed to stabilize droplets. Nevertheless, for a precise explanation of these behaviors, the knowledge of the critical micellar concentration would be necessary.

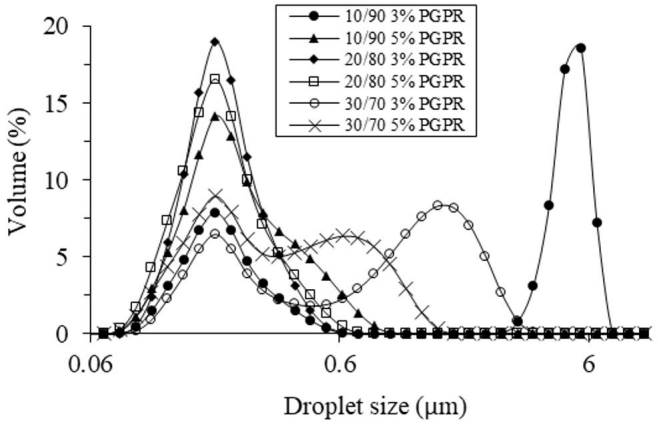

Figure 2. Droplet size distributions of W/O emulsions.

Table 1. Surface-based average droplet size ($D_{3,2}$) of W/O emulsions.*

W/O Ratio	$D_{3,2}$ (μm)	
	3 g PGPR/100 g oil	5 g PGPR/100 g oil
10/90	4.52 ± 0.23^{aA}	0.42 ± 0.04^{bB}
20/80	0.25 ± 0.02^{cA}	0.31 ± 0.04^{cA}
30/70	1.53 ± 0.08^{bA}	0.85 ± 0.05^{aB}

*Different lowercase letters in the same column and different uppercase letters on the same line indicate significant differences between averages according to Tukey test ($p < 0.05$).

For the purpose of comparison, the $D_{3,2}$ of W/O emulsions was calculated, and it ranged from 0.3 to 4.5 μm (Table 1), which is in the range of those found in the literature (Table 2). The increase in PGPR concentration from 3 to 5 g/100 g of oil resulted in a decrease ($p < 0.05$) in the $D_{3,2}$ for the W/O emulsions with 10/90 (4.5 to 0.4 μm) and 30/70 (1.5 to 0.9 μm) W/O ratios. Similarly, Márquez et al. (2010) observed that the increase of the PGPR concentration from 0.2 to 1% (in whole emulsion) in 20:80 W/O emulsions provoked a reduction in $D_{3,2}$ from 2.9 to 1.3 μm (Table 2), as in this work (Table 1).

Table 2. Examples of average droplet size values determined by others authors.

Emulsions*	D (μm)	References
Z-average droplet size of 20:80 W/O emulsions prepared with soybean oil with PRPG and water with either gelatin or sodium alginate, encapsulating MSKE.	0.12-0.17	Tepsongkroh et al. (2015)
D of 20:80 W/O emulsions prepared with medium chain triglycerides of caprylic/capric faty acids with PGPR, as O phase, and water, as W phase.	0.14-0.19	Fraj et al. (2017)
D of 10:90 – 30:70 W/O emulsions prepared with medium chain triglycerides with CR-310, as O phase, and water, as W phase encapsulating açaí berry extract.	0.13-0.20	Rabelo et al. (2018)
$D_{4,3}$ of 25:75 W/O emulsions prepared with corn oil with PGPR as O phase, and water with glycerol as W phase, enscapsulating MPPE.	0.38-0.84	Velderrain-Rodríguez et al. (2019)
$D_{v,0.5}$ of 20:80 W/O emulsions prepared with Miglyol 812 with PGPR, as O phase, and ethanol/water, as W phase, encapsulating *trans*-Resveratrol.	0.66	Matos et al. (2018)
Average droplet size of emulsions prepared with rapeseed oil with PGPR and DMG, and water with NaCl as W phase, measured by optical microscopy.	~1	Dridi et al. (2016)
$D_{4,3}$ of 30:70 W/O emulsion prepared with soybean oil and PGPR, as O phase, and water.	1.2	Ushikubo & Cunha (2014)
$D_{3,2}$ of 10:90 W/O emulsion prepared with sunflower oil and PGPR, as O phase, and water without salt, as W phase.	1.3-2.9	Márquez et al. (2010)
D of 80:20 W/O emulsions prepared with paraffin oil, ABIL EM 90 and bees wax, as O phase, and water, as W phase with Beta vulgaris extract, measured by optical microscopy.	1.6	Huma et al. (2020)
$D_{3,2}$ of 75:25 W/O emulsion prepared with soybean oil and PGPR, as O phase, and water, as W phase, containing Gallic acid solution.	2.0-3.5	Gomes et al. (2016)
$D_{3,2}$ of 75:25 W/O emulsion prepared with sunflower oil and PGPR, as O phase, and water, as W phase.	2.1	Okuro et al. (2019)
D of 10:90 W/O emulsions prepared with canola oil with GMS, as O phase, and water, as W phase, encapsulating green coffee extract.	3.2-8.5	Rahpeyma & Sekhavatizadeh (2020)

*PGPR: polyglycerol polyricinoleate; MPPE: mango peel phenolic-rich extract; MSKE: mango seed kernel extract; CR-321: Tetraglycerin monolaurate condensed ricinoleic acid esters; DMG: distilled mono-glycerides. GMS: Glycerol monostearate.

These behaviors were due to the effectiveness of PGPR for W/O emulsions, as observed by Tepsongkroh et al. (2015), who reported similar behavior for W/O produced with different concentrations of PGPR emulsifier. Furthermore, Matos et al. (2018) compared the $D_{3,2}$ values of W/O emulsions with different emulsifiers (PGPR, Span 80, Plurol oleique and Peceol) and observed that the W/O emulsion containing PGPR had the smallest $D_{3,2}$ value (Table 2), confirming that PGPR was a more effective emulsifier in the production and stabilization of W/O emulsions. Nevertheless, for the W/O emulsion with 20/80 ratio, the PGPR concentration did not affect ($p>0.05$) the $D_{3,2}$ values (Table 1). A possible explanation is that the presence some bioactive compound present in PLHE extracts could act as W/O stabilizer considering that W/O ratio (Tepsongkroh et al., 2015). However, further experiments are needed to better understand and explain these results.

Further, the W/O ratios also affected ($p < 0.05$) the $D_{3,2}$ values (Table 1). Considering that the expected behavior must be similar to that observed by Katsouli et al. (2017), even if working with nanoemulsions (NE): increasing W phase content in W/O NE led to higher droplet sizes; it can be considered that for emulsions containing 3 g PGPR/100 g oil, the $D_{3,2}$ increased from 0.25 to 1.53 μm when the W phase content increased from 20 to 30% (Table 1). The higher value (4.52 μm) for 10/90 emulsion containing 3 g PGPR/100 g oil was due to the insufficient stabilization of droplets, as explained above. For emulsions containing 5 g PGPR/100 g oil, overall, the $D_{3,2}$ increased with the W phase content and the higher value (0.85 μm) was observed in the 30/70 W/O emulsion (Table 1). But, Ushikubo & Cunha (2014), studying the effect of two W/O ratios on emulsions produced with PGPR as emulsifier, didn't find such effect {$D_{4,3}(30:70) = 1.15$ μm ≈ $D_{4,3}(60:40) = 1.19$ μm}. And, curiously, Okuro et al. (2019) produced a W/O emulsion with very high-water phase ratio (75:25) and emulsified by PGPR, and determined $D_{3,2}$ (2.1 μm, Table 2) in the same range of this work (Table 1), which means that the W/O ratio could not have so important effect on droplet size. Similar results were observed by Gomes et al. (2016), also working with 75:25 W/O emulsion and with Gallic acid solution as W phase. By the way, these authors determined higher $D_{3,2}$ values when using pure water as W phase.

Matos et al. (2018) determined $D_{v,0.5}$ = 0.66 μm for W/O emulsions produced with 20% (v/v) W phase (water/ethanol) containing *trans*-Resveratrol, and 80% of miglyol 812 containing 5% of PGPR, as O phase (Table 2). This value was similar to those determined in this study (Table 1).

Physical stability of W/O emulsions

The physical stability of W/O emulsions was studied at 20, 40 and 60 °C. This temperature range was interesting because these emulsions can be applied to thermal processed foods. For instance, it can be used to produce active films loaded with encapsulated PLHE in such emulsions, and film processing imply the preparation of film-forming solution in temperature above the room temperature (Tessaro et al. 2021). The good stability of these emulsions will guarantee, thus, that the PLHE will be well dispersed into biopolymer matrix.

The curves of the W/O emulsion's light transmission profiles, as determined by the change in laser transmission in a layer (33-35 mm) of W/O emulsion, were basically straight horizontal lines (Figure 3) and always demonstrated a low transmission (<10%) for all studied temperatures. This behavior was a consequence of the opacity in the whole sample, which was due to a uniform droplet distribution throughout the sample layer inside the cuvette (Figure 3), not destroyed by the high gravity force during analysis, confirming the high stability of these emulsions (Dammak & Sobral, 2017).

The instability Index (iI), calculated from the spectra presented in the Figure 3, has an arbitrary scale ranging from 0 (more stable) to 1 (less stable) (Figure 4). Regardless of temperature and the emulsion's formulation, the iI ranged from 0.009 to 0.063 (iI<<0.1), meaning that these systems were highly stable. Overall, iIs were correlated with $D_{3,2}$, but it was significant only for iI determined at 60 °C: $iI = 7.6 \times 10^{-3} D_{3,2} + 0.026$, $R^2 = 0.81$. Moreover, the high stability of W/O emulsions can be also associated to two factors: the viscosity of the lipid phase (and consequently, the high viscosity of the whole emulsion, as can be seen in 3.1.4) and the use of PGPR as emulsifier (Dickinson, 2011; Ilić et al., 2017; Tepsongkroh et al., 2015). According to Gomes et al. (2016), the good stability of the emulsions with PGPR could be attributed to the attachment of PGPR hydrophobic chains in the branched structure of the oil.

Ushikubo & Cunha (2014) studied the stability of W/O emulsions produced with different oils and emulsifiers and observed that emulsions produced with PGPR and soybean oil were the most stable. On the other side, Rabelo et al. (2018) studied the stability of nanoemulsions with different W/O ratio and Açaí extract (AE) concentrations by measuring its creaming index, and observed that samples with higher AE concentrations were able to stabilize the nanoemulsions systems, especially for 30:70 W/O. They explained these results considering that AE is rich in amino acids, such as methionine, threonine, and lysine; hence, they might play a role in stabilizing the nanoemulsions.

Although no significant differences were observed between emulsions containing 3 and 5 g PGPR/100 g oil for 20/80 W/0

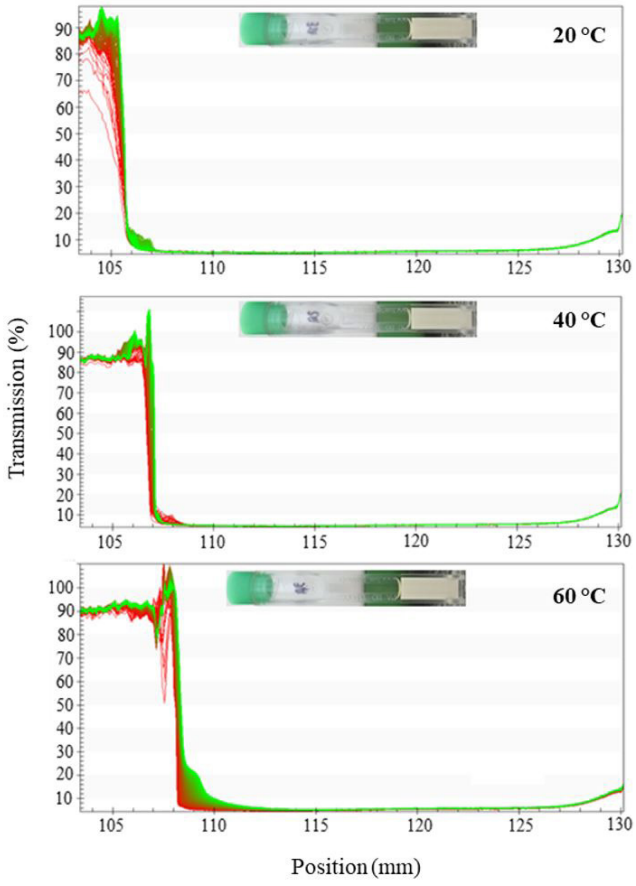

Figure 3. Profiles transmission of W/O emulsions determined using an analytical centrifuge (2.325 x g, time 3600 s) at 20, 40 and 60 °C (photo of cuvettes with samples after analysis were attached in figures). Red-green lines are the light transmition profiles measured during analysis for each time.

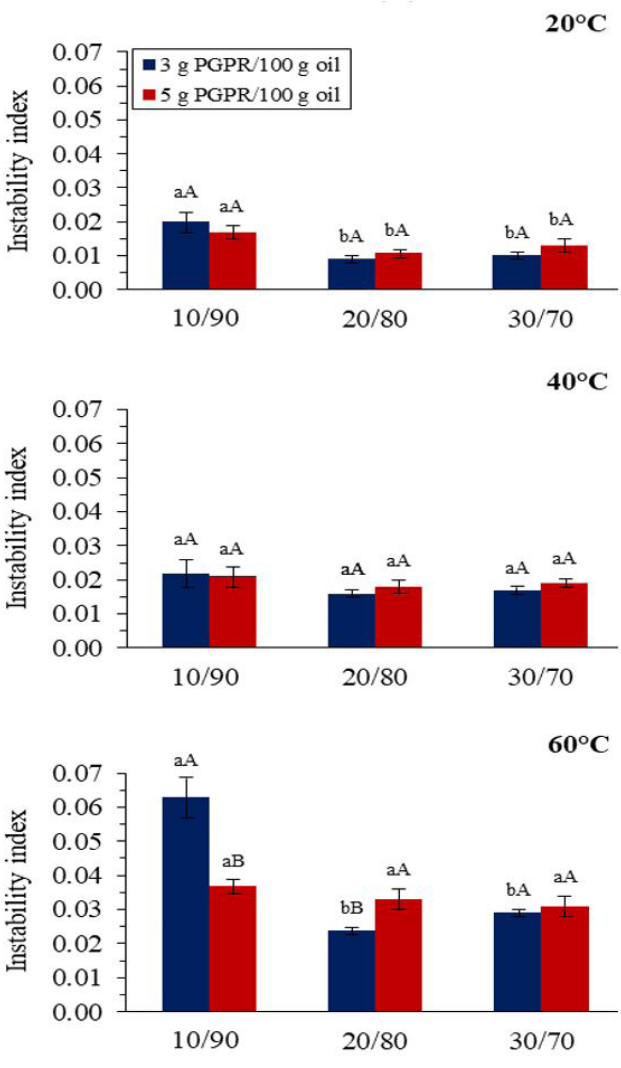

Figure 4. Instability index* of the W/O emulsions with different W/O ratios, at 20, 40 and 60 °C. *Different lowercase letters at the same concentration of emulsifiers and different uppercase letters in same ratio indicate significant differences between averages according to Tukey test ($p < 0.05$).

condition at 20 and 40 °C, a significant difference ($p<0.05$) was observed at 60 °C: 3 g PGPR/100 g oil produced a more stable 20/80 W/O emulsion (Figure 4). And, this can be important for eventual application of this emulsion on thermal processed food, for instance. This W/O emulsion showed iI of 0.009 (20 °C), 0.016 (40 °C) and 0.024 (60 °C) (Figure 4). Therefore, the emulsion with a 20/80 W/O ratio and 3 g PGPR/100 g oil was chosen as the most stable formulation because they presented the well distributed droplet size (unimodal), the lowest $D_{3,2}$ (0.25 ± 0.02 mm), and the highest physical stability at 60 °C.

Confocal laser scanning microscopy (CLSM)

The micrographs obtained using the CLSM allowed observation of a drop of PLHE dispersed in the oil phase (Figure 5). Due to the limited sensitivity of the microscope, it was not possible to observe the smallest drops of PLHE. The red color at the PLHE-oil interface corresponds to the excitation of rhodamine B present in the PLHE. This showed that the active compounds present in PLHE, predominantly polyphenols, tended to remain at the PLHE-oil interface after the process of emulsification.

In fact, it is known that some antioxidant compounds, such as polyphenols, can remain at the water-oil interface of emulsions, interacting with both phases contributing to the reduction of the interfacial tension between them (Katsouli et al., 2017, Velderrain-Rodríguez et al., 2019). That is: the PLHE may have some contribution to the high stability of the W/O emulsions produced. Gomes et al. (2016) determined lower sedimentation index (higher stability) for emulsions prepared with Gallic acid solution as W phase compared with this result for emulsion with pure water, as W phase. According to these authors, the Gallic acid could increase emulsions stability in two ways: a) changing the polarity of the phases leading to increased solubility between them or b) penetrating the interfacial film as a surfactant causing increased flexibility of the interface. The result presented in the Figure 5 corroborates with this last explanation (b).

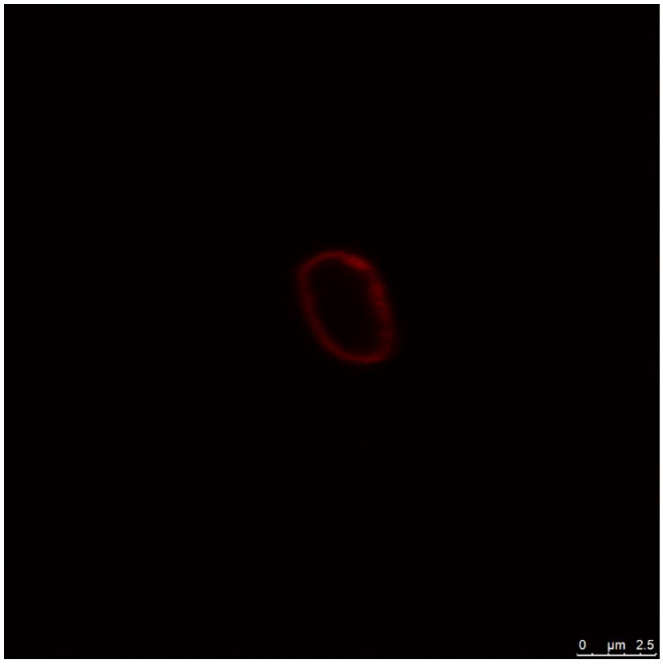

Figure 5. Confocal laser scanning micrographs of the W/O emulsion with the more stable formulation and stained with Rhodamine B. Source: own authorship.

Some physical properties of W/O emulsion with the more stable formulation

The best W/O emulsion presented a density equal to 0.937 ± 0.001 g/cm^3, lower than that of water due to the presence of the oil phase. Considering the soybean oil density (ρ_O = ~ 0.92 g/cm^3) (De Almeida et al., 2013), and the water density (ρ_W = 0.999 g/cm^3), the emulsion density (ρ) can be calculated using the principle of volume additivity ($1/\rho = W_O/\rho_O + W_W/\rho_W$) as 0.935 g/cm^3, very close of the experimental value. The contribution of PGPR and PLHE can be neglected because its concentrations in the whole emulsion were very low.

The pH of this emulsion was 5.12 ± 0.01, due to the presence of some acids in the PLHE whose pH is 4.12 ± 0.02. An acidic pH (4.2-5.5) in W/O emulsion containing *Moringa* leafs extract in W phase at various storage conditions was also observed by Ali & Akhtar (2014). Similar values were determined by Huma et al. (2020). The pH value determined in this work is acceptable for its use as a food additive or even in the pharmaceutical and cosmetic industries, since human skin has a pH between 4.5 and 6.0, and cosmetics must have a pH in this range (Ali & Akhtar, 2014).

The refractive index of this emulsion was 1.640 ± 0.004, which is typical of a mixture with phases with different refractive indices values (water = 1.333; soybean oil = 1.467) (Chantrapornchai et al., 2001), as in the case of emulsions, which consequently appear to be opaque (Figure 1). This result is in accordance with the low light transmittance through the sample, as can be observed in Figure 3.

Regarding the emulsion viscosity, it was observed that best W/O emulsion behaves as a Newtonian fluid (Figure 6). Its viscosity was calculated ($R^2 > 0.99$) as 104.6 ± 0.6 mPa.s. This value was higher than that of pure water (~1 mPa.s) and even that of soybean oil (~60 mPa.s) at 25 °C (Ilić et al., 2017), because the droplets contributed to increasing the viscosity

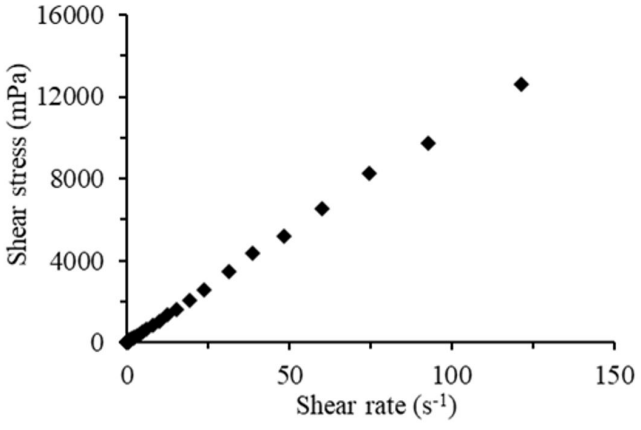

Figure 6. Flow curve of the W/O emulsions, at 25 °C.

of the system, according to the Krieger Dougherty relation (Shrestha et al., 2011). Tepsongkroh et al. (2015) observed that the increase of PGPR concentration, from 2 to 8%, increased the viscosity of 20:80 W/O emulsions from ~90 to ~116 mPa.s, that is, in the range of that determined in this work.

Nevertheless, a non-Newtonian behavior has been observed by Ushikubo & Cunha (2014), working with 30:70 W/O emulsions prepared with soybean oil and PGPR, and water, and by Rahpeyma & Sekhavatizadeh (2020), working with 10:90 W/O emulsions prepared with canola oil with GMS, and water containing green coffee extract. Okuro et al. (2019) also observed a non-Newtonian behavior working on 75:25 W/O emulsions emulsified by PGPR.

The high viscosity found in this work also could have contributed to the high stability of W/O emulsion (McClements, 2004), and could be interesting for application of this emulsion in dressing sauces, for example, even if this product must be thermal treated until 60 °C because it is very stable in this condition.

4 Conclusions

This study showed that it is feasible to encapsulate the PLHE in a stable W/O emulsion system using PGPR as emulsifier and ultrasound equipment to homogenize. The W/O emulsions showed small and very homogeneous droplet sizes and a very high physical stability and Newtonian behavior. Overall, these properties were affected by the concentrations of the emulsifier (PGPR) and phase ratios. In addition, the analyzed physical properties of the more stable primary emulsion have shown that it can have potential applications in the food, pharmaceutical and cosmetic industry.

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