

ORIGINAL ARTICLE

Development, characterization and stability of microemulsions of bacaba, *Oenocarpus bacaba* oil

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ABSTRACT

Advances in nanotechnology, combined with the use of natural products, represent a promising research field. Brazil is a country of a rich biodiversity, especially in the Amazon forest. Fruits commonly used by local communities, such as bacaba (*Oenocarpus bacaba*), are potentially important for prospection of industrial applications of natural products. In nanotechnology, microemulsions stand out for providing a modified release to conveyed substances. This work aimed to develop microemulsions of bacaba oil, characterize them and evaluate their stability. We determined the HLB (hydrophile-lipophilic balance) of bacaba oil for formulation development. Six formulations were selected from pseudoternary diagrams, which indicated the proportions of surfactants, aqueous phase and bacaba oil. The viability of these formulations was evaluated through stability tests. We provided the rheological characterization of the formulations, evaluated their potential antioxidant activity through the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, and determined the hydrodynamic diameter of the droplets. The microemulsions were stable throughout the test period. Droplet diameter was below 200 nm, and the microemulsions were characterized as newtonian fluids, presenting an increase in antioxidant activity when compared with the diluted oil. Our results confirm the potential of bacaba oil in microemulsions formulation as a suitable carrier for active compounds.

KEYWORDS: microemulsion, Amazon, native fruit, nanotechnology, carriers

Desenvolvimento, caracterização e estabilidade de formulações microemulsionadas de óleo de bacaba, *Oenocarpus bacaba*

RESUMO

Os avanços no âmbito da nanotecnologia, aliados ao uso de produtos naturais, representam uma área promissora de pesquisa. O Brasil é um país de rica biodiversidade, especialmente na floresta amazônica. Frutos utilizados comumente nas comunidades locais, como a bacaba (*Oenocarpus bacaba*), são potencialmente interessantes para prospecção de aplicação industrial de produtos naturais. Na área da nanotecnologia, as microemulsões se destacam por prover uma liberação modificada das substâncias veiculadas. Este trabalho teve como objetivo desenvolver formulações microemulsionadas de óleo da bacaba, caracterizá-las e avaliar sua estabilidade. Determinamos o EHL (equilíbrio hidrófilo-lipófilo) do óleo da bacaba para o desenvolvimento das formulações. A partir de diagramas pseudoternários, que indicaram as proporções de tensoativos da fase aquosa e do óleo de bacaba, foram selecionadas seis formulações. A viabilidade dessas formulações foi analisada por meio de testes de estabilidade. As formulações passaram por caracterização reológica, avaliação do potencial de atividade antioxidante através da capacidade de redução do radical DPPH (2,2-difenil-1-picrilhidrazil), e determinação do diâmetro hidrodinâmico de suas gotículas. As microemulsões mostraram-se estáveis durante o período de teste e apresentaram diâmetro de gotículas abaixo de 200 nm, foram caracterizadas como fluidos newtonianos e apresentaram um aumento da atividade antioxidante quando comparadas com o óleo diluído. Nossos resultados indicam o potencial do óleo de bacaba em formulação microemulsionada para veiculação de diversos princípios ativos.

PALAVRAS-CHAVE: microemulsão, Amazônia, fruto nativo, nanotecnologia, carreadores

CITE AS: Torres, M. de P.R.; Espendor, R.V. de F.; Bonaldo, S.M.; Ribeiro, E.B.; Valladão, D.M. de S. 2019. Development, characterization and stability of microemulsions of bacaba, *Oenocarpus bacaba* oil. *Acta Amazonica* 49: 246-255.

INTRODUCTION

The Amazon rainforest holds about one third of the world's biological diversity (Albagli 2001). The use of this biodiversity in new technologies constitutes a great tool of scientific advancement and development of the industry in Brazil (Albagli 2001; Funari and Ferro 2005). Bacaba (*Oenocarpus bacaba* Mart.) is an Amazonian palm tree with a fruit that produces oil traditionally used in cooking. The fruit has great economic and nutritional potential, as its oil is rich in unsaturated fatty acids, mainly oleic and palmitic, and phenolic compounds (Balick 1986; Queiroz and Bianco, 2009; Finco *et al.* 2012; Pereira *et al.* 2013; Santos *et al.* 2017). Traditionally, the oil is extracted by boiling the fruit pulp (Cymerys 2005). However, the heat might lead thermosensitive substances to degradation, which calls for other methods that do not use high temperatures in the oil extraction process (Roy *et al.* 2006; Pinto *et al.* 2018). Studies concerning the biochemical activity of bacaba oil are scarce, but it has already been shown to have antioxidant and cardioprotective potential (Pinto *et al.* 2018).

Nanotechnology is a subject of research in several industries, due to the need of new carriers for active compounds, to convey natural substances that are difficult to incorporate in traditional forms, or to enhance productivity in agriculture (Bonifácio *et al.* 2014; Dasgupta *et al.* 2015). Nanostructured formulations can increase the selectivity and effectiveness of active compounds, as their delivery structures can surpass biological barriers that traditional formulations do not. They also convey protection against degradation, and reduce side effects by controlling the release of several substances, including plant extracts, which allows the association of two market trends, such as natural products and nanotechnology (Oliveira *et al.* 2004; Bonifácio *et al.* 2014; Katz *et al.* 2015; Costa and Santos 2017). Microemulsions present some advantages when compared to other nanostructured formulations (Bonifácio *et al.* 2014), since they are thermodynamically stable, isotropic and transparent systems of two immiscible liquids (usually water and oil), dispersed into each other in the form of nanometric droplets, stabilized by a interfacial surfactant/cosurfactant film, obtained without the need of energy addition (Lawrence and Rees 2012; Fanun 2012; Callender *et al.* 2017). These characteristics make microemulsions cheaper, easier and quicker to develop.

Thus, the aim of this work was to develop microemulsified formulations with bacaba oil, to characterize them and to evaluate their stability in order to determine their potential capacity as carriers for the delivery of active compounds.

MATERIAL AND METHODS

HLB determination

Oenocarpus bacaba oil was purchased from the company "Mundo dos Óleos", Brasília, Brazil. All the formulations described in here were prepared using oil from the same batch.

According to the manufacturer, the oil was extracted by cold pressing and presented corresponding quality characteristics. We prepared a series of emulsions containing bacaba oil with increasing HLB (hydrophilic-lipophilic balance). The surfactant mixture fractions of Span 80 (sorbitan monostearate) and Tween 80 (polysorbate 80), and the ratio [w/w (weight/weight)] of the surfactants to obtain the HLB (from six up to 12), were calculated. The samples for each HLB were prepared in triplicate. The aqueous and oily phases were prepared separately, then mixed and kept under manual stirring and heating in an electric water bath at 75 °C for 15 min. After having been removed from the water bath, the emulsions were kept under manual stirring for fifteen minutes at room temperature. The emulsions were analyzed under a microscope (BEL® Photonics, Italy) at 40x magnification, and the globules were photographed (one picture for each replicate, adding up to three pictures for each HLB value) for determination of their area using the Image J® software. Mean droplet size, standard deviation and coefficient of variation in function of globule area were calculated (Zanin *et al.* 2002; Fiori *et al.* 2017).

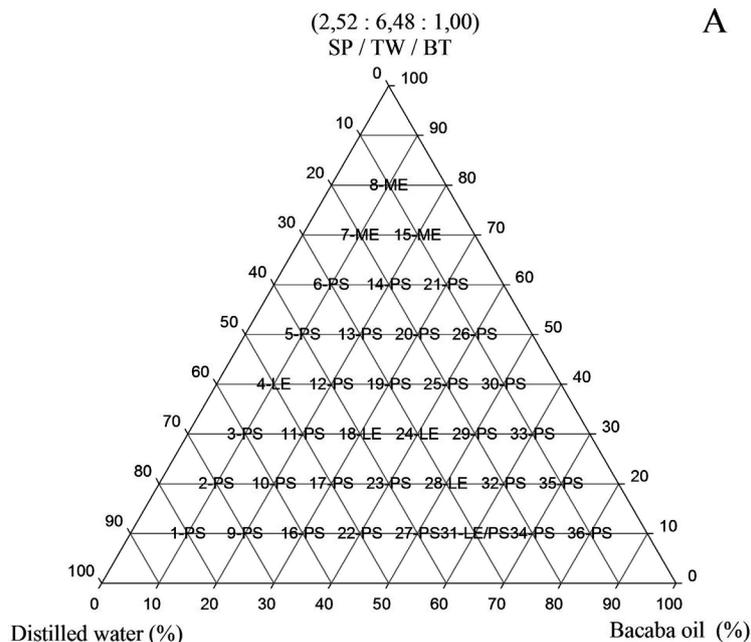
System composition

The microemulsified systems were developed using bacaba oil, distilled water, sorbitan monostearate (Span 80 - SP), HLB = 4.3; polysorbate 80 (Tween 80 - TW), HLB = 15.0; and butan-1-ol (BT). The reagents used in the study were analytical grade and purchased from Sigma®, USA. Butan-1-ol (BT) was used at a ratio of 10% (fraction = 1) in the surfactant mixture. BT is one of the best cosurfactants for the preparation of microemulsions (Kumar *et al.* 2014; Boonme *et al.* 2006; Wilk *et al.* 2009) and its concentration in our formulations is considered residual and non-toxic (USP 2007). The HLB of SP and TW was used to calculate the mix relation of surfactants and cosurfactants, to achieve an HLB corresponding to the oily phase (Ribeiro *et al.* 2015).

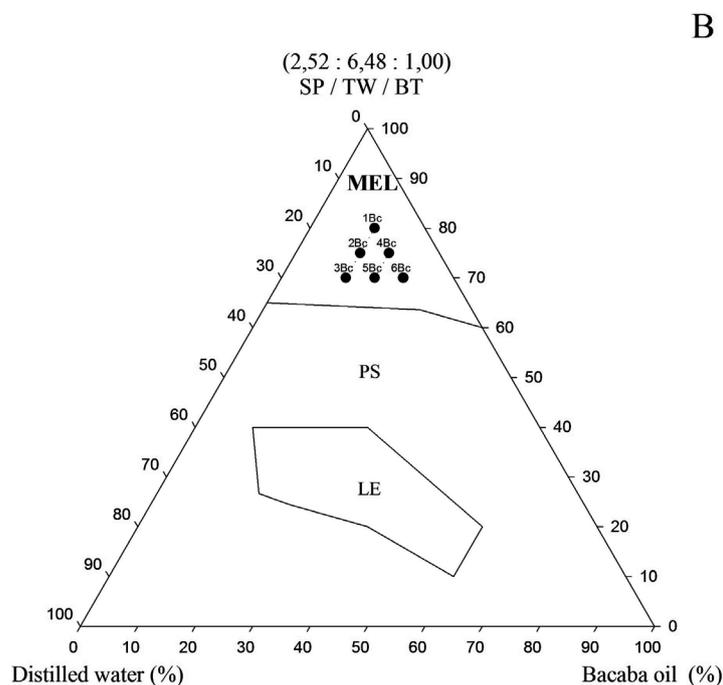
System development

Thirty six formulations were developed using pre-established quantities of the components, ranging from 10% to 80%, in order to construct a pseudoternary diagram. The samples were prepared and visually rated after 72 hours at 25 °C in the following categories: microemulsion (ME), liquid emulsion (LE), and phase separation (PS). Titrations were performed to obtain the bounding points, regions and classification of different systems to form the diagram. Surfactant/oil mixtures were prepared in the mass ratios of 1:9 up to 9:1, then distilled water was added in quantities between 0.05 and 0.10 mL, with constant agitation. After homogenization of each mixture, they were visually classified.

Pseudoternary diagrams were obtained from the sample and titration data using the SigmaPlot® version 8.0 software. The superior vertex of the diagrams (Figure 1) represents



A



B

Figure 1. (A) Pseudoternary diagram containing all 36 points for microemulsions of *Oenocarpus bacaba* oil and their respective classifications. (B) Region diagram showing the microemulsion region (MEL) containing the six selected microemulsions. LE = liquid emulsion; PS = phase separation

100% surfactant/cosurfactant mix, the lower right represents 100% oily phase and the lower left represents 100% aqueous phase. After acquisition of the pseudoternary phase diagram, the microemulsion region was determined. From this region, microemulsions with different compositions were selected to achieve representative samples of the studied systems.

Stability analysis

Microemulsion stability was analyzed according to Fiori *et al.* (2017) and Gustmann *et al.* (2017). For preliminary stability, the formulations selected from the pseudoternary diagram were submitted to alternate heating/cooling cycles of 24 hours, at 40 ± 1 °C and 5 ± 1 °C, respectively, during 14

days. Physicochemical characteristics of the formulations were measured before and after the test. The pH was determined with a pH meter (DLA-PH, Del Lab, Brazil) calibrated with standard pH 7 and pH 4 buffer solutions. Electrical conductivity was measured using a conductivity meter (MCA-150, Tecnopon, Brazil) calibrated with a 0.1 mol/L KCL solution. The refraction index was measured with a digital Abbe refractometer (WYA-2S, Polax, Brazil).

For accelerated stability tests, microemulsions selected from the previous test were submitted to three different conditions, in triplicate: 5 ± 1 °C, 25 ± 1 °C and 40 ± 1 °C. The test lasted 90 days and the physiochemical characteristics were assessed at the beginning of the test and every 30 days.

Rheological characterization

Rheological parameters were measured for each formulation, before and after the accelerated stability test, according to Fiori *et al.* (2017) and Gustmann *et al.* (2017) using a Modular Compact Rheometer (MCR 102, Anton Paar® Germany GmbH, Ostfildern, Germany). The tests were conducted under controlled conditions at 25 °C and statistically significant differences within samples before and after the test were evaluated using analysis of variance (ANOVA), followed by multiple comparisons by Tukey test. Statistical significance was set at $p < 0.05$.

Dynamic light scattering (DLS)

Dynamic light scattering analysis were performed using a Zetasizer Nano (Z90, Malvern® Instruments, United Kingdom) with excitation at 632.8 nm, according to Fiori *et al.* (2017) and Gustmann *et al.* (2017). Colloidal structures of the formulations were prepared for analysis by the dynamic light scattering technique, to evaluate the hydrodynamic diameter of the dispersed system.

Antioxidant activity

We determined the potential antioxidant activity using DPPH (2,2-diphenyl-1-picryl-hydrazila) radical scavenging adapted from Elmasta *et al.* (2006) and Li *et al.* (2012). Measurements were determined in a spectrophotometer (CIRRUS 80MB, Femto®, São Paulo, Brazil). The microemulsions were diluted in methanol, and the oil in a solution of methanol:chloroform (1:1). The microemulsions selected from the previous tests were tested along four control formulations (pure oil and oil in 10, 15 and 20% dilution, according to its percentage in the formulations). Absorbance readings were performed at the wavelength of 517 nm using respective white controls. To evaluate the free radical scavenging activity, a calibration curve was build for each formulation, pure oil and oil dilutions. The percentage of DPPH radical inhibition of each point in the curves was calculated by the following equation: % inhibition of DPPH = $[(A_0 - A_1) / A_0 \times 100]$, where A_0 = control absorbance, and A_1 = absorbance of the sample. The IC50 (half maximal inhibitory concentration) value was calculated using the equation provided by the curve.

RESULTS

HLB 7 and 12 had similar mean droplet size (Table 1), HLB 7 being a bit smaller, and HLB 12 having lower values of standard deviation and coefficient of variation, which indicates a greater homogeneity of the globules and greater proximity to the ideal HLB. Therefore, two pseudoternary diagrams were constructed, one considering the HLB value of the oil phase as seven and the other as 12. The HLB 12 diagram formed more microemulsions, showing that HLB 12 was the most adequate, being selected for subsequent analyzes.

The obtained surfactant fractions were 2.52 and 6.48 for SP and TW, respectively, resulting in a surfactant mix relation of 1.0:2.52:6.48 (BT:SP:TW), which was used in the pseudoternary diagram.

The resulting HLB 12 diagram presented 36 points with divergent characteristics (Figure 1a), from which three were visually classified as microemulsions, considering their translucence. After the titrations, it was possible to determine a microemulsion region limit on the diagram (Figure 1b), and, from this region, six different formulations (1Bc, 2Bc, 3Bc, 4Bc, 5Bc and 6Bc) were selected, which presented different amounts of each phase (at least 10% of oil and water), aiming to represent the diversity of the microemulsion region. The selected formulations contained at least 70% of the surfactant mixture, 10% of the aqueous phase, and an oil phase in a range between 10% and 20%.

The six formulations were macroscopically stable after centrifugation and were clear and homogeneous, with no sign of phase separation. The pH values before and after the preliminary stability test ranged between 6.75 and 7.35. The electric conductivity and refraction index showed a small variation (Table 2).

Table 1. Droplet dimension of the series of emulsions with *Oenocarpus bacaba* oil used to determine the oil's HLB. Diameter values are the mean \pm SD.

HLB	Diameter (nm)	Coefficient of variation
6.0	0.0325 \pm 0.1326	4.0752
6.5	0.0186 \pm 0.0873	4.7041
7.0	0.0065 \pm 0.0065	0.9988
7.5	0.0361 \pm 0.1520	4.2056
8.0	0.0588 \pm 0.2156	3.6681
8.5	0.0076 \pm 0.0084	1.1062
9.0	0.0355 \pm 0.1535	4.3307
9.5	0.0354 \pm 0.1604	4.5363
10.0	0.0507 \pm 0.1913	3.7722
10.5	0.0355 \pm 0.1574	4.4327
11.0	0.0227 \pm 0.1151	5.0792
11.5	0.0066 \pm 0.0091	1.3707
12.0	0.0066 \pm 0.0056	0.8552

Table 2. Physicochemical parameters of the six selected formulations of *Oenocarpus bacaba* oil microemulsions after preliminary stability tests (14 days). no = no phase separation. Values are the mean ± SD of three replicates.

Formulation	1Bc	2Bc	3Bc	4Bc	5Bc	6Bc
Centrifugation	no	no	no	no	no	no
pH	7.35 ± 0.11	7.20 ± 0.11	6.93 ± 0.03	7.14 ± 0.06	7.07 ± 0.05	7.17 ± 0.08
Conductivity (µScm ⁻¹)	9.83 ± 0.63	17.66 ± 2.63	35.46 ± 0.82	9.34 ± 0.09	20.66 ± 0.58	9.22 ± 0.36
Refraction index	1.4550 ± 0.0006	1.4490 ± 0.0016	1.4429 ± 0.0002	1.4552 ± 0.0004	1.4492 ± 0.0002	1.4554 ± 0.0002
After heating-cooling cycles						
pH	7.16 ± 0.23	6.90 ± 0.09	6.75 ± 0.03	7.00 ± 0.02	6.91 ± 0.03	7.01 ± 0.06
Conductivity (mScm ⁻¹)	6.69 ± 3.94	16.13 ± 1.85	32.46 ± 1.55	8.22 ± 0.22	17.53 ± 0.44	8.57 ± 0.67
Refraction index	1.4555 ± 0.0062	1.4497 ± 0.0021	1.4441 ± 0.0003	1.4559 ± 0.0003	1.4499 ± 0.0013	1.4558 ± 0.0005

In the accelerated stability test, the pH of the microemulsions varied slightly, settling around 7 (Table 3). Mean conductivity in all formulations remained above 1.3 µScm⁻¹ after the 90-day test period (Table 3), characterizing them as O/W (oil in water) microemulsions, and indicating stability and non inversion of the system phases. The refraction index of the formulations also remained constant over time (Table 3).

The formulations displayed a flow curve with linear ascending and descending behavior, and viscosity values that did not change with the increase of the shear rate, characterizing them as Newtonian fluids. Formulations 2Bc, 3Bc, 4Bc and 5Bc maintained statistically similar equivalent stability profiles (ANOVA 90 days: F = 0.952; p = 0.533) (Figure 2), while 1Bc and 6Bc differed significantly (p < 0.01) and were therefore not included in Figure 2. Formulations 2Bc, 3Bc and 5Bc had statistically similar viscosity values at the beginning and end of the stability analysis (Figure 3), while 1Bc, 4Bc and 6Bc differed significantly (p < 0.01) and were therefore not included in Figure 3 (ANOVA 90 days: F = 29.724; p < 0.0001 – 2Bc: p > 0.05, 3Bc: p > 0.05 and 5Bc: p > 0.05). Therefore we concluded that formulations 2Bc, 3Bc and 5Bc were the ones with greater rheological stability over time.

Mean droplet size ranged between 130 and 250 nm (Figure 4). The PDI values ranged between 0.18 and 0.33, indicating homogeneity of the globules in the formulations. The hydrodynamic diameter of all six final formulations was dependent on the proportion of the constituents, since there was a statistical difference among them (p < 0.001).

The oil with no dilution had the lowest IC50, and, therefore, the best antioxidant activity. All formulations had IC50 values indicative of better antioxidant activity than their respective oil dilutions (Figure 5).

Table 3. Physicochemical parameters of the six selected formulations of *Oenocarpus bacaba* oil microemulsions during the accelerated stability test (determined at 0, 30, 60 and 90 days). Values are the mean ± SD of three replicates.

Formulation	pH			
	0	30	60	90
1Bc	7.47 ± 0.10	7.19 ± 0.25	7.33 ± 0.27	7.07 ± 0.38
2Bc	7.35 ± 0.11	7.04 ± 0.33	7.18 ± 0.28	6.94 ± 0.35
3Bc	7.19 ± 0.06	6.88 ± 0.32	7.18 ± 0.35	6.80 ± 0.53
4Bc	7.41 ± 0.10	7.14 ± 0.26	7.35 ± 0.28	7.16 ± 0.37
5Bc	7.32 ± 0.18	7.17 ± 0.15	7.28 ± 0.23	7.05 ± 0.24
6Bc	7.47 ± 0.19	7.31 ± 0.16	7.39 ± 0.13	7.23 ± 0.30
Conductivity (µScm ⁻¹)				
1Bc	7.89 ± 0.68	9.05 ± 1.07	8.34 ± 0.95	9.78 ± 4.28
2Bc	15.63 ± 1.80	16.90 ± 2.21	18.14 ± 5.73	29.74 ± 25.59
3Bc	28.37 ± 3.13	30.11 ± 4.72	25.28 ± 9.40	30.03 ± 12.03
4Bc	7.83 ± 0.96	9.01 ± 1.50	6.84 ± 1.74	8.14 ± 1.34
5Bc	15.20 ± 0.25	15.52 ± 3.56	12.45 ± 4.98	14.33 ± 6.22
6Bc	7.15 ± 0.10	7.41 ± 2.21	5.61 ± 3.05	6.93 ± 3.13
Refraction Index				
1Bc	1.4553 ± 0.0007	1.4544 ± 0.0011	1.4565 ± 0.0014	1.4577 ± 0.0054
2Bc	1.4495 ± 0.0012	1.4488 ± 0.0009	1.4585 ± 0.0093	1.4559 ± 0.0108
3Bc	1.4439 ± 0.0020	1.4438 ± 0.0017	1.4479 ± 0.0087	1.4484 ± 0.0090
4Bc	1.4545 ± 0.0013	1.4545 ± 0.0013	1.4574 ± 0.0019	1.4584 ± 0.0050
5Bc	1.4544 ± 0.0137	1.4508 ± 0.0028	1.4539 ± 0.0042	1.4531 ± 0.0047
6Bc	1.4552 ± 0.0005	1.4567 ± 0.0030	1.4601 ± 0.0052	1.4593 ± 0.0040

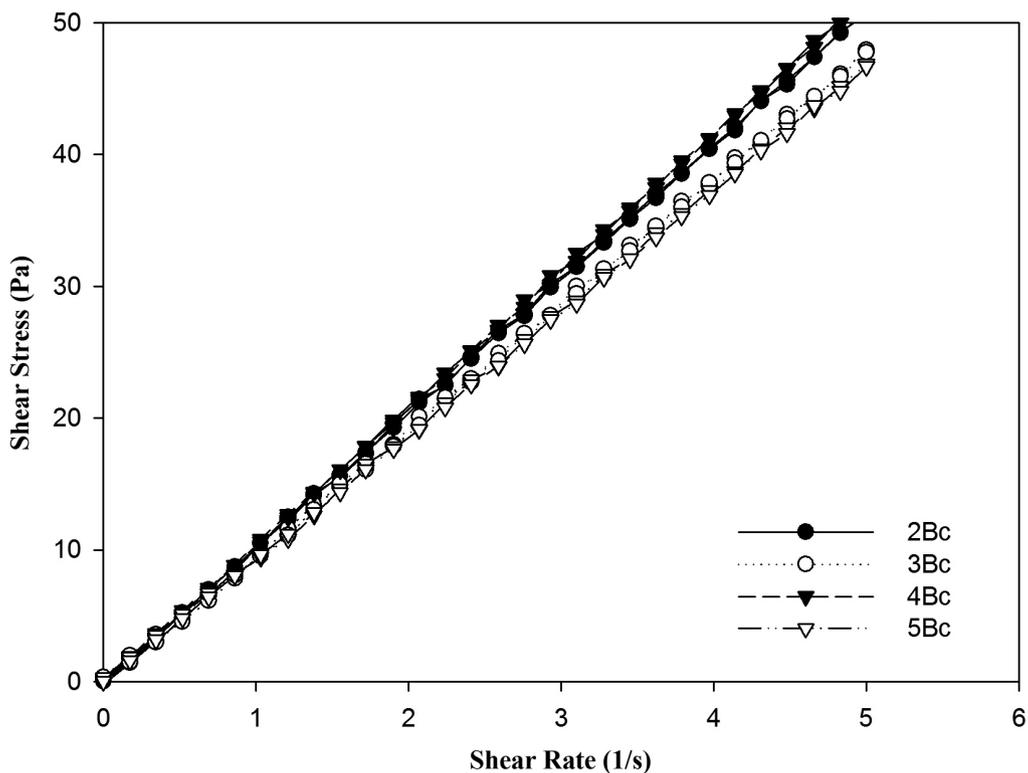


Figure 2. Flow curve of the selected microemulsions of *Oenocarpus bacaba* oil before and after the accelerated stability test, considering only the microemulsions that did not differ significantly before and after the test.

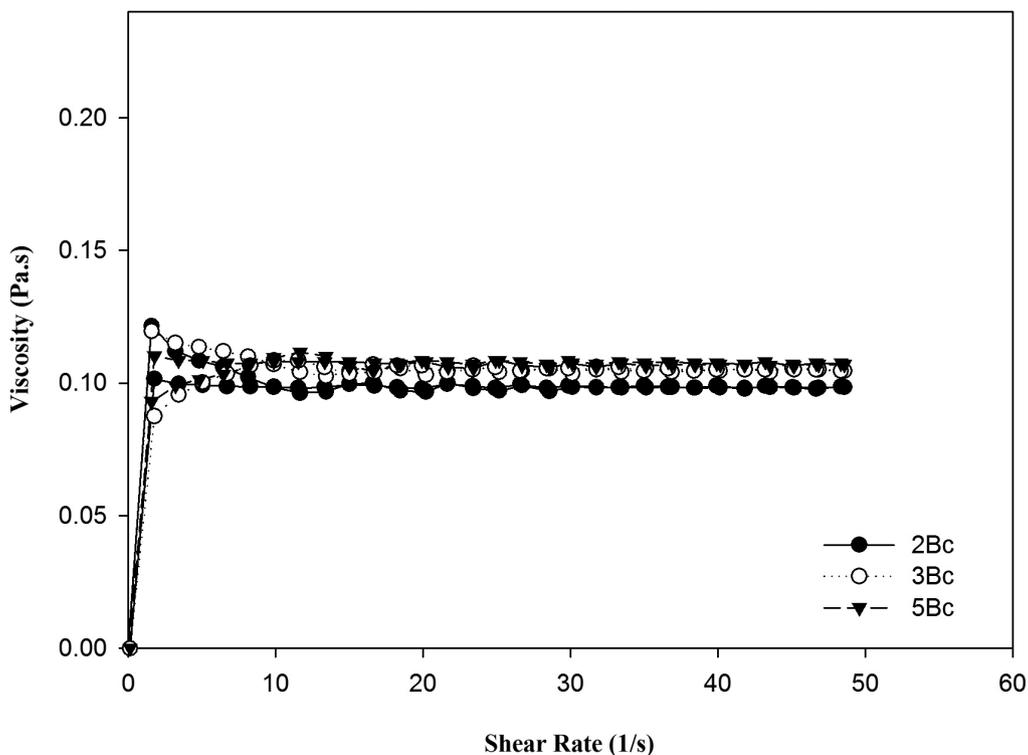


Figure 3. Viscosity curve of the microemulsions of *Oenocarpus bacaba* oil before and after the accelerated stability test, considering only the microemulsions that did not differ significantly before and after the test.

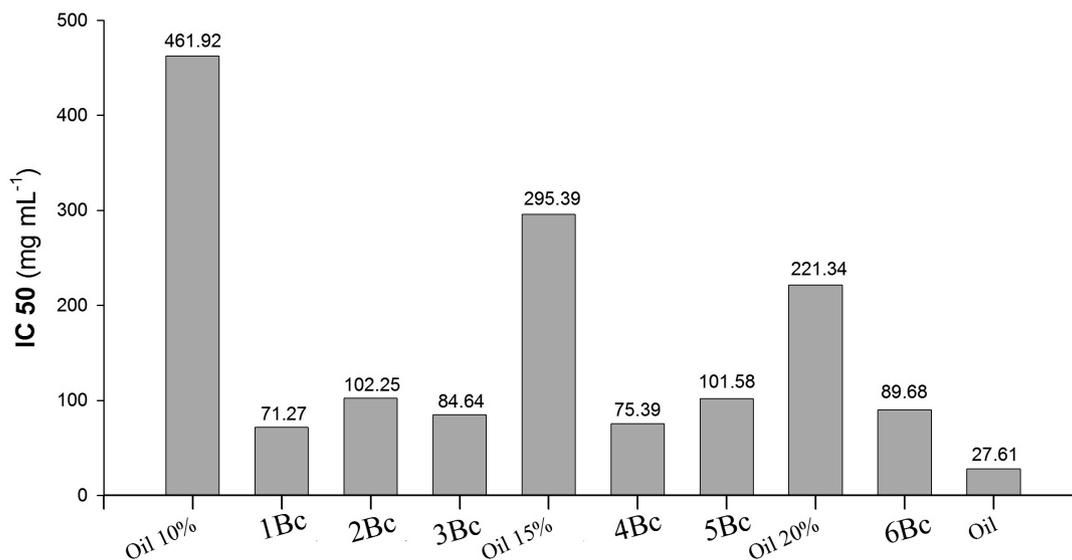


Figure 4. Potential antioxidant activity (IC₅₀) of the microemulsions of *Oenocarpus bacaba* oil approved in the stability tests, compared with the values for pure oil and its dilutions (10, 15 and 20%).

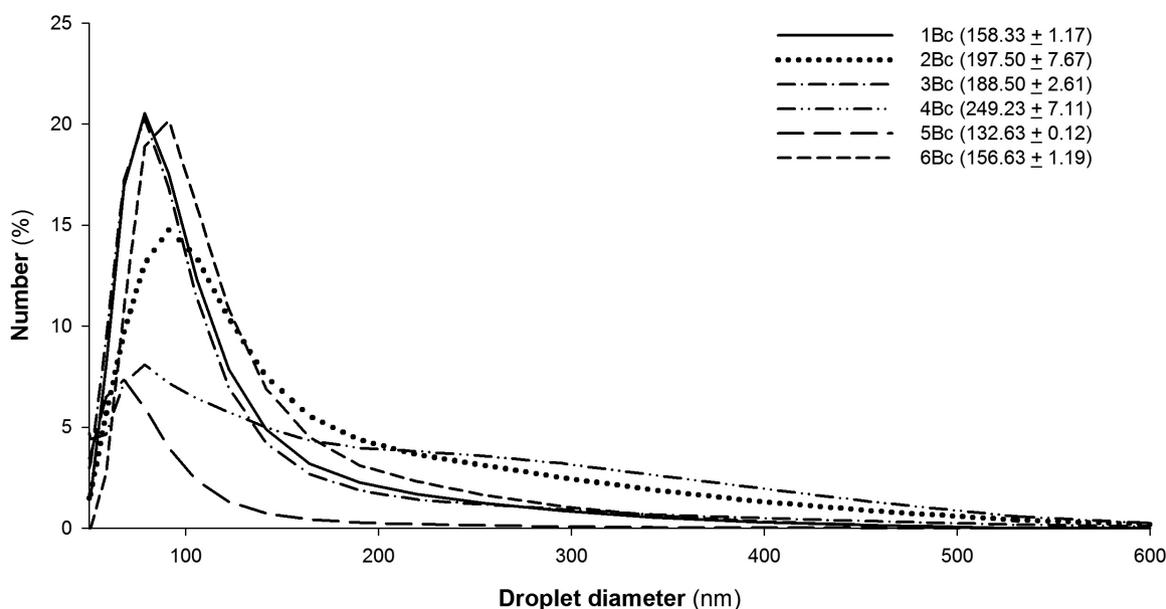


Figure 5. Droplet hydrodynamic diameter of the microemulsions of *Oenocarpus bacaba* oil approved in the stability tests. In the graph legend, values in brackets after the microemulsion code are the mean followed by the standard deviation.

DISCUSSION

The HLB obtained for bacaba oil in our sample was the same as that for olive oil (Frangé and Garcia 2009), which may be due to a similarity in fatty acid composition of both oils, with oleic acid in largest proportion (Santos *et al.* 2017; Wani *et al.* 2018).

The high surfactant concentration in the selected formulations coincides with that employed in other studies

of microemulsion prospection (Ribeiro *et al.* 2015; Boonme *et al.* 2006; Prajapati *et al.* 2012; Fiori *et al.* 2017). The high proportion of surfactant results from the need to reduce the surface tension between the oily and aqueous phase, to enable smaller droplet sizes and provide stability to the formulation, the basic characteristics of viable microemulsions (Fanun 2012; Pessoa *et al.* 2015).

The formulations were characterized as O/W microemulsions, because $1.3 \mu\text{Scm}^{-1}$ is the electrical

conductivity of water, and the values above represent water as the outside phase in the microemulsion system (Pessoa *et al.* 2015; Lanes *et al.* 2016).

The range of pH values in our formulations are indicative of stability, since variability in this parameter can indicate the presence of impurities, hydrolysis and/or decomposition of the system constituents, which cause instability (Pianovski *et al.* 2008). Moreover, pH 7 is considered the most adequate to different routes of administration (Franco and Bochi 2013). Likewise, the constance of our refraction indices over time are indicative of stable, translucent and homogeneous microemulsion systems, since unstable and inhomogeneous mixtures are usually turbid (Ferreira *et al.* 2006).

The Newtonian behavior of our formulations corroborates other studies on microemulsions containing oil extracted from natural products (Fiori *et al.* 2017; Torres *et al.* 2018). Microemulsions with a Newtonian profile tend to show stability over a long period of time (Ribeiro *et al.* 2015; Pessoa *et al.* 2015; Lanes *et al.*, 2016), however, the composition of the system may influence this characteristic in the formulations, since other studies with microemulsions containing different surfactants resulted in a non-Newtonian profile (Jiao and Burgees 2003).

The droplet size in our formulations (in the order of nanometres) was consistent with that obtained in other studies (Fiori *et al.* 2017; Torres *et al.* 2018).

The potential antioxidant activity of our pure oil was similar to that of açai (*Euterpe oleraceae*) oil (Rufino *et al.* 2011). The higher activity of the formulations relative to the oil dilutions was probably due to the incorporation of the oil into the formulation, which may influence its behavior (Hamed *et al.* 2012; Chaiyana *et al.* 2014). Although the antioxidant activity of the formulations was higher than that of the diluted oil, it was not as high as that of microemulsions with olive oil (Chaiyana *et al.* 2016). The choice of the carrier can either increase or decrease the potential antioxidant activity of the microemulsion, thus the choice of surfactant is important, since these substances may or may not present antioxidant activity when isolated (Kim *et al.* 2009; Pérez-Rosés *et al.* 2014).

CONCLUSIONS

Bacaba oil microemulsions developed with a greater amount of water (2Bc, 3Bc and 5Bc) were stable and increased the antioxidant potential of bacaba oil when compared with their respective oil dilutions. The water amount may facilitate the incorporation of hidrofilic substances to the microemulsion. The three selected bacaba oil microemulsions have potential as carriers in the pharmaceutical, cosmetic, food and/or agriculture industry.

ACKNOWLEDGMENTS

The financial support from the Brazilian agencies CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMAT (Fundação de Amparo à Pesquisa do Estado de Mato Grosso; process nr. 224179/2015) is gratefully acknowledged.

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RECEIVED: 30/07/2018

ACCEPTED: 05/05/2019

ASSOCIATE EDITOR: Valdir Veiga Junior



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