

Development, Characterization and Stability of Bacaba Peel Extract Microemulsion Systems

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ABSTRACT

Bacabeira (Oenocarpus bacaba MART.), a palm tree native from Amazon, has flavonoids found in its fruit peel with antioxidant activity, interesting to incorporate and stabilize in formulations in order to create drug vehicles with advantages. Thus, the aim of this work was to incorporate bacaba peel extract in microemulsions to assess its stability. All formulations contained pre-established quantities of distilled water, capric/caprylic triglycerides, Span 80® and Tween 80®, with 5 % of extract at 0.1 g mL-1 added. From the 72 tested formulations, eight produced microemulsions and were subjected to centrifugation, physicochemical characterization and stability studies, with four sustaining their physical stability. All samples showed Newtonian profile and linear viscosity. The droplets size did not exceed 325 nm of hydrodynamic diameter. Test for antioxidant activity showed satisfactory. Therefore, it was possible to develop microemulsions from peel bacaba with stability that is suitable for their use as drug vehicles.

Keywords: Bacaba; Stability Study; Microemulsion.

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B razil has a notable biodiversity, with new species discovered every year (Andrade 2006). The Amazon region is highlighted due its important scenario for bio prospecting, once the last decade was marked by the consciousness of the importance of natural sources and their use, and it was noticed an increase in the number of native plants in that region used as food, in the traditional medicine, in search for new drugs, in the cosmetic industry, to obtain biofuel, and others (Escriche et al. 1999; Oliveira et al. 2006). In this context, stand out the fruits, although accessible for the regional consumption, their commercial use is least or absent. Nutritional and health attributes of many of these fruits are unexplored and their scientific research is yet insufficient (Sousa et al. 2016).

In the fruit abundance around Amazon region there is bacabeira (Oenocarpus bacaba Mart.) that produces an eatable purple fruit, called bacaba, which is locally consumed as natural juice or processed as beverages, jams and ice creams (Finco et al. 2012). It was attributed to its fruit peel antioxidant activity due to the presence of phenolic compounds, such as anthocyanin, a flavonoid that acts as chromophore agent in leaves, flowers, fruits and stems, varying among purple, red, pink, orange and blue, what indicates to be responsible for its purple color (Canuto et al. 2010; Finco et al. 2012; Teixeira et al. 2008).

Antioxidant compounds extracted from plants, as anthocyanins, are of great interest to protect the skin from damages caused by excessive solar radiation, such as premature aging and skin cancer, besides decreasing the risk of developing some non-communicable chronic diseases, when ingested (Nichols & Katiyar 2010; Panapisal et al. 2012; Silva et al. 2010; Svobodová et al. 2003). Thereby, the nutritional, pharmaceutical and cosmetic potential regarding this fruit become interesting due to its popular use.

The seek for new vehicles, in a sense of establishing alternatives for the delivery of active ingredients, solubilization of lipophilic active ingredients, as well the increase of physical and chemical stability of labile molecules and minimizing side effects have been increasing in order to overcome difficulties of administration of bioactive molecules and to create a vehicle for pharmaceutical formulations (Gusmann et al. 2017; Tiuman et al. 2011). In this context, studies (Leanpolchareanchai et al. 2014; Panapisal et al. 2012; Zorzi et al. 2016) have been emerging in an attempt to carry plant extracts in microemulsions to incorporate and stabilize antioxidants, as the phenolic compounds, for topic application.

Microemulsions are characterized as oil and water dispersions, stabilized by surfactants and cosurfactants that form an interfacial film, they are thermodynamically stable, macroscopically homogeneous and translucent (Fanun 2012; Fiori et al. 2017; Lawrence & Rees 2000). Their advantages

as systems for topical drug administration are: high stability, higher drug solubility, easy manufacture and improvement of percutaneous penetration of molecules (Date et al. 2006; Panapisal et al. 2012).

Therefore, the use of microemulsions may be an alternative formulation as a carrier for delivery of different actives principles. Then, the aimed of this work was to develop microemulsions containing bacaba peel extract and to evaluate its stability.

EXPERIMENTAL

BACABA PEEL EXTRACT

All fruits were picked in the rural area of Sinop – MT, they were washed, sanitized and rinsed, and therefore they were submerged in ultra pure water at 40 °C for 40 minutes in order to manually remove the peels, which were further kept in oven with forced circulation of air at 40 °C for 24 hours to remove the water. Furthermore, the peels were milled and stocked in freezer at -20 °C.

The bacaba peel extracts were prepared with two different solvents: ethanol 70 % (v/v) and propylene glycol. The extraction was done using 1 g of peel for every 25 mL of solvent (1:25) and left standing still for 24 hours in the fridge. Afterwards, the extracts were filtered and stocked in the refrigerator.

SYSTEM COMPOSITION

For the development of the formulations it was used: distilled water, capric/caprylic triglyceride (TCC, Henrifarma®, Brazil), sorbitan monooleate – Span 80® (SP, Sigma-Aldrich®, Brazil), polysorbate 80 – Tween 80® (TW, Synth®, Brazil) and bacaba peel extract at 0.1 g mL-1.

DEVELOPMENT OF THE MICROEMULSION SYSTEMS

All formulations were prepared using pre-established amounts of distilled water, TCC, SP and TW, with concentrations ranging from 10 to 80 %. Afterwards, 5 % of bacaba peel extract was added to all formulations.

After 72 hours of preparation at 25 °C, all formulations were visually classified as microemulsion (ME), liquid emulsion (LE), gel emulsion (GE) and phase separation (PS). With this data it was built a pseudo-ternary phase diagram through SigmaPlot version 8.0 software.

From the pseudo-ternary phase diagram it was determined the amount of TCC, SP and TW that formed the microemulsion (ME) area, object of this study.

PHYSICOCHEMICAL CHARACTERIZATION

All formulations that formed ME were preselected and subjected to physicochemical characterization after 24 h of their preparation. Aliquots were centrifuged at 3600 rpm with Quimis® centrifuge (Brazil) at room temperature, and then assessed their pH, electric conductivity and refractive index (Fanun 2012; Gustmann et al. 2017).

STABILITY STUDY

Due to evaluate the preliminary stability, all samples were exposed to alternate cycles of temperature, at 5 ± 1 °C and 40 ± 1 °C, every 24 h and after 14 days of cycles it was identified the thermally stable systems. The accelerated stability trials were performed with the most stable formulations from the preliminary stability study, being exposed to different temperatures (5 ± 1 °C, 25 ± 1 °C and 40 ± 1 °C) for 90 days, having their physicochemical properties evaluated every 30 days. All trials were in triplicate.

RHEOLOGICAL CHARACTERIZATION

The rheological characterization was performed by Modular Compact Rheometer – MCR 102 (Anton Paar®, Germany) with 600 μ L of each ME placed on the reading surface. During the readings, TruGapTM supported at 0,099 mm continually controlled the space measurement. T-ReadyTM conquered the accurate temperature control, and the measurement cell was a Toolmaster TM CP 50. All data and graphs were managed by SigmaPlot 8.0 software. Established parameters, fundamentals of shearing stress control (τ) with 0-5 Pa for the ascending curve and 5-0 Pa for the descending curve were

used to build the flow and viscosity curves. It was performed 75 readings per test, at 25 °C and isothermal conditions.

DYNAMIC LIGHT SCATTERING (DLS)

Dynamic light scattering (DLS) technic was used to evaluate the particles average size of the ME formulations, with a colloidal suspension of the samples prepared from the formulation dilution in deionized water. The readings were obtained by Zetasizer Nano ZS (Malvern®, United Kingdom) at 632.8 nm.

DETERMINATION OF THE ANTIOXIDANT POTENTIAL BY DPPH (2,2-DIPHENYL-1-PICRYLHYDRAZYL) METHOD

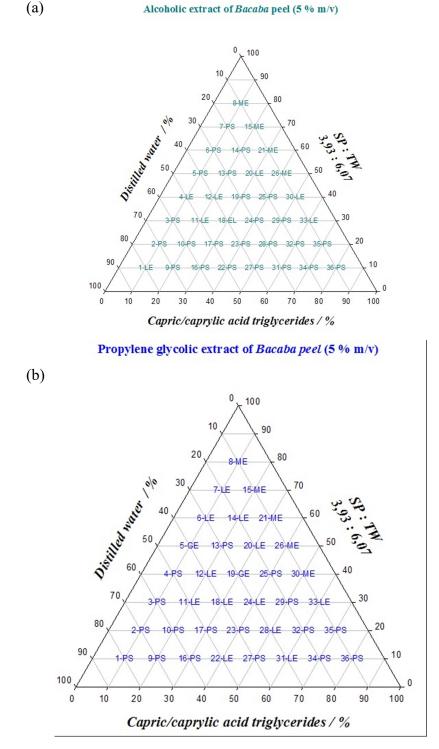
The antioxidant activity of the microemulsion systems was performed through the DPPH (2,2diphenyl-1-pricylhydrazyl) free radical method with reading in quartz (1 cm) in spectrophotometer (PG Instruments Ltd, T80 UV/VIS). The samples were serially diluted (10 – 50 mgmL-1) in methanol for the test. The DPPH radical inhibition percentage was calculated by the equation: % DPPH inhibition = $[(A0 - A1)/A0 \times 100]$, with A0 as standard absorbance and A1 as sample absorbance. For the determination of the ME concentration needed to reduce in 50 % the DPPH (IC50) radical, data were assembled in linear regression to obtain the line equation (Elmasta et al. 2006; Li et al. 2012).

RESULTS AND DISCUSSION

For the development of the systems, it was initially calculated the ideal surfactant proportion, according to the Hydrophilic Lipophilic Balance (HLB) of the formulation's oily phase, the TCC presents HLB of 10.8, with SP/TW of 3.93:6.07 as the adequate proportion of these surfactants. Once established surfactants, TCC, aqueous phase and bacaba peel extract at 5 % (in alcohol 70 % and propylene glycol), a total of 36 formulations were prepared for each solvent used in the extracts.

The pseudo-ternary phase diagrams used to classify macroscopically the formulations are in Figure 1. From the proportions given by the diagrams it was obtained a total of 72 formulations with conflicting balance characteristics.

Alcoholic extract of Bacaba peel (5 % m/v)



Source: Author

Figure 01. Pseudo-ternary diagrams of the 72 formulations in pre-established proportions of TCC, distilled water, surfactants and ethanolic extract 70 % (a), and propylene glycolic extract (b). Micro emsulsion (ME), liquid emulsion (LE), gel emulsion (GE) and phase separation (PS).

From the pseudo-ternary diagrams it was possible to observe the ME domain area, with identification of homogeneous and translucent systems with surfactant mixture concentrations over 40 % (Figure 1). From the 36 formulations with extract prepared in ethanol 70 %, four showed ME features, being called 8MA, 15MA, 21MA and 26MA (Figure 1(a)). The same occurred with the 36 formulations prepared with extract in propylene glycol, with the formulations named 8MP, 15MP, 21MP and 30MP (Figure 1(b)).

All nine formulations selected according to the ME area from the pseudo-ternary diagrams were subjected to centrifugation. The ME 8MA, 15MA, 21MA, 26MA, 8MP, 15MP, 21MP and 30MP showed macroscopic stability, remaining translucent and homogeneous, being considered normal (N) and 26MP showed phase separation. The results of the preliminary stability trials of the formulations in the beginning and at the end of 14 days are in Table 1.

	Preliminary Stability		
	Centrif	fugation	
Formulation	Time (days)		
	0	14	
8 MA	Ν	Ν	
15 MA	Ν	Ν	
21 MA	Ν	PS	
26 MA	Ν	PS	
8 MP	Ν	Ν	
15 MP	Ν	Ν	
21 MP	Ν	PS	
26 MP	PS	-	
30 MP	Ν	PS	
	р	Н	
Formulations	Time	(days)	
	0	14	
8 MA	6.94 ± 0.05	6.66 ± 0.08	
15 MA	6.89 ± 0.17	6.73 ± 0.10	
21 MA	6.83 ± 0.13	-	
26 MA	6.89 ± 0.10	-	
8MP	6.92 ± 0.04	6.64 ± 0.12	
15MP	6.82 ± 0.10	6.78 ± 0.08	
21 MP	6.78 ± 0.12	-	
30MP	6.74 ± 0.04	-	
	Conductiv	ity (µScm ⁻¹)	
Formulation	Time (days)		
	0	14	
8 MA	7.18 ± 1.47	5.80 ± 0.04	
15 MA	4.12 ± 0.10	2.52 ± 0.73	
21 MA	5.99 ± 0.66	-	

Table 01. Physicochemical parameters of the preliminary stability study of the formulations

26 MA	10.98 ± 1.41	-	
8 MP	4.61 ± 0.70	3.92 ± 0.51	
15 MP	1.83 ± 0.57	2.03 ± 0.13	
21 MP	3.59 ± 0.17	-	
30 MP	4.31 ± 0.03	-	

 $*Normal \left(N\right)$ and phase separation (PS).

Source: não sei

After 14 days of the preliminary stability study, the samples 21MA, 26MA, 21MP and 30MP showed visual heterogeneity, being withdrawn from the stability study. The other formulations presented stable pH, with very less variation after the test cycles. The electrical conductivity was sustained along the study, showing that all ME formulations were oil in water systems (O/W), once the value found exceeded the conductivity of the water (>1.3 μ Scm⁻¹).

Formulations 8MA, 15MA, 8MP and 15MP were subjected to accelerated stability study and results are in Table 2.

		p	H	
Formulation	Time (days)			
	0	30	60	90
8MA	7.04 ± 0.01	6.90 ± 0.12	6.93 ± 0.20	6.90 ± 0.11
15MA	7.09 ± 0.09	6.98 ± 0.06	6.99 ± 0.08	6.83 ± 0.04
8MP	7.05 ± 0.09	6.92 ± 0.05	6.90 ± 0.19	6.78 ± 0.25
15MP	7.03 ± 0.03	6.83 ± 0.17	6.92 ± 0.21	6.99 ± 0.52

Table 02. Physicochemical parameters during the accelerated stability study

Conductivity (µScm⁻¹)

Formulation -				
ronnulation -		Time	(days)	
-	0	30	60	90
8MA	5.80 ± 1.05	4.79 ± 1.10	3.99 ± 1.25	2.81 ± 0.02
15MA	5.58 ± 1.19	4.26 ± 1.86	4.06 ± 2.04	2.89 ± 1,24
8MP	2.84 ± 1.10	2.42 ± 0.57	4.07 ± 0.56	3.16 ± 0.27
15MP	2.78 ± 0.25	2.04 ± 0.28	3.27 ± 0.71	1.96 ± 0.34
Formulation	Refractive Index			

	Time (days)			
	0	30	60	90
8MA	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.005
15MA	1.45 ± 0.001	1.45 ± 0.002	1.45 ± 0.002	1.45 ± 0.003
8 MP	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.002	1.45 ± 0.001
15MP	1.45 ± 0.001	1.45 ± 0.001	1.45 ± 0.002	1.45 ± 0.00

Source: Author

At the end of the accelerated stability study the ME formulations showed themselves stable, with slight pH variation, kept between 6.78 and 7.09, suggesting no decomposition reactions. The conductivity of samples 8MA and 15MA decreased, most likely due to the evaporation of interstitial water (Bumajdad & Eastoe 2004), although there was no phase inversion. Samples 8MP and 15 MP showed mild conductivity variation, maintaining stable. Concerning the refractive index, there was no change during the test, remaining 1.45 for all formulations, supporting the stability of the systems. Therefore, in spite of the low variation of conductivity in some samples, the systems were stable, homogeneous and translucent throughout the stability study.

For the rheological characterization, flow and viscosity curves were used as a function of the shear rate (τ) (Figure 2). According to the flow curves (Figure 2(a)), it was possible to determine the Newtonian behavior of the formulations, due to the fact that the curves began at the origin and exhibited linear ascending and descending behavior. In addition, the viscosity curves (Figure 2 (b)) showed no variation as the shear rate increased, with values between 0.15 and 0.30 Pas, confirming the Newtonian behavior of the systems.

The tendency of the systems to Newtonian profile, sustained viscosity, even with shear rate increased, and the stability studies indicate a physical stability of the ME formulations (Gustmann et al. 2017). This behavior is in agreement with other authors (Cotrim et al. 2016; Gustmann et al. 2017; Ribeiro et al. 2015).

Lately, ME systems have become a practical platform of drug delivery in improving the target specificity, its therapeutic activity and reducing toxicity, highlighting the great potential of ME as a vehicle for a variety of drugs due to its several polarity domains (Fanun 2012). That way, obtaining systems with physical stability for their use as drug vehicles becomes of great importance in developing new products that may contribute with controlled and/or sustained release of active ingredients.

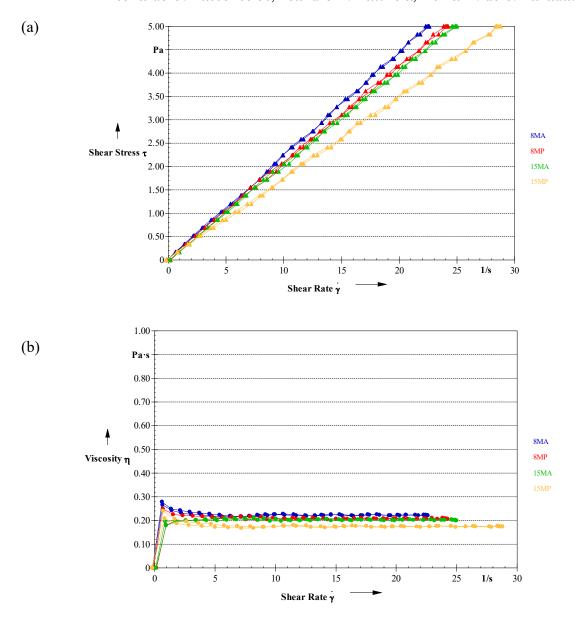


Figure 02. Flow (a) and Viscosity (b) curves of bacaba peel extract microemulsions (8MA, 15MA, 8MP and 15MP)

The droplet size and the distribution of the particle size of the ME internal phase were determined by dynamic light scattering (DLS) (Fryd & Manson 2012), and the droplets hydrodynamic diameter (HD) of formulations 8MA, 15MA, 8MP and 15MP were respectively 324.73; 300.07; 287.63 and 322.20 nm (Figure 3), and showed themselves independent of the constituents proportion, except formulation 8MP (p = 0.0053). The polydispersivity index (PDI) of the formulations was 0.35 + 0.01 (p = 0.1526), indicating homogeneity of the formulations' droplets.

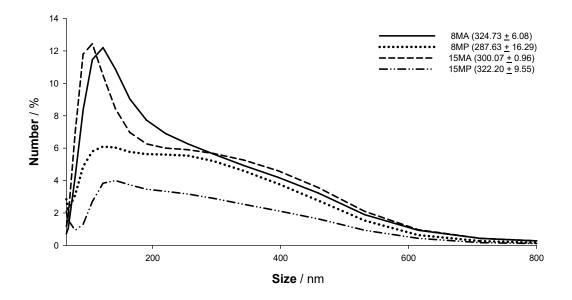


Figure 3. Hydrodynamic diameters of bacaba peel extract ME.

The droplets size was larger than ME from other studies (Fiori et al. 2017; Gustmann et al. 2017) that did not exceed 150 nm, however their HD maintained a nanometric scale, indicating that the systems were thermodynamically stable and were formed spontaneously (Constantinides 1995).

Short chain alcohols are usually added to several microemulsion systems as co-surfactants, with butanol as one of the best for ME formulations (Boonme et al. 2006; Kumar et al. 2014). In the developed systems it was not used a specific co-surfactant, however the extracts were prepared in alcohol, contributing to the ME formation by interacting with the surfactants and improving the interfacial film flexibility.

The results of antioxidant activity trials were expressed in IC_{50} , and ME 8MA, 15MA, 8MP and 15MP presented 62.12, 63.26, 137.76 and 118.60 mgmL⁻¹, respectively. Therefore, it is clear that systems with ethanol 70 % extract showed a better antioxidant activity, with lower amount of ME needed to reduce DPPH in 50 % than the systems containing propylene glycol extract. Ethanol in combination with water, due to their non-polar characteristic, are the solvents of choice for anthocyanin extraction (Araújo 2011), what may explain why ME with ethanol 70 % extract showed better antioxidant activity than ME with propylene glycol extract, in other words, ethanol 70 % extract was able to extract, incorporate and stabilize larger amount of antioxidants than propylene glycol extract.

The development of stable systems to incorporate and deliver drugs offers advantages, given that it allows active ingredients incorporation and cooperates preventing diseases for their antioxidant

activities (Fontes 2009; Nichols & Katiyar 2010; Panapisal et al. 2012; Svobodová et al. 2003). Its thermodynamic stability offers advantages over the unstable dispersions, such as suspensions and emulsions, having a way longer lifetime. Therefore, the development of microemulsion systems with potential antioxidant activity becomes a good choice for drugs and/or cosmetics incorporation in pharmaceutical and cosmetic industries.

CONCLUSION

The development of microemulsion systems with bacaba peel extract showed potential antioxidant activity, which combined with their physical and thermodynamic stabilities may turn them into drug or cosmetic active ingredients carrier. It was also observed that systems developed in ethanol 70 % showed higher antioxidant activity attributed to the greater affinity of anthocyanin to ethanol during its extraction, being the best option for the development of cosmetics and medicines.

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Desenvolvimento, Caracterização e Estabilidade de Sistemas Microemulsionados Contendo Extrato de Casca de Bacaba

RESUMO

Bacabeira (Oenocarpus bacaba MART.), uma palmeira nativa da Amazônia, possui flavonóides encontrados na casca de seu fruto com propriedades antioxidantes, interessantes para incorporar e estabilizar em formulações no intuito de criar veículos de fármacos com benefícios. Portanto, objetivo deste trabalho foi incorporar extrato de casca de bacaba em microemulsões para avaliar sua estabilidade e potencial antioxidante. As formulações contêm quantidades pré-estabelecidas de água destilada, triglicerídeo caprico/caprílico, Span 80® e Tween 80® sendo adicionados 5 % do extrato a 0.1 g mL-1. Das 72 formulações testadas, oito formaram microemulsões sendo submetidas à centrifugação, caracterização físico-química (pH, condutividade elétrica e índice de refração) e estudos de estabilidade, na qual quatro mantiveram sua estabilidade física. As amostras apresentaram perfil newtoniano e

viscosidade linear. O tamanho das gotículas não passou de 325 nm de diâmetro hidrodinâmico. Seu potencial antioxidante se demonstrou satisfatória. Portanto, os sistemas apresentaram estabilidade e potencial antioxidante, podendo ser utilizados para veiculação de fármacos.

Palavras-Chave: Bacaba; Estudo de Estabilidade; Microemulsão

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