

Original Article

# The potential antioxidant activity of incorporating bacaba (*Oenocarpus bacaba* Mart.) extract into a nanoemulsion system with baru oil

A potencial atividade antioxidante da incorporação do extrato casca de bacaba (*Oenocarpus bacaba* Mart.) em um sistema nanoemulsionado com óleo de baru

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## Abstract

The bacaba (*Oenocarpus bacaba* Mart.) peel corresponds to 15% of the whole fruit and is rich in antioxidants with potential application in product development. In nanotechnology, emulsified formulations such as nanoemulsions stand out for providing modified release and improving the bioavailability of conveyed substances. The aim of this work was to develop nanoemulsified systems from baru oil containing hydroalcoholic extract from the bacaba peel, evaluate their stability and antioxidant potential. After the HLB (Hydrophilic-lipophilic balance) determination of the baru oil, thirty-two formulations were developed, varying the proportions of surfactants, aqueous phase, and baru oil. Of those 32, 16 formed emulsified systems, and the ones with a higher amount of oil (20%) were incorporated with the BPE. The systems were submitted to stability studies to verify their viability. After that, several tests were performed, such as rheological characteristics, hydrodynamic diameter of the droplets, polydispersion index, zeta potential, and antioxidant potential by DPPH and ABTS<sup>•</sup> radical scavenging methods. After the studies, two samples remained stable and presented a non-Newtonian pseudoplastic profile with thixotropy, hydrodynamic diameter of less than 200 nm, monodispersity, and negative zeta potential. The BPE showed antioxidant potential, with superior activity when incorporated into the nanoemulsified system. A strong negative correlation was found between the two antioxidant methods, where both demonstrated the same profile of potential antioxidant activity for the extract and formulations. The studied formulation showed that the use of BPE is a viable alternative for the development of new products based on sustainable technologies.

**Keywords:** antioxidant potential, *dipteryx alata* vog., nanotechnology, *oenocarpus bacaba* mart., stability.

## Resumo

A casca da bacaba (*Oenocarpus bacaba* Mart.) corresponde a 15% de seu fruto sendo rica em compostos antioxidantes com potencial aplicação no desenvolvimento de produtos. Na nanotecnologia, formulações emulsionadas como as nanoemulsões destacam-se por fornecer a liberação modificada e melhorar a biodisponibilidade das substâncias veiculadas. O objetivo do trabalho foi desenvolver sistemas nanoemulsionados a partir do óleo de baru contendo extrato hidroalcolólico de casca de bacaba (BPE), avaliar sua estabilidade e potencial atividade antioxidante. Após a determinação do EHL (Equilíbrio hidrófilico-lipófilico) do óleo de baru, 32 formulações foram desenvolvidas variando as proporções de tensoativos, fase aquosa e do óleo de baru, sendo que 16 formaram sistemas emulsionados e aos com maior quantidade de óleo (20%) incorporou-se o BPE, preparado em álcool 70% (m/v) na proporção de 1:2 (m/v). Os sistemas foram submetidos aos ensaios de estabilidade para verificar sua viabilidade e, após isso, determinaram-se as características reológicas, diâmetro hidrodinâmico das gotículas, índice de polidispersão, potencial zeta e o potencial antioxidante pelos métodos de eliminação dos radicais DPPH e ABTS<sup>•</sup>. Após os estudos, 2 amostras mantiveram-se estáveis e apresentaram perfil não-Newtoniano pseudoplástico com tixotropia, diâmetro hidrodinâmico inferior a 200 nm monodispersividade e potencial zeta negativo. O BPE apresentou potencial antioxidante, com atividade superior quando incorporado ao sistema nanoemulsionado. Uma correlação negativa entre os dois métodos foi verificada, o que demonstraram o mesmo perfil de potencial atividade antioxidante para o extrato e formulações. A formulação desenvolvida mostrou que o aproveitamento do BPE é uma alternativa viável para o desenvolvimento novos produtos baseados em tecnologias sustentáveis.

**Palavras-chave:** potencial antioxidante, *dipteryx alata* vog., nanotecnologia, *oenocarpus bacaba* mart., estabilidade.

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## 1. Introduction

The industrial processing of fruits and vegetables is a potential producer of food by-products of limited commercial exploration, which results in economic and environmental problems. However, many of these by-products have nutritional value and may present bioactive compounds that perform important biological activities (Trigo et al., 2020). One of the ways to make use of these residues is to utilize them as raw materials in the development of different products, diminishing environmental impact and economic losses and adding value to a by-product that would be discarded.

Currently, the search for natural antioxidants derived from plants has generated a growing interest among consumers and the scientific community, due to their low toxicity when compared to synthetic antioxidants (Comunian et al., 2021). In this sense, bacaba (*Oenocarpus bacaba* Mart.) emerges as a potential option. Native to the Amazon biome, the bacaba fruit is used in the preparation of beverages by processing its pulp, leaving the peel as a by-product. This peel is considered a source of antioxidants and can be applied in different industrial fields, such as food, pharmaceuticals, and cosmetics (Santos et al., 2021; Baldissera et al., 2023). The problem with antioxidant compounds lies on their stability, which is influenced by factors such as light, temperature, and pH, which can lead to degradation, making their use difficult (Corrêa et al., 2020).

Nanotechnology is a promising tool used in the development of versatile and effective formulations, such as nanoemulsions (NEs), which contribute to the stabilization of different bioactive compounds and can increase their antioxidant activity and prolong their release, increasing the effectiveness and stability of the product (Manikanika and Jaswal, 2021).

Thus, the development of nanoemulsified systems containing the bacaba peel extract turns to be a promising alternative in the food, pharmaceutical, and/or cosmetic fields through the efficient use of waste as raw material, promoting strategies based on safe, economic, and sustainable technologies. Within this context, the aim of this work was to develop nanoemulsified systems from baru oil containing hydroalcoholic extract from bacaba peel.

## 2. Materials and Methods

### 2.1. Obtaining the baru oil and the hydroalcoholic extract from the bacaba peel

The baru oil was extracted by ultrasound-assisted extraction according to Raiser et al. (2020), analyzed, and provided to the Quality Control Laboratory (LaCQ) at the Federal University of Mato Grosso - UFMT, Sinop Campus.

The bacaba (*Oenocarpus bacaba* Mart.) fruits were collected in the municipality of Sinop - Mato Grosso and taken to the LaCQ where they were sanitized and then submerged in distilled water at 40 °C for 40 minutes to enable manual removal of the peels. The removed peels were kept in a forced convection drying oven at 40 ± 1 °C for 24 hours to remove excess water. After drying, the peels were ground and stored in a freezer at -20 °C until the moment of its use.

The ground bacaba peel was weighed, and an ethanolic solution (70% w/v) was added in the proportion 1:2 (m/v). The ultrasound-assisted extraction was performed on an ultrasonic bath operating at 45 kHz and temperature of 35 °C. The resulting extracts were vacuum filtered using Whatman n° 1 filter paper and concentrated on a rotary evaporator at 50 ± 1 °C and dry extract stored in amber bottle under refrigeration (5 ± 1 °C).

### 2.2. Determination of the hydrophilic-lipophilic balance (HLB)

The HLB determination of the baru oil was performed by preparing a series of emulsions obtained with the surfactants SP (EHL = 4.3) and TW (EHL = 15.0), in order to obtain defined and scaled HLB values (Zanin et al., 2002). After 24 hours of preparation, the emulsions were visually analyzed and the size of the formed globules was determined using the *ImageJ*® software and a microscope (BEL® Photonics, Piracicaba, SP, Brazil) with a 40x objective lens and a 10x micrometer scale eyepiece. (Fiori et al., 2017). The HLB value found for the oil resulted from a mixture of surfactants capable of providing a stable emulsion with the smallest droplet diameter, and was obtained through the following Equation 1:

$$(SP \times HLBSP) + (TW \times HLBTW) = \text{requiredHLB} \quad (1)$$

Where: *SP* = percentage (%) of sorbitan monooleate (Span 80) in the emulsion; *HLB SP* = Span 80 hydrophilic-lipophilic balance value (HLB = 15.0); *TW* = percentage (%) of polysorbate 80 (Tween 80) in the emulsion; *HLB TW* = Tween 80 hydrophilic-lipophilic balance value (HLB = 4.3); *required HLB* = HLB value required by the oil phase.

### 2.3. Systems development

Based on the HLB results for the baru oil, formulations were developed by the construction of the pseudoternary phase diagram, using the point formulation and water titration methods. A total of 36 points were obtained, which, associated with the titration results, provided the region domains (microemulsion, liquid emulsion, gel emulsion, and phase separation). Using the liquid emulsion region as reference, 32 new formulations were prepared by the low-energy phase inversion method, varying the proportion of surfactants between 5 and 40%.

The oily phases composed of baru oil and surfactants, and the aqueous phases were heated separately at 70 ± 1 °C, then the aqueous phase was slowly added to the oily phase under stirring. The systems were stirred in an ultrasonic processor (VCX-130, Sonics & Materials®, Newtown, CT, USA) for 1 hour, the time at which they reached room temperature (25 ± 1 °C) and were left to rest for 24 hours for latter visual classification. The systems were classified as liquid emulsions (LE) and phase separation (PS).

An optical microscope was used to analyze the LE and select the formulations with the smallest globule size, and then incorporate the BPE. The BPE was added to the formulations, at different concentrations (0.5, 1.0 and 1.5% w/w).

For the next tests, the samples were prepared again and divided in two groups, one without the bacaba peel extract (NC) and the other with the extract (NBPE).

#### 2.4. Characterization of the systems

The physicochemical characterization was carried out to verify possible processes of alteration and/or degradation of the systems. The formulations were analyzed after 24 hours of preparation, as well as during the preliminary stability tests and during the accelerated stability. The tests performed were macroscopic evaluation (color, odor, appearance), centrifugation at 3600 rpm for 30 minutes at  $25 \pm 1$  °C, pH determination, electrical conductivity and refractive index (Torres et al., 2019).

#### 2.5. Stability studies

The formulations were submitted to preliminary stability for a period of 14 days, with alternating temperature cycles ( $5 \pm 1$  °C /  $40 \pm 1$  °C) every 24 hours. At the end of the cycles, the samples were left at room temperature for 24 hours to then evaluate the physicochemical parameters. The formulations that showed stability were prepared again and submitted to the accelerated stability test.

For the accelerated stability test, the formulations were subjected to different temperature conditions ( $5, 25, 40 \pm 1$  °C) and exposure to light radiation at  $25 \pm 1$  °C. The formulations were analyzed every thirty days during a period of 90 days. All tests were performed in triplicate (Torres et al., 2019).

#### 2.6. Rheological characterization

The rheological parameters were measured in a rheometer (MCR 102, Anton Paar® GmbH, Ostfildern, BW, Germany) using the Rheoplus V3.61 Software, with permanent control of the measurement gap with a 0.099 mm TruGap™ support, a Toolmaster™ CP 50 measuring cell, and precise temperature control with the T-Ready™ feature. Assays were performed according to Ribeiro et al. (2020), using 600 µL of the formulation placed on the surface of the reading plate. For the flow and viscosity curves, the shear stress ( $\tau$ ) was established to vary from 0 to 5 Pa for the upward curve and from 5 to 0 Pa for the downward curve. These measurements were performed under isothermal conditions at 25 °C, comprising 75 readings per analysis.

#### 2.7. Dynamic light scattering (DLS) and zeta potential ( $\zeta$ )

Mean globule size and polydispersity index (PDI) were determined by dynamic light scattering (DLS), while zeta potential ( $\zeta$ ) was obtained by electrophoretic measurements (Zetasizer Nano ZS – Malvern Instruments®, Malvern, Worcestershire, UK) at a temperature of 25 °C. For both tests, samples were prepared in a 1:100 (v/v) dilution of the formulations with distilled water and evaluated in triplicates.

#### 2.8. Evaluation of antioxidant potential by the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS<sup>•</sup> (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging methods

The antioxidant potential was evaluated by the DPPH and ABTS<sup>•</sup> methods according to the methodology described by Rufino et al. (2007a, b). Stock solutions of bacaba peel extract at a concentration of 200 mgL<sup>-1</sup> and of formulations at a concentration of 4000 mgL<sup>-1</sup> (equivalent to 20 mgL<sup>-1</sup> of extract in the NBPE) were prepared with methanol.

From these solutions, dilutions were prepared to obtain different concentrations for the extract and formulations.

For the DPPH method, a methanolic solution of the free radical (6 mM) was added to extract dilutions in a concentration range from 4.0 to 24 mgL<sup>-1</sup> and from 333.3 to 2000 mgL<sup>-1</sup> for the formulations (corresponding to 1.6 to 10 mgL<sup>-1</sup> of extract in the NBPE). After 30 minutes in the absence of light, the absorbance was read in a spectrophotometer (CIRRUS 80 MB, Femto®, São Paulo, SP, Brazil), at the wavelength of 517 nm, using methanol as blank. A calibration curve was constructed for each formulation, extract, and DPPH. Using the equations provided by the curves, the results were expressed in IC<sub>50</sub> (mgL<sup>-1</sup>) which corresponds to half the maximum inhibitory concentration and in milligram of extract or formulation per gram of DPPH (mgg<sup>-1</sup>).

For the ABTS<sup>•</sup> radical assay, a 7 mM ABTS stock solution prepared with distilled water was mixed with a potassium persulfate solution. The formed radical was diluted in ethanol until obtaining an absorbance of  $0.700 \pm 0.05$  at a wavelength of 734 nm. Then, it was added to the sample dilutions, providing concentration ranges from 1.2 to 3.6 mgL<sup>-1</sup> for the peel extract and from 333.3 to 1666.7 mgL<sup>-1</sup> for the formulations (corresponding to 1.6 to 8.3 mgL<sup>-1</sup> of extract concentration in the NBPE). After 6 minutes of reaction, the spectrophotometric determination was performed at a wavelength of 734 nm, using ethanol as a blank. Antioxidant potential was calculated in relation to the trolox activity, and results were expressed as mM of trolox per gram of extract or formulation (mM Trolox.g<sup>-1</sup>).

A calibration curve of Trolox in the concentration range between 100 and 2000 µM was constructed and presented the line equation  $y = -0.000296x + 0.6130$  and a coefficient of determination of 0.9997. All tests were performed in triplicate.

#### 2.9. Statistical analysis

Test results were represented as mean  $\pm$  relative standard deviation (% RSD). Pearson's correlation coefficient was performed using the software OriginPro, v 8.5 (OriginLab®).

### 3. Results

The baru oil provided by UFMT's LaCQ followed the quality criteria required by the oil legislation (Brasil, 2021).

The dry bacaba peel extract (BPE) was brown colored. Its yield of 1.65% was calculated as the weight of the obtained extract after dried and concentrated.

The baru oil HLB presented a value of 7.0 and the analysis of the emulsion by the *ImageJ*® software revealed an average droplet size of  $0.055 \pm 0.188$  mm<sup>2</sup>.

Out of the 32 developed systems, 16 formed liquid emulsions (LE) (F1 to F16) with a milky white appearance and varying percentages of oil and surfactants. The other 16 presented phase separation (PS). For the incorporation of the BPE, the formulations with 20% of oil, the smallest apparent globule diameter, and concentrations of 5, 10, and 20% of surfactants were selected. These formulations presented globule sizes that couldn't be estimated by the optical microscope.

To incorporate the extract into the formulations, its solubilization was tested in the oil phase and in the aqueous phase at different concentrations. Total solubilization of the extract occurred using 0.5% of the extract in the aqueous phase.

Based on this data, the selected formulations were called NC 1, 2, and 3 (formulations without extract) and NBPE 1, 2, and 3 for those with BPE, which was solubilized in the aqueous phase. The extract incorporation did not change the color of the formulations. NC and NBPE went through preliminary stability tests and the results are shown in (Table 1).

After the 14 days of preliminary stability, only the 1 NC and 1 NBPE formulations remained stable, without changes in their organoleptic characteristics, being prepared again and submitted to the accelerated stability study for a period of 90 days (Table 2).

The NBPE showed phase separation when exposed to light radiation and high temperatures ( $40 \pm 1$  °C) indicating that light and temperature are crucial in its storage. The average pH values at  $25 \pm 1$  °C showed little variation, stabilizing around 4.9 for NBPE and 5.6 for NC. The formulations showed constant values of refractive index over time.

**Table 1.** Parameters evaluated in the preliminary stability test (centrifugation, organoleptic characteristics, pH, electrical conductivity, and refractive index) of the NC (1, 2, and 3) and NBPE (1, 2, and 3) formulations.

Formulation	Days	1		2		3	
		NC	NBPE	NC	NBPE	NC	NBPE
Centrifugation	Before	N	N	N	N	N	N
	After	N	N	PS	PS	PS	PS
Appearance, color, odor	Before	N	N	N	N	N	N
	After	N	N	M	M	M	M
pH	Before	6.37	4.77	5.95	5.00	5.83	5.10
	After	6.33	4.77	-	-	-	-
Electrical conductivity ( $\mu\text{Scm}^{-1}$ )	Before	142.9	585.1	116.7	528.2	107.6	439.1
	After	114.9	456.0	-	-	-	-
Refractive index	Before	1.381	1.378	1.391	1.390	1.399	1.399
	After	1.378	1.383	-	-	-	-

N = Normal; M=Modified; PS = Phase Separation; (-) = excluded from the trial.

**Table 2.** Physicochemical parameters evaluated during the accelerated stability test of the NC and NBPE formulations.

Samples	D	Temp. °C	Color, odor	pH	RI	EC ( $\mu\text{Scm}^{-1}$ )	LR	
NC	1	25 ± 1	N	5.97 ± 0.34	1.375 ± 0.254	84.0 ± 2.99	N	
		30	5 ± 1	N	6.31 ± 1.28	1.375 ± 0.230	96.8 ± 3.61	
			25 ± 1	N	5.70 ± 0.35	1.374 ± 0.115	86.8 ± 0.46	N
	60		40 ± 1	N	5.27 ± 0.37	1.375 ± 0.208	97.5 ± 0.97	
			5 ± 1	N	6.33 ± 0.79	1.375 ± 0.063	91.0 ± 4.12	
			25 ± 1	N	5.41 ± 2.22	1.374 ± 0.001	115.1 ± 0.63	N
	90		40 ± 1	N	5.14 ± 0.89	1.375 ± 0.026	102.0 ± 2.83	
			5 ± 1	N	6.17 ± 1.25	1.378 ± 0.098	103.0 ± 1.65	
			25 ± 1	N	5.44 ± 3.80	1.378 ± 0.194	129.1 ± 3.11	N
NBPE	1	40 ± 1	N	4.96 ± 1.92	1.379 ± 0.406	114.1 ± 2.11		
			25 ± 1	N	5.00 ± 0.01	1.375 ± 0.200	514.4 ± 0.46	N
			5 ± 1	N	5.00 ± 0.30	1.374 ± 0.125	502.8 ± 0.25	
	30		25 ± 1	N	4.99 ± 0.20	1.375 ± 0.158	501.2 ± 0.54	M
			40 ± 1	M	-	-	-	
			5 ± 1	N	4.93 ± 0.40	1.374 ± 0.033	415.3 ± 0.66	
	60		25 ± 1	N	4.87 ± 1.74	1.374 ± 0.066	435.1 ± 0.17	-
			40 ± 1	-	-	-	-	
			5 ± 1	N	4.87 ± 0.47	1.374 ± 0.131	452.5 ± 0.09	
90		25 ± 1	N	4.80 ± 1.73	1.374 ± 0.252	450.0 ± 0.28	-	
		40 ± 1	-	-	-	-		
		5 ± 1	-	-	-	-		

D = Days; Temp.= Temperature; RI = Refractive index; EC = electrical conductivity; LR = Light radiation; N = Normal; M = modified; (-) = Excluded from the assay. Values expressed as mean ± relative standard deviation (n = 3).

Changes in electrical conductivity values were observed for all formulations, however, the average conductivity values remained above  $1.3 \mu\text{Scm}^{-1}$  after 90 days of tests, classifying the formulations as oil-in-water (O/W) type systems.

The rheological characteristics of NC and NBPE were determined considering the flow curves (Figure 1) and viscosity (Figure 2) as functions of the shear rate.

The flow curves begin at their origin and present nonlinear upward and downward behavior, indicating that the formulations behave as non-Newtonian pseudoplastic fluids, with thixotropic characteristics. The viscosity curves presented a decreasing behavior with the increase of shear rate.

The results of globules size, PDI, and zeta potential showed that the formulations with and without extract had a hydrodynamic globules diameter of 142.9 and 198.0 nm, PDI of 0.37 and 0.32 with zeta potential of  $-34.7$  and  $-35.1$  mV, respectively.

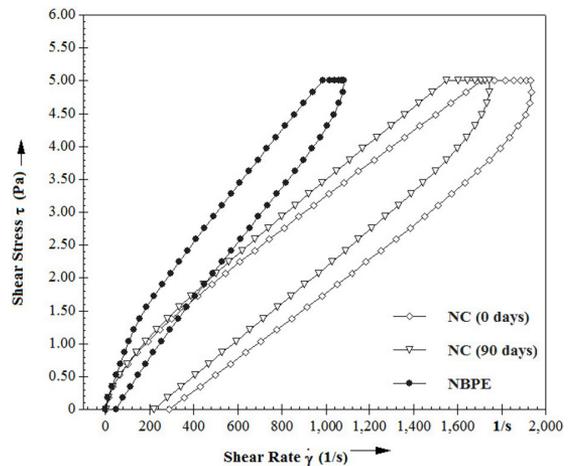
The results of the antioxidant potential for the BPE and the NC and NBPE formulations by the DPPH and ABTS<sup>+</sup> radical scavenging methods are shown in (Table 3). BPE showed antioxidant activity, while NC did not reduce DPPH and ABTS<sup>+</sup> radicals, showing no activity. In both assays, NBPE showed better antioxidant activity than the extract.

Pearson correlation coefficient for the DPPH and ABTS<sup>+</sup> assays showed a strong negative correlation between  $\text{IC}_{50}$  ( $\text{mgL}^{-1}$ ) and mM trolox per gram of extract/formulation ( $\text{mM Trolox.g}^{-1}$ ) values ( $-0.9342$ ), as well as for values in milligram of extract/formulation per gram of DPPH ( $\text{mgg}^{-1}$ ) and mM Trolox. $\text{g}^{-1}$  ( $-0.9769$ ).

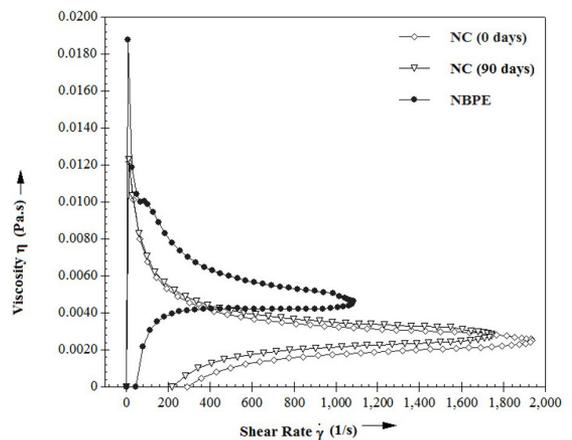
#### 4. Discussion

Bacaba is a species from the Amazon region that has been conquering the regional and national markets. This increase in demand has benefited the economy, especially in the state of Pará, where large areas are planted with the species, which contributes to the income for individuals in its production chain (Souza et al., 2021). However, along with the increase in production comes an increase in waste, both the seed and the peel, which can lead to environmental problems. In this sense, the use of the peels for the preparation of an extract, even though it presents a low yield, as well as its application in the development of a product is an alternative for its use in order to minimize these impacts, besides increasing its economic value.

Furthermore, the advantage of the extract obtained from the fruit peels is the presence of phenolic compounds, such as anthocyanins (Corrêa et al., 2019).



**Figure 1.** Flow curve of the selected nanoemulsion with bacaba peel extract (NBPE). Flow curve of the nanoemulsion before and after the accelerated stability test (NC – 0 and 90 days).



**Figure 2.** Viscosity curve of the selected nanoemulsion with bacaba peel extract (NBPE). Viscosity curve of the nanoemulsion before and after the accelerated stability test (NC – 0 and 90 days).

**Table 3.** Evaluation of the antioxidant potential of BPE and NC and NBPE formulations by the DPPH and ABTS<sup>+</sup> radical scavenging methods.

Samples	DPPH $\text{IC}_{50}$ ( $\text{mgL}^{-1}$ )	DPPH ( $\text{mgg}^{-1}$ )	ABTS <sup>+</sup> ( $\text{mMg}^{-1}$ )
BPE	$15.60 \pm 0.75$	$78.00 \pm 0.75$	$643.69 \pm 4.10$
NC	$24300 \pm 0.00$	$6075 \pm 0.00$	$0.42 \pm 0.00$
NBPE	$1714.67 \pm 1.87$	$428.67 \pm 1.87$	$4.41 \pm 2.23$
NBPE*	$8.14 \pm 0.98$	$2.03 \pm 0.98$	$933.88 \pm 1.27$

NBPE\* = Antioxidant potential as a function of extract concentration in the nanoemulsion. Values expressed as mean  $\pm$  relative standard deviation ( $n = 3$ ).

The baru oil, which is rich in unsaturated fatty acids (Siqueira et al., 2016) and was used as the oil phase in the systems, presented an HLB of 7.0. In the HLB determination using TW and SP, this value presented the best droplet size, morphology, and creaminess, agreeing with Maruno (2017) and Moraes et al. (2018), who found similar values (6.0-7.0).

The choice of the systems for the incorporation of the extract, from the 16 liquid emulsions, was based on the highest proportion of oil since these would be the ones with the highest probability of phase separation. Thus, associated with the oil, it was verified that the percentage of surfactants and the amount of incorporated extract could influence the final product. The variation in the concentrations of surfactants was based on the concept that in systems that use low energy, relatively high concentrations are often required for effective nanoemulsion formation, which may limit its use in various applications. On the other hand, the slightly oily characteristic of the extract may lead to the need for a higher percentage of surfactants. Thus, the concentrations of extract and surfactants varied.

The extract was solubilized in the oily and aqueous phases, with partial solubilization in the oil phase and total solubilization in the aqueous phase. Regarding the amount of extract, concentrations higher than 0.5% caused the destabilization of the formulations, with phase separation in all concentrations of surfactants suggesting that the oily characteristic of the extract increased the amount of the oil phase, destabilizing it. Thus, the concentration of the extract was set at 0.5% and three formulations containing baru oil (20%) and different proportions of surfactants (5, 15 and 20%) were subjected to preliminary stability.

Out of the three formulations submitted to preliminary stability, two coalesced and only the formulation with the smaller proportion of surfactants remained stable after the test. Coalescence happens due to the increase in surface tension and Gibbs free energy in the system, which may be related to the different characteristics of the surfactants used, as well as their percentage in the formulations.

The formulations 1 NC and 1 NBPE subjected to accelerated stability were classified as O/W systems, with electrical conductivity values higher than the conductivity of distilled water ( $1.3 \mu\text{Scm}^{-1}$ ), indicating stability and absence of phase inversion of the systems.

The pH control is a fundamental parameter to determine the chemical stability of the systems, that is, the decrease of these values indicates the occurrence of degradation reactions such as oxidation, hydrolysis, among others (Maruno, 2017). The pH of the formulations remained in the range of 4.9 - 5.6, values that are suitable for formulations for topical use, with no need for pH adjustments since the pH of the skin can vary between 4.5 - 7.0 depending on the application place (Moraes et al., 2018).

The refractive index values did not show variations, corroborating the stability of the systems, while the electrical conductivity values showed that the incorporation of the extract collaborated with the transfer of ions to the continuous phase of the formulation, increasing the values in this parameter, which can be explained by the presence of minerals in the bacaba peel (Corrêa et al., 2019).

In the accelerated stability test, the group submitted to a temperature of  $5 \pm 1 \text{ }^\circ\text{C}$  presented results similar to those of  $25 \pm 1 \text{ }^\circ\text{C}$ , remaining stable. On the other hand, in the groups submitted to a temperature of  $40 \pm 1 \text{ }^\circ\text{C}$ , as well as exposure to light, there was a loss of stability for NBPE, showing that luminosity and temperature are decisive for the stability of the extract and the formulation. The loss of stability under these conditions suggests that antioxidants, such as the anthocyanins present in bacaba peel, have suffered degradation since they are compounds susceptible to variations in temperature, pH, and luminosity (Corrêa et al., 2020; Mattioli et al., 2020).

The rheological analyses and flow and viscosity curves presented characteristic profiles to the formulation. Throughout the stability study, gradual and homogeneous changes indicate that the formulations are stable over time and conditions to which they were submitted to.

Physical stability is an essential parameter for the development of active release systems. Systems with extreme modifications in the flow or viscosity curves demonstrate inefficiency and instability and, therefore, cannot be approved. Additionally, the adding of a natural active ingredient in the formulation influences the rheological profile and stability. Changes may occur in stable formulations, but it is important that the profile is maintained, which was observed in the NC and NBPE formulations. Once the system presents stability, the viscosity profile can be adjusted later on, according to the formulation application. At this point, it was verified that each system presented a rheological profile that provides important information about its stability and applicability.

The size of less than 200 nm and low PDI (0.3) of the formulations globules is characteristic of monodisperse systems and indicated that the systems presented homogeneity, while the incorporation of the extract positively favored the globule diameter in the NBPE since it presented lower values than the NC, which may be associated with its greater solubility in the phases of the system (Islam et al., 2016). Lower values were obtained by Maruno (2017) in nanoemulsions with baru oil, but the percentage of oil phase was lower (10%), in addition to a higher proportion of surfactants and use of co-surfactants.

The zeta potential of the formulations showed values greater than  $|30 \text{ mV}|$  indicating the presence of negative charges on the surface of the globules, responsible for the repulsion between them, providing steric and electrostatic stability to the formulations and ensuring that there was no coalescence between the formed droplets (Ribeiro et al., 2020).

Concerning the antioxidant activity using the DPPH and ABTS<sup>+</sup> methods, it was found that BPE can be considered a promising source of antioxidants. When compared to the fruit, the BPE presented better results than those obtained by Abadio Finco et al. (2012) ( $0.70 \text{ mgmL}^{-1}$ ) and lower antioxidant activity compared to Canuto et al., (2010) ( $3.1 \mu\text{molL}^{-1}$ ). The peel presented better results than those found by Corrêa et al. (2019) ( $1.07 \text{ mgmL}^{-1}$ ), who worked with acidified peel extract. These variations in antioxidant activities may be related to experimental conditions such as type of solvent, pH, and temperature, as well as the extraction technique and the utilized part of the fruit (Silva et al., 2021).

As for the formulations, it was found that the incorporation of the extract provided an antioxidant activity to the system since the NC did not show such potential. Furthermore, it was possible to observe that the BPE incorporated into the formulation at a concentration of 0.5% presented antioxidant potential superior to that obtained by the crude extract.

The analysis by the ABTS<sup>+</sup> method confirmed the results obtained through the DPPH method. Although the methods present different analysis mechanisms, they showed the same behavior of antioxidant potential for the formulations and the extract. Pearson's correlation test showed a strong negative correlation between the DPPH and ABTS<sup>+</sup> methods, proving the relationship between the tests, that is, the lower the DPPH values, the higher the ABTS<sup>+</sup> values.

Similarly, studies performed with extracts of *Plinia peruviana* (Poir.) Govaerts (Mazzarino et al., 2018), *Achyrocline satureioides* (Lam.) A.D. (Zorzi et al., 2016) found that the incorporation of extracts into nanoemulsions was favored by the better solubilization of the components in the developed systems. Furthermore, the incorporation of BPE in a nanoemulsion system was advantageous when compared to microemulsion systems, as observed by Corrêa et al. (2020) who obtained lower results of antioxidant activity in microemulsions, which may have been influenced by the large amount of surfactants, as well as the larger diameter of the droplets of the systems.

Thus, the incorporation of BPE in a nanoemulsified system contributed to the increase of its solubility, antioxidant capacity, and reduction of the globules diameter, which favors the delivery of bioactive compounds to the site of action. Therefore, the development of a nanoemulsified system containing bacaba peel extract proved to be a promising alternative for the use of this by-product, since it showed promising results when compared to isolated antioxidant compounds such as resveratrol (Li et al., 2018) and quercetin (Das et al., 2020).

## 5. Conclusion

It was possible to incorporate the bacaba peel extract in a nanoemulsified system containing baru oil that showed stability and potential antioxidant activity. The developed formulation opens possibilities for the use of residues rich in bioactive compounds with potential biological activity that would be discarded, motivating the efficient use of by-products as raw material for the development of food, pharmaceutical, and cosmetic products, contributing to the search for a solution to optimize the reduction in waste generation and resource depletion, promoting new strategies based on sustainable technologies.

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## References

- ABADIO FINCO, F.D., KAMMERER, D.R., CARLE, R., TSENG, W.H., BÖSER, S. and GRAEVE, L., 2012. Antioxidant activity and characterization of phenolic compounds from bacaba (*Oenocarpus bacaba* Mart.) fruit by HPLC-DAD-MS(n). *Journal of Agricultural and Food Chemistry*, vol. 60, no. 31, pp. 7665-7673. <http://dx.doi.org/10.1021/jf3007689>. PMID:22788720.
- BALDISSERA, L., DEBIASI, B.W., AGOSTINI, J.S., ANDRIGHETTI, C.R., SUGUI, M.M., RIBEIRO, E.B. and VALLADÃO, D.M.S., 2023. Use of bacaba peel for the development of hydroelectrolytic beverages and their consumer acceptance. *Brazilian Journal of Pharmaceutical Sciences*, vol. 59, pp. e21762. <http://dx.doi.org/10.1590/s2175-97902023e21762>.
- BRASIL, 2021 [viewed 15 December 2021]. *Resolução RDC nº 481, de 15 de Março de 2021. Dispõe sobre os requisitos sanitários para óleos e gorduras vegetais* [online]. Diário Oficial da República Federativa do Brasil, Brasília. Available from: <https://www.in.gov.br/web/dou/-/resolucao-rdc-n-481-de-15-de-marco-de-2021-309012789>
- CANUTO, G.A.B., XAVIER, A.G.O., NEVES, L.C. and BENASSI, M.T., 2010. Caracterização físico-química de polpas de frutos da Amazônia e sua correlação com a atividade anti-radical livre. *Revista Brasileira de Fruticultura*, vol. 32, no. 4, pp. 1196-1205. <http://dx.doi.org/10.1590/S0100-29452010005000122>.
- COMUNIAN, T.A., SILVA, M.P. and SOUZA, C.J.F., 2021. The use of food by-products as a novel for functional foods: their use as ingredients and for the encapsulation process. *Trends in Food Science & Technology*, vol. 108, pp. 269-280. <http://dx.doi.org/10.1016/j.tifs.2021.01.003>.
- CORRÊA, B.M., BALDISSERA, L., BARBOSA, F.R., RIBEIRO, E.B., ANDRIGHETTI, C.R., AGOSTINI, J.S. and VALLADÃO, D.M.S., 2019. Centesimal and mineral composition and antioxidant activity of the Bacaba fruit peel. *Bioscience Journal*, vol. 35, no. 2, pp. 509-517. <http://dx.doi.org/10.14393/BJ-v35n2a20198-41868>.
- CORRÊA, B.M., PICCOLI, A.F.P., BARBOSA, F.R., RIBEIRO, E.B., ANDRIGHETTI, C.R., VASCONCELOS, L.G., BATTIROLA, L.D. and VALLADÃO, D.M.S., 2020. Development, characterization and stability of bacaba peel extract microemulsion systems. *Fronteiras*, vol. 9, no. 2, pp. 88-102. <http://dx.doi.org/10.21664/2238-8869.2020v9i2.p88-102>.
- DAS, S.S., VERMA, P.R.P. and SINGH, S.K., 2020. Screening and preparation of quercetin doped nanoemulsion: characterizations, antioxidant and anti-bacterial activities. *Lebensmittel-Wissenschaft + Technologie*, vol. 124, pp. 109141. <http://dx.doi.org/10.1016/j.lwt.2020.109141>.
- FIORI, K.P., TORRES, M.P.R., SCHONS, J.I., RIBEIRO, E.B., NOGUEIRA, R.M., VASCONCELOS, L.G., ANDRIGHETTI, C.R., JACINTO, M.J. and VALLADÃO, D.M.S., 2017. Microemulsion of Brazil nut oil as a natural product to improve superoxide release in human phagocytes. *Química Nova*, vol. 40, no. 9, pp. 1051-1057. <http://dx.doi.org/10.21577/0100-4042.20170113>.
- ISLAM, M.D.T., STRECK, L., PAZ, M.F.C.J., SOUSA, J.M.C., ALENCAR, M.V.O.B., MATA, A.M.O.F. and CARVALHO, R.M., 2016. Preparation of phytol-loaded nanoemulsion and screening for antioxidant capacity. *International Archives of Medicine*, vol. 9, pp. 1-15. <http://dx.doi.org/10.3823/1941>.
- LI, T., HUANG, J., WANG, Q., XIA, N. and XIA, Q., 2018. Resveratrol and linseed oil co-delivered in O/W nanoemulsions: preparation and characterization. *Integrated Ferroelectrics*, vol. 190, no. 1, pp. 101-111. <http://dx.doi.org/10.1080/10584587.2018.1457339>.
- MANIKANIKI, J.K. and JASWAL, S., 2021. Role of nanotechnology in the world of cosmetology: a review. *Materials Today: Proceedings*, vol. 45, no. 2, pp. 3302-3306. <http://dx.doi.org/10.1016/j.matpr.2020.12.638>.

- MARUNO, M., 2017. Characterization and stability studies on vegetable nanoemulsions obtained by low energy process. *Journal of Nanomedicine & Nanotechnology*, vol. s8, pp. 1-8. <http://dx.doi.org/10.4172/2157-7439.S8-003>.
- MATTIOLI, R., FRANCIOSO, A., MOSCA, L. and SILVA, P., 2020. Anthocyanins: a comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. *Molecules (Basel, Switzerland)*, vol. 25, no. 17, pp. 3809. <http://dx.doi.org/10.3390/molecules25173809>.
- MAZZARINO, L., PITZ, H.S., VOYTENA, A.P.L., TREVISAN, A.C.D., RIBEIRO-DO-VALLE, R.M. and MARASCHIN, M., 2018. Jaboticaba (*Plinia Peruviana*) extract nanoemulsions: development, stability, and in vitro antioxidant activity. *Drug Development and Industrial Pharmacy*, vol. 44, no. 4, pp. 643-651. <http://dx.doi.org/10.1080/03639045.2017.1405976>. PMID:29139323.
- MORAES, C., ANJOS, J.L.V., MARUNO, M. and ROCHA-FILHO, A.A., 2018. Development of Lamellar Gel Phase Emulsion Containing Baru Oil (*Dipteryx Alata* Vog.) as a prospective delivery system for cutaneous application. *Asian Journal of Pharmaceutical Sciences*, vol. 13, no. 2, pp. 183-190. <http://dx.doi.org/10.1016/j.ajps.2017.09.003>. PMID:32104391.
- RAISER, A.L., TORRES, M.P.R., RIBEIRO, E.B. and VALLADÃO, D.M.S., 2020. Characterization of Munguba oil obtained by ultrasound. *Ciência Rural*, vol. 50, no. 12, pp. 1-9. <http://dx.doi.org/10.1590/0103-8478cr20191028>.
- RIBEIRO, E.B., MARCHI, P.G.F., HONÓRIO-FRANÇA, A.C., FRANÇA, E.L. and SOLER, M.A.G., 2020. Interferon-gamma carrying nanoemulsion with immunomodulatory and anti-tumor activities. *Journal of Biomedical Materials Research. Part A*, vol. 108, no. 2, pp. 234-245. <http://dx.doi.org/10.1002/jbm.a.36808>. PMID:31587469.
- RUFINO, M.S.M., ALVES, R.E., BRITO, E.S., MORAIS, S.M., SAMPAIO, C.G. and PÉREZ-JIMÉNEZ, J., 2007a [viewed 15 December 2021]. *Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH* [online]. Fortaleza: EMBRAPA, 4 p. Comunicado Técnico, no. 127. Available from: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/426953/metodologia-cientifica-determinacao-da-atividade-antioxidante-total-em-frutas-pela-captura-do-radical-livre-dpph>
- RUFINO, M.S.M., ALVES, R.E., BRITO, E.S., MORAIS, S.M., SAMPAIO, C.G. and PÉREZ-JIMÉNEZ, J., 2007b [viewed 15 December 2021]. *Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS<sup>•+</sup>* [online]. Fortaleza: EMBRAPA, 4 p. Comunicado Técnico, no. 128. Available from: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/426954/metodologia-cientifica-determinacao-da-atividade-antioxidante-total-em-frutas-pela-captura-do-radical-livre-abts>
- SANTOS, O.V., VIANA, A.A., SOARES, S.D., VIEIRA, E.L.S., MARTINS, M.G., NASCIMENTO, F.C.A. and TEIXEIRA-COSTA, B.E., 2021. Industrial potential of Bacaba (*Oenocarpus Bacaba*) in powder: antioxidant activity, spectroscopic and morphological behavior. *Food Science and Technology (Campinas)*, vol. 26, pp. 1-7. <http://dx.doi.org/10.1590/fst.62820>.
- SILVA, L.D., SARI, R., DIEDRICH, C., PEREIRA, C., LIMA, V.A., OLDONI, T.L.C., PERIN, E.C. and CARPES, S.T., 2021. Extraction, characterization and antioxidant properties of phenolic compounds in açai juçara (*Euterpe edulis* Mart.) from Atlantic Forest. *Brazilian Journal of Food Technology*, vol. 24, pp. e2020193. <http://dx.doi.org/10.1590/1981-6723.19320>.
- SIQUEIRA, A.P.S., CASTRO, C.F.S., SILVEIRA, E.V. and LOURENÇO, M.F.C., 2016. Chemical quality of baru almond (*Dipteryx Alata* oil). *Ciência Rural*, vol. 46, no. 10, pp. 1865-1867. <http://dx.doi.org/10.1590/0103-8478cr20150468>.
- SOUZA, C.N.C., BEGOT, L.H., PARAENSE, V.C. and FREITAS, A.D.D., 2021 [viewed 15 December 2021]. Cadeia produtiva da Bacaba (*Oenocarpus bacaba* Mart.) Barreiras, ALMEIRIM-PA. *Revista Contribuciones a las Ciencias Sociales* [online], vol. 14, no. 1, pp. 1-16. Available from: <https://revistacontribuciones.com/ojs/index.php/clcs/article/view/28>
- TORRES, M.P.R., ESPRENDOR, R.V.F., BONALDO, S.M., RIBEIRO, E.B. and VALLADÃO, D.M.S., 2019. Development, characterization and stability of microemulsionated formulations of Bacaba, *Oenocarpus Bacaba* oil. *Acta Amazonica*, vol. 49, no. 3, pp. 246-255. <http://dx.doi.org/10.1590/1809-4392201802702>.
- TRIGO, J.P., ALEXANDRE, E.M.C., SARAIVA, J.A. and PINTADO, M.E., 2020. High value-added compounds from fruit and vegetable by-products—characterization, bioactivities, and application in the development of novel food products. *Critical Reviews in Food Science and Nutrition*, vol. 60, no. 8, pp. 1388-1416. <http://dx.doi.org/10.1080/10408398.2019.1572588>. PMID:30740995.
- ZANIN, S.M., MIGUEL, M.D., CHIMELLI, M.C. and OLIVEIRA, A.B., 2002. Determinação do Equilíbrio hidrófilo-lipófilo (EHL) de óleos de origem vegetal. *Visão Acadêmica*, vol. 3, no. 1, pp. 13-18. <http://dx.doi.org/10.5380/acd.v3i1.494>.
- ZORZI, G.K., CAREGNATO, F., MOREIRA, J.C.F., TEIXEIRA, H.F. and CARVALHO, E.L.S., 2016. Antioxidant effect of nanoemulsions containing extract of *Achyrocline Satureioides* (Lam) D.C.: *Asteraceae*. *AAPS PharmSciTech*, vol. 17, no. 4, pp. 844-850. <http://dx.doi.org/10.1208/s12249-015-0408-8>. PMID:26361953.