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Obtaining and preliminary stability assessment of emulsions containing açaí oil (*Euterpe oleracea*) as a bioproduct for cosmetic application

Obtenção e avaliação da estabilidade preliminar de emulsões contendo óleo de açaí (*Euterpe oleracea*) como bioproduto para aplicação cosmética

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Açaí oil (*Euterpe oleracea*) is a valuable bioproduct native to the Amazon region, widely used in the food, pharmaceutical, and cosmetic sectors due to its antioxidant, anti-inflammatory, and anti-aging properties, playing an essential role in the sustainable use of biological resources. This study aimed to develop and assess the preliminary stability of emulsions containing açaí oil. The oil was characterized using the acidity index (AI), saponification, iodine, nuclear magnetic resonance spectroscopy (NMR), fatty acid profile by gas chromatography (CG), relative density, pH, and organoleptic properties. Two emulsions were obtained, F1 and F2, and evaluated in 6 cycles of thermal stress, each cycle consisting of 24 hours at 45 ± 2 ºC and 24 hours at 5 ± 2 ºC; the control was kept at 25 ± 2 ºC, at the end of the cycles were evaluated the organoleptic properties, pH and density. The açaí oil obtained values of AI (1.48mg NAOH/g), iodine (65.58 gl2), saponification (199.54 mg), and density (0.914 g/mL), and the NMR and GC showed the presence of oleic, linoleic, palmitoleic and palmitic acids as priorities. During the preliminary stability test, there were no changes in pH (p= 0.8281 and 0.7497) and density (p= 0.7177 and 0.5166) for F1 and F2, respectively. Therefore, formulation F1 presented promising characteristics for application in cosmetology, requiring further recommended stability tests.
Keywords: phytocosmetics, control quality, amazon active.

O óleo de açaí (*Euterpe oleracea*) é um bioproduto valioso nativo da região amazônica, amplamente empregado nos setores alimentício, farmacêutico e cosmético devido as suas propriedades antioxidantes, anti-inflamatória, e antienvelhecimento, desempenhando um papel essencial na bioeconomia e na utilização sustentável de recursos biológicos. Este estudo teve como objetivo caracterizar o óleo de açaí e desenvolver emulsões a partir dele, visando analisar sua estabilidade preliminar. A caracterização do óleo foi realizada através do índice de acidez, saponificação, iodo, espectroscopia por ressonância magnética nuclear, perfil de ácidos graxos por cromatografia gasosa, densidade relativa, pH e propriedades organolépticas. A partir deste, foram obtidas duas emulsões, F1 e F2, sendo avaliados em 6 ciclos de estresse térmico, cada ciclo composto de 24 horas em 45 ± 2 ºC e 24 horas em 5 ± 2 ºC, a amostra controle foi mantida em 25 ± 2 ºC, ao final dos ciclos foram avaliados as propriedades organolépticas, pH e densidade. O óleo de açaí obteve valores de IA (1,48mg NAOH/g), iodo (65,58 gl^2), saponificação (199,54 mg) e densidade (0,914 g/mL), o RMN e o CG evidenciou a presença dos ácidos oleico, linoleico, palmitoleico e palmítico como prioritários. Durante o teste de estabilidade preliminar não houveram alterações em relação ao pH (p= 0,8281 e 0,7497) e densidade (p= 0,7177 e 0,5166), para F1 e F2, respectivamente. Entretanto, F2 apresentou separação de fases durante o ciclo de estresse térmico. Portanto, a formulação F1, apresentou características promissoras para serem aplicadas na cosmetologia, sendo necessário próximos testes de estabilidade preconizados.
Palavras-chave: fitocosméticos, controle de qualidade, ativo amazônico.

1. INTRODUCTION

Vegetable oils have a high economic and nutritional potential, which has attracted the interest of various scientific areas, including the manufacture of pharmaceuticals, food, renewable fuels, and cosmetics. Açaí (*Euterpe oleracea*), a species native to the Amazon region, stands out for its role in the food market. Still, its secondary metabolites are widespread in the literature, significant levels of phenolic compounds (55.4mg. $100g^{-1}$) and anthocyanins characterize its purple color,

however, it is possible to quantify tocopherols, sterols and carotenoids [1, 2] and the presence of fatty acids, making it enjoyable to use as a topical application system [3].

Emulsions are thermodynamically unstable due to the union of two immiscible liquids, in which an emulsifying agent mediates the action [4]. Açaí oil drops can form the internal phase of an emulsion, making it an effective emollient, antioxidant, anti-aging, anti-inflammatory, and antimicrobial [1, 5-6]. It is possible to find in the literature the use of açaí in various formulations applied to emulsified systems, as in the patents for açaí emulsions for larvicidal activity (BR102020013253A2), antioxidant compositions containing açaí oil (BR102019004137A2), and the use of açaí as a hair oil (KR10-2402021B1). This suggests that the species should be included in various segments and represent a strong transfer of technology to the industrial sector [2].

The National Health Surveillance Agency (ANVISA) requires that, when placed on the market, the product comply with the guidelines for obtaining the safety of cosmetic products [7]. The study of stability seeks to provide information that guarantees the quality of the product, maintaining its properties throughout its useful life, emphasizing the importance of quality as a characteristic for protecting and promoting people's health. Analyzing the stability of a cosmetic formulation is crucial because it will maintain the product's shelf life, keeping its physical and chemical characteristics and microbiological conditions within the specified and previously approved limits.

To evaluate the development of a pharmaceutical formulation, it is crucial to understand all its aspects to guarantee safety and verify compliance with the quality control standards established by ANVISA, which implies a thorough analysis of the physical, chemical, and organoleptic factors that make up the formulation. Thus, manufacturing formulations such as emulsions require a careful stability analysis to ensure their safe use. It is necessary to perform a preliminary assessment of the formulation's stability under specific temperature conditions, enabling the most favorable conditions to be determined for maximum product viability [8]. This study aims to develop cosmetic emulsions containing açaí oil and assess their preliminary stability. The best formulation can be used as a topical cosmetic that presents possible antioxidant activity by adding açaí oil.

2. MATERIAL AND METHODS

2.1 Material

The açaí (extracted from the fruit pulp), batch VPS 169-002/072023, was purchased from Amazon Oil (Ananindeua, Pará, Brazil). 150 mL was fractionated into hermetically sealed amber bottles for the characterization analyses. The reagents were deuterated chloroform (ExodoCientifica, São Paulo, Brazil), phenolphthalein (Synth, Diadema, Brazil), sodium hydroxide (ExodoCientifica, São Paulo, Brazil), and a solution of ethyl ether and ethyl alcohol 2:1 (ACS Cientifica, Rio de Janeiro, Brazil).

2.2 Characterization of açaí oil

2.2.1 Acidity Index

The Acidity Index (AI) of açaí oil was determined following the protocol established by the Adolfo Lutz Institute (2008) [9] and realized in triplicate. The procedure began by weighing 2 grams of the oil sample in a 125 mL Erlenmeyer flask. A solution of ethyl ether and ethyl alcohol in a 2:1 volume/volume ratio was then prepared to dissolve the sample. Two drops of phenolphthalein were added as an indicator. The solution was titrated with 0.1 mol. L^{-1} sodium hydroxide (NaOH) until a pinkcolor appeared and persisted for approximately 30 seconds. The AI was calculated using Equation 1 as a reference:

$$IA = \frac{Vxfx\ 5{,}61}{P(g)} \quad \text{(eq.1)}$$

Where: V = number of mL of sodium hydroxide solution used in the titration;
P = number of g of sample;
f = correction factor of the solution obtained from titration with a standard solution.

2.2.2 Saponification index

The saponification index (SI) was determined using the indirect method, according to the methodology described by the Adolfo Lutz Institute (2008) [9], using the calculation established by Equation 2, realized in triplicate and determined by:

$$IS = \frac{3 \text{ x } 56{,}1 \text{ x } 1000}{\text{MW x } 3 + 92{,}09 - (3 \text{ x} 1\ 8)} \quad \text{(eq.2)}$$

Where: MW= Molecular weight of fatty acids (g/mol);
3 = Number of fatty acids pertriacylglycerol;
56,1 = Molecular weight of KOH (g/mol);
1000 = Conversion from g to mg;
92,09 = Molecular weight of glycerol (g/mol);
18 = The molecular weight of water.

2.2.3 Iodine index

The iodine index was obtained following the guidelines of the American Oil Chemists' Society (AOCS), according to protocol Cs c-85 [10]. Determination was carried out by gas chromatography, and the index (in triplicate) was calculated using Equation 3, given by:

Iodine index = (% palmitoleic acid x 0,950) + (% oleic acid x 0,860) + (% linoleic acid x 1,732) + (% linolenic acid x 2,616) (eq. 3)

2.2.4 Nuclear Magnetic Resonance Spectroscopy - NMR

The ^{1}H, ^{13}C Nuclear Magnetic Resonance (NMR) spectra provided a comprehensive and detailed analysis of the molecular structure, enriching the understanding of the atomic interactions present in the sample. The signals were acquired on a Bruker spectrometer, model Ancend™, operating at 400 and 100 MHz, respectively. 100µL of the sample dissolved in 500µL of deuterated chloroform ($CDCl_3$) was used for the analysis. TopSpin 3.6.0 software was used to control and process the data. The raw frequency signals (FIDs) were subjected to the Fourier transform using a Line broadband (LB) of 0.3 Hz. The spectra were manually processed, baseline corrected, and calibrated using the residual $CDCL_3$ solvent signal as an internal [11].

2.2.5 Fatty acid profile by gas chromatography

The fatty acid profile was analyzed according to the official standard method recommended by the AOCS (2013) [10]. A gas chromatograph coupled to a mass spectrometer - GC-MS model trace 1300 MS-ISQ Single Quadrupole (ThermoScientific - Waltham, USA) was used, equipped with a ZB-5HT 30m x 0.25mm x 1µm capillary column (Phenomenex - Torrance, USA). The chromatographic conditions adopted were helium (He) as the carrier gas at a flow rate of 1mL/min, and the injection volume of the oil sample was 1µL in Splitless mode. The injector and detector temperatures were set at 220 ºC and 280 ºC, respectively. For chromatographic separation, a gradient was applied with an initial temperature of 60 ºC for 1 minute, followed by an increase to 250 ºC, maintained for 10 minutes. The individual fatty acid peaks were identified by comparing the retention times with those of unknown fatty acid standard mixtures. This process ensured precision and reliability in determining the constituents present in the oil,

providing detailed data on the composition of the fatty acid profile. The results were expressed as a relative percentage of the total fatty acids.

2.2.6 pH analysis

The pH was analyzed using a model PHS3BW potentiometer (Bel engineering®, Italy), calibrated in a standard pH 4.0 and pH 7.0 solution from the equipment; all analyses were performed in triplicate [7, 11, 12].

2.2.7 Determination of relative density

The relative density of the oil was determined at room temperature 25ºC by dividing its mass (g) by its volume (mL); all analyses were performed in triplicate [7, 11, 12].

2.3 Development and stability testing of emulsions

2.3.1 Obtaining emulsions

The study consisted of a laboratory experiment to develop two emulsion formulations with different concentrations of the active ingredient, açaí oil, adapted from Pinheiro et al. (2023) [12]. Classic emulsions used in anti-aging and antioxidant treatments are excellent vehicles for vegetable oils. In this sense, two macroemulsions or classic emulsions (o/w), F1 and F2 (Table 1), were developed. The formulations were packaged in transparent polyethylene tubes with non-hermetic sealing lids. The formulations were synthesized at the quality control laboratory of Amazon University, campus Ananindeua.

Table 1: Classic emulsions containing açaí oil (Euterpe oleracea).

Components	Formulações	
	F1	F2
Açaí oil	3%	5%
Anionic base lotion*	97%	95%

*: Aqua, cereal Alcohol, Sodium Ceteary Sulf, Mineral Oil, Sorbitol Cyclopentasiloxane, Imidazolidinyl Urea, Buthylhydroxytolueno, Disodium EDTA, Methylisothiazolinone, Methylchloroisothiazolinone.

2.3.2 Centrifugation

The formulations underwent the centrifugation test, which was essential for analyzing the responses to the experiment. 5g of the preparations were weighed into test tubes and centrifuged at 3.000 rpm for 30 minutes at 25°C. After centrifugation, the samples were visually assessed to see any physical instability, such as phase separation, precipitation, or sediment formation [7].

2.3.3 Preliminary stability assessment of emulsions

The preliminary stability test subjected the F1 and F2 formulations to six heat stress cycles. For each cycle, the samples were kept in an oven (FANEN, Belém, Brazil) at a temperature of 45 ±2°C for 24 hours and then in a refrigerator (Consul, Belém, Brazil) at a temperature of 5 ±2°C, totaling 12 consecutive days. The control was the same samples kept at room temperature (25 ±2°C). At the end of each freeze-thaw cycle, the samples were thoroughly analyzed in triplicate using quality control tests, assessing organoleptic characteristics, pH, and density [7, 11, 13].

2.3.4 Emulsion quality control tests

The color was determined using software (Color picker and helper), which uses the L*a*b system to indicate color, version 1.2.0 free license. The organoleptic properties were assessed qualitatively in terms of appearance and odor. Each perception was carefully checked to determine whether the samples remained N (Normal, unchanged) or LM (Slightly modified, separated, precipitated, or cloudy), following the guidelines set out in ANVISA's Cosmetic Product Stability Guide [7].

Density was calculated by dividing mass (g) by volume (L). The total amount of the formulation was weighed on an analytical balance, and the mass value was recorded [7].

The pH was analyzed using a model PHS3BW potentiometer (Bel engineering®, Italy), calibrated using a standard pH 4.0 and pH 7.0 solution from the equipment [7].

After carrying out the quality control tests, the results were subjected to analysis of variance (ANOVA) using BioStat® software version 5.3 free licenses, which applies dependent or independent variables for each study criterion. Through this test, the F value will be obtained to determine the degree of dispersion between the analysis data and the P value to determine the significance of the study findings, using a 95% confidence level and a 5% margin of error, thus considering a value of p=0.05 [8].

3. RESULTS AND DISCUSSION

3.1 Characterization of açaí oil

The physicochemical properties of vegetable oils are intrinsically linked to their fatty composition, requiring specific tests to gain an in-depth understanding of these characteristics. Various tests have been used to extract relevant data on the nature of açaí oil, playing an essential role in quality control [8, 12]. These procedures are indispensable for detecting possible degradation or falsification, guaranteeing the integrity and authenticity of the product [11].

The results of the physicochemical characterization of açaí oil are detailed in Table 2, providing valuable information for analyzing the quality and stability of this oil. According to the methodology of Adolfo Lutz (2008) [9], the physical-chemical characterization of vegetable oils is carried out using traditional techniques, such as titrimetric methods, which are essential for determining acidity and peroxide indices [13]. In addition, more advanced techniques provide detailed data on the composition of these oils, which is why it is important to emphasize that the physico-chemical parameters of vegetable oils are directly linked to their fatty composition [13].

Table 2: Physicochemical characteristics of açaí oil.

Parameters	Results
Acidity index	1.48 ± 0.04 mg NaOH/g
Saponification index	199.50 ± 0.03 mg KOH/g
Iodine index	65.58 ± 0.01 gl^2/100g
Relative density	0.91 ± 0.02 g/mL
pH	6.00 ± 0.01

The acidity index is a necessary parameter and one of the most important for assessing the quality of vegetable oil. It indicates the level of hydrolytic rancidity resulting from the degradation of triacylglycerols, which increases free fatty acids. This alteration causes sensory changes in the oil, such as variations in color and odor, which consequently affect the products [14].

The saponification reaction establishes the degree of deterioration and stability, verifying that the oil's properties align with specifications and checking for possible adulteration containing unsaponifiable substances. As described in Table 2, the saponification index found was (199.5 mg NaOH/g), which is in line with the value found by Contente et al. (2020) [14],

documented in the literature. It reinforces the authenticity of the oil evaluated, ruling out any signs of adulteration or irregularity.

Another critical parameter is the iodine index, a measure of the extent of unsaturation in the chemical structures of vegetable oils. The greater the amount of unsaturation, the greater the oil's capacity to absorb iodine. This index is directly related to the number of double bonds in the sample. A higher iodine index indicates a more unsaturated oil, which can be less stable due to its susceptibility to oxidation. This study found a value equal to (65.58 gl^2 /100g) suggesting no degradation of the unsaturated fatty acids in the oil during the analysis process [13].

The density was 0.914g/mL, close to the figures found by Contente et al. (2020) [14]. Density is essential for ensuring the stability and consistency of formulations and guaranteeing the products' durability. It is also fundamental for maintaining integrity and effectiveness over time.

The oil's pH, measured at 6.0, is similar to the skin's pH, a critical factor for its stability. As the literature suggests, this similarity supports a gentle application, minimizing potential disturbances to the skin barrier. Our findings provide confidence in the oil's potential for gentle application, reducing the risk of alterations in the skin's protective capacity or irritations [7, 12].

Açaí oil (Figure 1) is a combination of triacylglycerols that include unsaturated fatty acids such as oleic, linoleic, and palmitoleic acid, the concentrations of which are detailed in Table 2. Figure 2 shows the 1H NMR spectrum of the oil with expansions highlighting the main hydrogen signals characteristic of the oil; each signal represents the atom detected in the sample, the signal located at 7.25 ppm corresponds to the deuterated chloroform ($CDCL_3$) solvent used in the analysis, the signals situated between 5.0 and 5, 5 ppm show precisely the location of the unsaturation of the oil's fatty acids in the spectrum, demonstrating its quality, the signals located at 4.0 and 4.5 ppm correspond to the unsaturated bonds in the triglyceride chain of açaí oil, another important signal is the saturation site located between peaks 1.0 and 1.5 ppm (Figure 2), the high index shows the amount of CH_2 in the same chain. In this case, to confirm the correct assignments between these signals belonging to the H proton, due to the proximity of the signals, we opted for unbinding as an integration method to determine the proportion of saturated and unsaturated fatty acids in both positions [11].

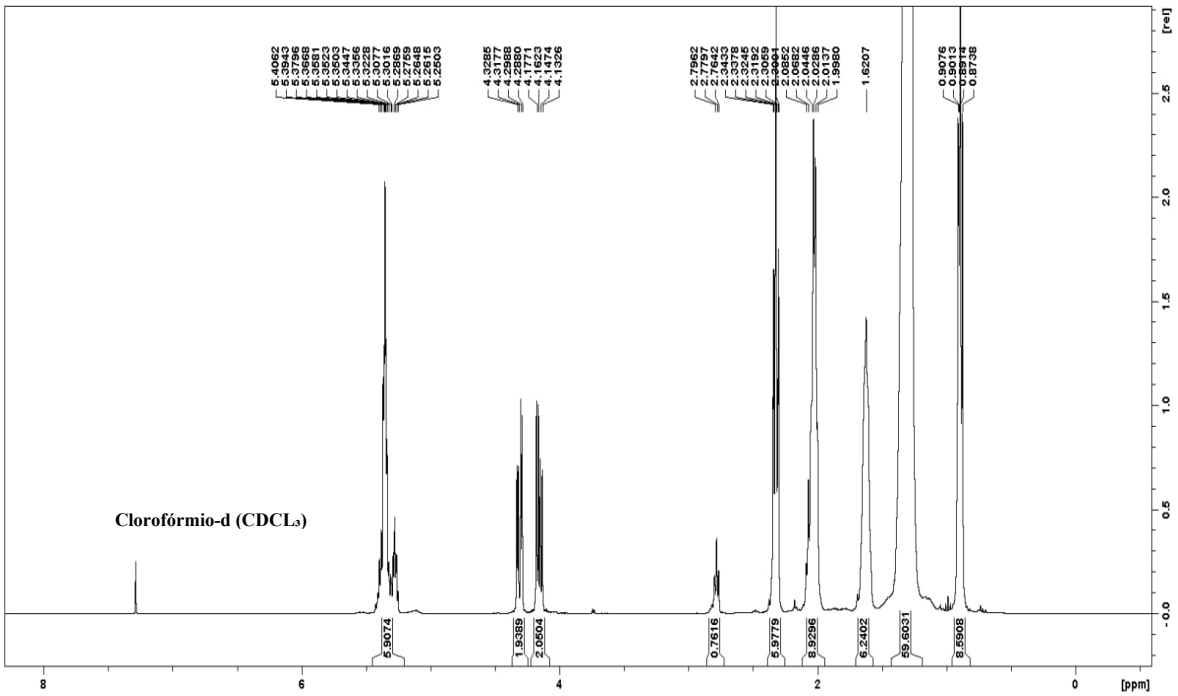

Figure 1: 1H NMR spectrum of açaí oil.

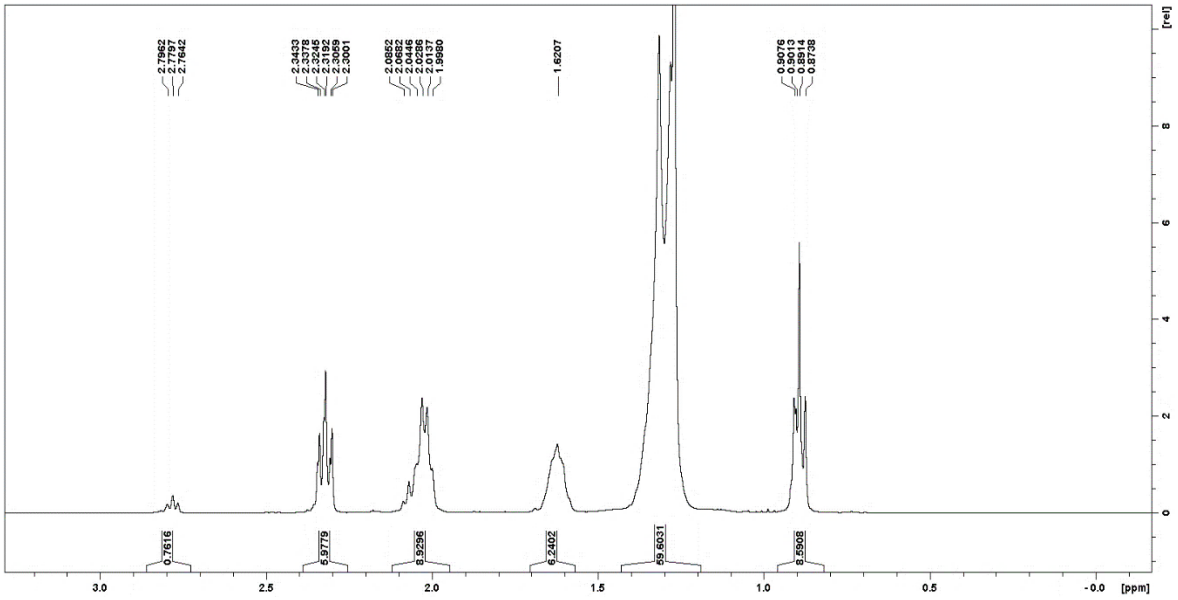

Figure 2: NMR spectra of fatty acids present in açaí oil.

NMR spectroscopy of vegetable oils is fundamental to understanding these natural products' chemical composition and molecular structure. The technique offers a detailed analysis of the constituents present in vegetable oils, providing valuable information on their quality, authenticity, and properties [15]. According to studies by Pinheiro et al. (2022) [11], using NMR, it is possible to identify the different types of fatty acids, unsaturations, functional groups, and other components in oils. The technique allows for the characterization of fatty acids, in this case, showing the presence of oleic, linoleic, palmitoleic, and palmitic acids, providing data on the quantity of each of these acids present in the analyzed sample.

Furthermore, NMR can be used to assess oil purity, detect the presence of impurities, identify possible adulteration, and even monitor changes. It stands out as a non-invasive and non-destructive tool, providing a practical approach to analyzing oils and its ability to reveal essential molecular details [12, 16].

The chromatogram illustrated in Figure 3 shows the profile of fatty acids that make up açaí oil. Consisting mainly of unsaturated fatty acids (66.12%), with oleic acid (30.75%) being the main component, followed by linoleic acid and palmitoleic acid (13.31% and 6.41%) respectively, it was also possible to identify the presence of saturated acids, with palmitic acid (28.96%) standing out. This evidence is borne out by the studies carried out by Silva and Rogez (2013) [13], highlighting oleic acid as the main unsaturated component of the oil. This lipid in the epidermis is vital in protecting and maintaining the skin barrier, preventing dehydration due to water loss. Linoleic acid can help restore the skin in various dermatological disorders when applied topically, as already highlighted in the literature [13]. As for palmitic acid, cited by Contente et al. (2020) [14], it is recognized for its use as an emulsifying agent and facilitator of skin penetration in topical formulations [7].

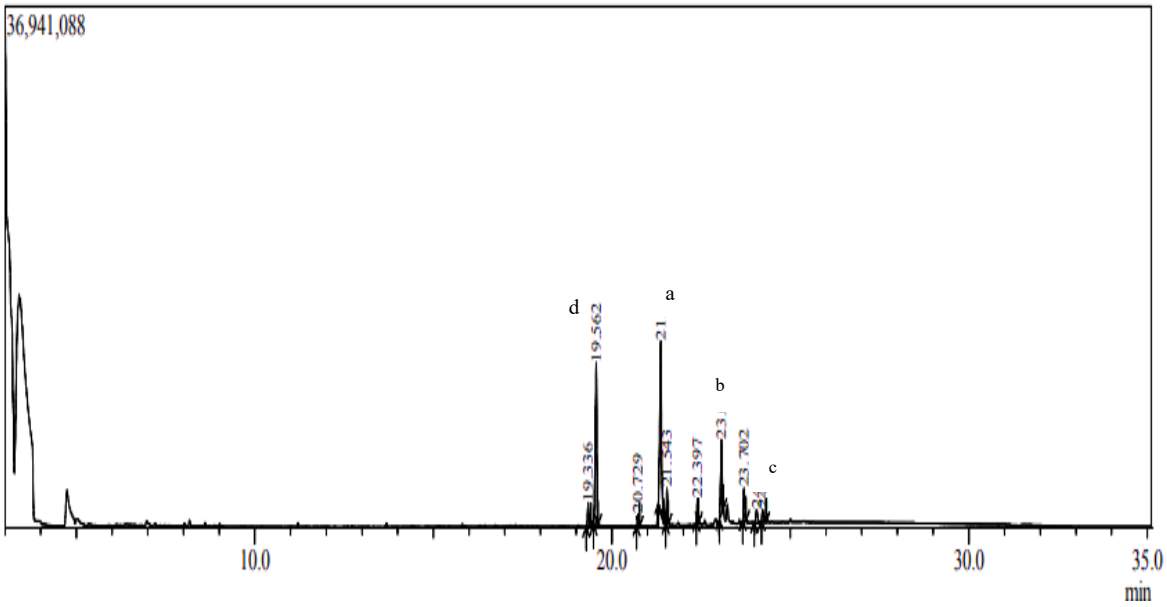

Figure 3: GC-MS chromatogram of açaí oil.

The fatty acid profile is a fundamental criterion for the authenticity of vegetable oil, and this method is generally determined by gas chromatography [14]. Studies indicate that this analysis allows changes in the oil's chemical composition to be identified, as evidenced by variations in the molar ratio between the different fatty acids in the triacylglycerol structure [17]. Considering these benefits, exploiting açaí oil in the composition of emulsions is feasible, taking advantage of its potential from both a pharmaceutical and cosmetic point [7, 14, 18]. The main composition of the percentages of unsaturated and saturated fatty acids found in the aliquot of oil analyzed can be seen in Table 3. These findings corroborate the fatty acid composition of açaí as oleic acid (47.58-64.19 %), palmitic acid (21.15-24.06 %), linoleic acid (12.31-22:30 %), palmiteleic acid (3.43-6.94 %), stearic acid (1.05-4.10 %) and myristic acid (0.07-0.19 %) [2]. It is worth noting that this composition can vary depending on the part of the plant used to extract the açaí oil.

Table 3: Fatty acid composition of açaí oil by gas chromatography.

Fatty acid	Percentual %
Oleic acid	30.75
Linoleic acid	13.31
Palmitoleic acid	6.41
Palmitic acid	28.96

3.2 Development and stability of emulsions containing açaí oil

Formulations F1 and F2 exhibited a greenish color, a straightforward appearance, free of air bubbles, and without any alterations to the characteristics of each sample. Following the Stability Guide [7], the delivery of cosmetic product safety is fundamental to formulation, guaranteeing the stability needed to guide production. Stability plays a key role in the manufacturing guide. Stability tests elucidate the requirements that impact the sensitivity of products, determining the ideal temperatures for proper storage of each item [7, 8].

Centrifugation generates stress in the sample, potentially precipitating instabilities and indicating the need for adjustments to its composition. However, per the stability guide for cosmetic products established [7], the color, odor, and appearance remained unchanged during the test.

Organoleptic aspects play a crucial role in stability studies, requiring attention to any signs of alterations. Color, odor, and appearance changes can indicate stability problems, directly affecting the product's quality and efficacy [3, 8, 14].

When analyzing the organoleptic characteristics of the F1 and F2 formulations (Table 4), each with different concentrations of oil and base lotion, variations in coloring were observed at each cycle, directly associated with the active ingredient concentrations. It was found that the higher the oil concentration, the more intense the coloring. F1 showed no color, appearance, or phase separation changes during the thermal stress stages. However, F2 showed phase separation during the last freeze-thaw cycle. This loss of stability may be related to the high density of the oil or the concentration of the oil added to the formulation [7].

Table 4: Color analyses of the F1 and F2 formulations.

Sample	F1					F2				
	L	a	b	A	O	L	a	b	A	O
Control	79.22	-10.95	44.29	N	N	75.57	-11.63	53.61	N	N
Cycle 1	85.75	-12.57	46.76	N	N	51.37	-5.32	55.01	N	N
Cycle 2	73.22	-7.23	32.35	N	N	67.70	-7.66	41.67	N	N
Cycle 3	73.70	-9.58	47.66	N	N	69.05	-8.77	47.25	N	N
Cycle 4	71.65	-10.82	50.33	N	N	77.16	-12.46	48.00	N	N
Cycle 5	63.07	-6.24	40.42	N	N	61.13	-7.34	58.01	N	N
Cycle6	73.22	-9.80	42.09	LM	N	74.97	-10.87	52.00	S	N

Legend: C: color; O: odor; A: appearance; N: normal without alteration; LM: slightly separated. Classification according to the ANVISA Cosmetic Product Stability Guide [7].

No significant changes were observed in pH values throughout the analysis cycles, remaining between 5.0 and 6.0 for the minimum and maximum readings, respectively (Table 5). These results are considered safe for skin and body application, represent no risk, and align with the stability criteria. In some cases, variations in hydrogen potential, according to the Brazilian Pharmacopoeia (2024) [19], may indicate the influence of factors that affect the stability of the formulation, such as the presence of impurities, decomposition, or inadequate storage conditions [16]. Therefore, carrying out the pH test is crucial to guarantee the stability and efficacy of a formulation. The pH of the emulsion remained within the acceptable range, which is an essential element in testing the formulation analyzed since a low pH can cause discomfort, such as hypersensitivity [7, 12, 14, 19].

The quantitative values relating to the density of the formulations developed were thoroughly analyzed in terms of mean and standard deviation. Examining the results obtained on the formulations' densities can reveal crucial information on the physical composition and homogeneity of the products in question [19].

Table 5: Organoleptic analyses of formulations containing açaí oil

	pH (X ± DP)		Density (X ± DP)	
Samples	**F1**	**F2**	**F1 (g/mL)**	**F2 (g/mL)**
Control	6.00 ± 0.01	6.00 ± 0.01	1.32 ± 0.01	1.32 ± 0.01
Cycle 1	5.00 ± 0.01	6.00 ± 0.01	1.39 ± 0.11	1.24 ± 0.05
Cycle 2	5.67 ± 0.57	5.50 ± 0.71	1.30 ± 0.05	1.23 ± 0.14
Cycle 3	5.00 ± 0.01	4.00 ± 0.01	1.30 ± 0.06	1.33 ± 0.02
Cycle 4	6.00 ± 0.01	5.00 ± 0.01	1.03 ± 0.12	1.12 ± 0.06
Cycle 5	6.00 ± 0.01	6.00 ± 0.01	1.26 ± 0.05	1.06 ± 0.05
Cycle 6	5.00 ± 0.01	5.00 ± 0.01	1.17 ± 0.14	1.12 ± 0.04

The mean density provides a representative central measure, while the standard deviation indicates the dispersion or variability of these values. These parameters are fundamental for

understanding the consistency and uniformity of formulations and are essential in developing cosmetic products [8, 12]. This statistical analysis is crucial for validating and optimizing formulations, guaranteeing high-quality and consistent products. Therefore, the quantitative assessment of density, expressed using the mean and standard deviation (described in Table 5), plays a vital role in understanding the uniformity and stability of the formulations developed, contributing significantly to improving and validating cosmetic products [14].

The values obtained by applying ANOVA to pH ($p=0.7497$) and density ($p=0.5166$) showed no significant difference between the results. These data indicate that there was no variance throughout the freeze-thaw cycles, where the pH and density parameters corroborate the guidelines of the cosmetic product stability guide [7, 8].

4. CONCLUSION

The development of emulsions with açaí oil involved two distinct phases: the first comprised the study of the oil's physicochemical properties and chemical constitution. In contrast, the second and main phase focused on obtaining and characterizing the emulsions, with açaí oil as the main active ingredient. The açaí oil (*Euterpe oleracea*) used in this study had a rich constitution of oleic, linoleic, palmitoleic, and palmitic acids. Molecular analysis by nuclear magnetic resonance (NMR) confirmed the localization of the acyl chains in the spectrum during the sample's analysis time; the interaction between the peaks highlighted the presence of these compounds, with no evidence of the formation or presence of other components. The gas chromatography (GC) fatty acid profile showed a predominance of unsaturated acids such as oleic, linoleic, palmitoleic, and saturated palmitic acid, which are the majority components. No results showed any signs of impurity in the raw material. The physicochemical parameters of the emulsions developed were oily, without lumps, and greenish-like results already described in the literature and within the acceptable values according to legislation. During the stability test, we observed no changes in the organoleptic properties, pH, and density of F1 compared to the control sample.

In contrast, F2 exhibited phase separation during the last stability cycle. This indicates that the F1 formulation possesses promising characteristics suitable for cosmetology. Therefore, we must conduct the following stability tests recommended by current legislation. We can package them in transparent plastic containers with lids.

5. ACKNOWLEDGMENTS

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