Evaluation of the quality of Amazonian butters as sustainable raw materials for applications in bioproducts

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Abstract
A new consumer profile for pharmaceutical and cosmetic products has motivated research into natural raw materials in the development of “green” products such as herbal medicines and biocosmetics. However, various limitations have been encountered in the marketing of these products, for example the quality control of the natural raw materials used by the industrial market. This study aims to evaluate the sensory and physicochemical parameters of murumuru (Astrocaryum murumuru Mart.), bacuri (Platonia insignis Mart.), tucuma (Astrocaryum vulgare Mart.), and ucuuba (Virola sebifera Aubl.) butters for applications in pharmaceutical and cosmetic bioproducts. The acidity and saponification as well as the iodine and peroxide indexes were evaluated and fatty acid profiles for the samples obtained by GC-MS. The sensory properties of the butters showed the appearance of solid to soft cream, color (yellow, brown, buttercup, and ochre), and characteristic odor. The melting temperatures of all butters ranged between 31 ºC and 49 ºC. The acidity, saponification, iodine and peroxide indexes for the butters were of 5.82 – 17.73 mg (NaOH or KOH) g⁻¹, 181.10 – 573.55 mg KOH g⁻¹, 2.78 – 44.96 gl² 100 g⁻¹, and 1.39 – 9.30 meq kg⁻¹, respectively. From analyses of the fatty acid profiles, the major components identified were lauric acid in murumuru (40%) and ucuuba butters (73%), myristic acid in tucuma butter (53%), and palmitic acid in bacuri butter (42%). In general, the results of the analyses differed from the specifications of the supplier reports and official compendia. These findings highlight the importance of quality control in natural raw materials to ensure their functionality in pharmaceutical and cosmetic bioproducts.

Keywords: Amazonian butters. Fatty acids. Biocosmetics. Herbal medicines. Physicochemical properties.

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1 INTRODUCTION

In recent years, scientific reports have highlighted the increasingly harmful effects of synthetic excipients present in pharmaceutical, food and cosmetic products on human health\(^1,2\). As a result, there has been a significant increase in natural products on the market, which has led Research and Development (R&D) to look more closely at the use of renewable and biodegradable raw materials\(^3,4\).

In this context, the Brazilian Amazon has been distinguished for its natural reservoir of active principles and excipients\(^5\). The functionalities of natural ingredients as emollients, hydrators and other biological agents can be an alternative to synthetic components, thereby enhancing the value of pharmaceutical and cosmetic products\(^5\). This new market for health and beauty, known as the “green market”, aims at providing a sustainable production chain\(^6,7\).

Amazonian butters, such as murumuru (Astrocaryum murumuru Mart.), bacuri (Platonia insignis Mart.), tucuma (Astrocaryum vulgare Mart.), and ucuuba (Virola sebifera Aubl.) can be used as ingredients in biocosmetics and pharmaceutical products\(^8-10\). Murumuru butter has high oil content (~ 40%) with unsaturated fatty acids. It is used as a food supplement as well as in cosmetics e.g. soaps and bases\(^11\). Bacuri butter has high potential as a natural antioxidant source\(^12\). Tucuma butter, rich in vitamin E, is used in creams and sweets\(^13\). Ucuuba butter is mainly composed of saturated fatty acids and it has been used in the production of cosmetics\(^14\). Moreover, studies have also highlighted anti-hyperglycemic\(^15\) and antioxidant activity in tucuma pulp oil\(^16\).

It is important to highlight the change in focus of the Amazonian production chain from its original use in commodities. In this context, R&D strategies have been carried out in order to produce technological applications with high added value. These strategies have included bioproducts. For example, murumuru butter has been used in the development of liquid crystalline systems for sustained release of bioactive substances in the skin and mucous membranes\(^9\). Américo et al.\(^8\) demonstrated a better in vivo performance of a liquid crystalline system for topical use containing murumuru butter and ethanolic extract of jucá (Libidibia ferrea Mart. ex Tul.) than the commercial ointment of allantoin in a study investigating wound healing.

Due to current relevant use of the sustainable raw materials in pharmaceutical and cosmetic bioproducts, possible adulteration or inadequate handling of oils and fats, this current study aims to evaluate the sensory and physicochemical properties and fatty acid profiles of Amazonian butters (murumuru, bacuri, tucuma and ucuuba) from methodologies established for the characterization and quality control of natural raw materials that are in compliance with the minimum criteria required by the Brazilian Health Regulatory Agency (Anvisa).

2 MATERIAL AND METHODS

Murmururu, bacuri, tucuma, and ucuuba butters were obtained from a specialized company of vegetable raw materials located in the city of Belém, Brazil. The butters were extracted from plant seeds, manufactured in April 2019 and their expiration date was 24 months. All solvents and chemicals used in this study for analyses of the butters samples were of analytical grade.

2.1 Sensory and physicochemical properties of the butters

The determination of sensory characteristics followed the methodology recommended by the Brazilian Pharmacopoeia for evaluation of appearance, color and odor of the butters\(^17\). The physicochemical properties were performed (melting point, acidity index, saponification index, iodine index, peroxide index, and fatty acid composition). The results were compared with those of the supplier reports and official compendia.

The determination of the melting point followed the methodology applied by the Aldolfo Lutz Institute (008 / IV)\(^18\). The tests were carried out according to the capillary method using a calibrated electrical device (melting point PMF II, MS Tecnopon), supplied with an electrically
heated metal chamber and adapted to a microscope for observation of the melting process. The locks were introduced at a height of 3 cm in glass capillaries containing the samples, with one end closed. The capillaries were left in the refrigerator for a few hours before analysis to avoid premature melting. The readings were performed on the device with the aid of a thermometer and ranged between −10 °C and 200 °C, with divisions of 1 °C. The analyses were performed in triplicate. The results were compared with those of the supplier reports.

For acidity index (AI), an aliquot of approximately 1.0 – 2.0 g of each sample (n = 3) was weighed in a conical flask and then melted at 30 °C – 50 °C. In order to solubilize the raw materials, 50 mL of 96% ethanol and ethyl ether (1:1 v/v) was added to the murumuru and bacuri butters, and 25 mL of a mixture of 96% ethanol and ethyl ether (1:2 v/v) to the tucuma and ucuuba. Three drops of 1% phenolphthalein alcoholic solution were then added as an indicator and titration, using 0.1 M KOH or 0.1 M NaOH solution was performed. The mixture was stirred consistently until a persistent pink color was observed for at least 30 s. The blank test was performed under the same conditions without the sample. The volume of titrant used and the calculation of the acidity index (mg KOH g⁻¹ or mg NaOH g⁻¹) was in accordance with the following equation 1:

\[ AI = \frac{V \times f \times M \times 28.2}{W} \]  
(Eq.1)

where, \( V \) = volume of 0.1 M KOH or 0.1 M NaOH titrant used (mL), \( f \) = correction factor of titrant solution, \( M \) = molarity of the NaOH or KOH solution, and \( W \) = weight of the sample (g). The results were compared with those of the supplier reports.

For saponification index (SI), approximately 2.0 – 4.0 g of each sample (n = 3) was weighed and melted at temperatures below 50 °C. 25 mL of 0.5 M KOH methanolic solution was then added to samples of murumuru and bacuri and 50 mL of the 4% (w/v) KOH alcoholic solution to tucuma and ucuuba. The vertical flow condenser was adapted to a water bath until boiling, for 30 – 60 min. 0.5 mL of phenolphthalein solution was then added and titrated with 0.5 M HCl solution until the pink color disappeared. The blank test was performed under the same conditions without the sample. The calculation of the saponification index (mg KOH g⁻¹) was obtained from the following equation 2:

\[ SI = \frac{(B - A) \times f \times 26.06}{W} \]  
(Eq.2)

where, \( A \) = volume of 0.5 M HCl titrant used (mL), \( B \) = volume of 0.5 M HCl used in the titration of the blank (mL), \( f \) = correction factor of 0.5 M HCl solution, \( W \) = weight of the sample (g). The saponification index determines the amount of potassium or sodium hydroxide needed to neutralize free acids and saponify the esters in 1 g of substance (mg KOH g⁻¹). The results were compared with those of the supplier reports.

For iodine index (II), 0.2 g of the samples of murumuru and bacuri (n = 3) were weighed in a conical flask. After, it was added 15 mL of chloroform and 25 mL of iodine chlorine solution (Wijs solution). The well protected flask was capped for 30 min and frequently shaken. 10 mL of a KI solution (100 g L⁻¹) and 100 mL of water were then added to flask and titrated with 0.1 M Na₂S₂O₅·5H₂O solution, stirred vigorously until the yellow color disappeared. 5 mL of starch solution 1% was added and the titration continued. The Na₂S₂O₅·5H₂O solution was dropped in steadily until the color disappeared. The blank test was performed under the same conditions without the sample. To determine the iodine index i.e. g of iodine per 100 g of fat (gI₂ 100 g⁻¹), the following equation 3 was applied:

\[ II = \frac{(B - A) \times M \times 12.69}{W} \]  
(Eq.3)

where, \( A \) = volume of 0.1 M Na₂S₂O₅·5H₂O titrant used (mL), \( B \) = volume of 0.1 M Na₂S₂O₅·5H₂O solution used in the titration of the blank (mL), \( M \) = molarity of the Na₂S₂O₅·5H₂O solution (mol
The sensory analyses of the murumuru, bacuri, tucuma, and ucuuba butters. It was observed that all butters rendered similar results when compared with those of the supplier reports, except for the color parameter of the murumuru and tucuma which showed a yellowish color instead of beige/white or ice white, respectively.
All samples were melted below 50 °C. The mean melting point temperatures of the murumuru and bacuri were 32 °C ± 0.58 °C and 42 °C ± 0.58 °C, respectively, while the melting points of tucuma and ucuuba were 31 °C ± 1.00 °C and 49 °C ± 0.58 °C, respectively.

Table 2 shows the results for the acidity and saponification as well as the iodine and peroxide indexes for Amazonian butters. The acidity indexes of the butters, excepting tucuma, were higher than those described by supplier reports (9.90 − 17.70% in oleic acid) and by Brasil21 (< 5% in oleic acid).

The results obtained for the saponification index (Table 2) were higher than the values found in their supplier reports for murumuru and bacuri, considering the range of 200 – 300 mg KOH g⁻¹ as predicted by the Brazilian Pharmacopoeia17, whereas tucuma and ucuuba butters were in accordance with their specification.

The iodine indexes for murumuru (11.49 gl² 100 g⁻¹) and bacuri (44.96 gl² 100 g⁻¹) butters were lower than those described in their supplier reports. Although, the supplier report provided an iodine index of 14 gl² 100 g⁻¹ for this butter, despite its fatty acid composition reading as 100% saturated. The results of the peroxide indexes obtained in this study for all butters were lower than those presented in the supplier report (10 – 15 meq kg⁻¹) and Brazilian Pharmacopoeia (< 15 meq kg⁻¹).

From the chromatographic analyses of the butters, it was possible to characterize them according to their fatty acid composition (Table 3 and Supplementary Material A1-A4). A total of 15 fatty acids were quantified (g 100 g⁻¹ of butter).

Murumuru butter showed the majority of its fatty acids as lauric and myristic, representing about 70% of the total mass, while bacuri butter presented palmitic and oleic as its major fatty acids (> 70% of the total mass), corroborating with the results of their supplier report.

The fatty acid profile of the tucuma butter extracted from almonds shows the major acids as myristic (53.26%), lauric (19.63%) and other fatty acids such as palmitic (6.94%), elidic (5.80%), and myristic acid derivate (4.05%). These results differ when compared to those found in the supplier report, in which lauric acid showed a percentage of 44 to 55% and myristic acid of 22 to 30% of the total mass of fatty acids.

Regarding the fatty acid composition of ucuuba butter, the evidence suggests a predominance of saturated fatty acids such as lauric acid (73.30%). The composition of this butter also includes myristic (11.18%) and caprylic acids (11.63%). These values do not corroborate with the specifications provided in the supplier report, in which the majority is myristic (72 - 76%), lauric (16 - 20%), and palmitic acids (7 - 9%).
Table 3. Fatty acids profile of the Amazonian butters. NS: not specified.

| Fatty acid          | Composition | Murumuru | Bacuri | Tucuma | Ucuuba |
|---------------------|-------------|----------|--------|--------|--------|
| Caprylic acid       | C 8:0       | NS       | NS     | NS     | 11.63% |
| Capric acid         | C 10:0      | NS       | NS     | 1.46%  | 3.80%  |
| Lauric acid         | C 12:0      | 40.00%   | 1.55%  | 19.63% | 73.30% |
| Myristic acid       | C 14:0      | 29.30%   | 1.24%  | 53.26% | 11.18% |
| Palmitoleic acid    | C 16:1      | NS       | 10.79% | NS     | NS     |
| Palmitic acid       | C 16:0      | 8.90%    | 42.23% | 6.94%  | NS     |
| Linoleic acid       | C 18:2      | 3.90%    | 3.90%  | 1.48%  | NS     |
| Oleic acid          | C 18:1      | 11.40%   | 28.80% | NS     | NS     |
| Stearic acid        | C 18:0      | 5.60%    | 2.52%  | 1.29%  | NS     |
| Elidic acid         | C 18:1(trans-9) | NS     | NS     | 5.80%  | NS     |
| Eicosanoic acid     | C 20:0      | 0.61%    | 1.36%  | NS     | NS     |
| Behenic acid        | C 22:0      | 0.16%    | NS     | NS     | NS     |
| Montanician acid    | C 28:0      | NS       | 0.12%  | NS     | NS     |
| Palmitic acid derivate | C 36:0 | NS       | 3.81%  | NS     | NS     |
| Myristic acid derivate | C 14:0 | NS       | NS     | 4.05%  | NS     |

4 DISCUSSION

Sensory analyses are recommended by official compendia in order to certify the identity of raw materials and the evaluation of each product. These tests measure perceptible characteristics important to the consumer, such as appearance, color and odor, which are decisive factors in their acceptance. The murumuru and tucuma butters showed a specific yellowish color instead of beige/white or ice white, respectively. The color is one of the first aspects noted for evaluating a product. Color changes from a specified pattern indicate an alteration in the material, which may result in rejection of the product by the consumer. Changes in the physical and chemical properties may also influence the buyer.

It is worth mentioning that the quality of these plant-based products is also related to the cultivation, harvesting, extraction methods and storage conditions of the raw plant material. These parameters can influence the chemical composition of the butters. Therefore, it would be important to invest in cultivation logistic to ensure the physicochemical quality. The handling and packaging for the removal of fractions of the product and their exposure to light and oxygen can result in changes to the physical and chemical stability of the plant material.

Oils and butters are lipophilic substances formed mainly by fatty acids, which may be free or associated with mono- and diacylglycerols, triglycerides, tocopherols, and steroids. The classification of lipids in oils and fats depends on their melting point, and hence, the type and organization of these fatty acids is important. Oils are liquid and butters are pasty at 25 °C.

It is worth observing that both bacuri and ucuuba butters showed different melting temperatures in relation to their specifications, suggesting an alteration in their fatty acid composition. Pereira et al. demonstrated that murumuru and tucuma kernel fat was completely melted at 35 °C, corroborating with our results.

The pure bacuri fat melted around 53 °C, but when blended with Brazil nut oil tended to melt around 46 °C. This finding may explain the higher melting point found for this butter. Ucuuba butter showed the highest melting point. This differed significantly from the work of Pardaual et al. which identified a melting point of 37 °C, suggesting possible contamination/adulteration in this butter. Fats with lower melting points are liquid at body temperature, which it interesting for the oral, vaginal and buccal administration of bioproducts. On the other hand, butters with higher melting temperatures had lower plasticity, which could be useful for room temperature storage.
There are no specific monographs for Amazonian butters, so data for oils and fats in general were used as well as oils similar to the chemical composition of the butters. The determination of the acidity index indicates the deterioration in fats and oils. This is denoted by the presence of free fatty acids, which usually accompany the rancidity process, and may come from the hydrolysis of the esters present in their composition. This reaction may be accelerated by heating or exposure to light. Thus, the acidity index assesses the conservation status of oils and fats.

The acidity index results for the butters, excepting tucuma, registered higher than their specifications. This increase may have occurred due to high temperature and humidity, characteristics of the Amazon region climate, increasing the free acidity of the butters. Galvão reported acidity index values higher than those specified by Anvisa for vegetable oils and butters.

Pereira et al. found acidity indexes of 5.16 and 20.98 mg KOH g \(^{-1}\) for murumuru and bacuri butters, respectively. The higher acidity index for bacuri butter corroborates with our results. This may have occurred due to inadequate handling and storage of the raw material. A decomposition process, either by hydrolysis, oxidation or fermentation, in general, alters the concentration of hydrogen ions, influencing the pH of the formulation, and hence, changing drug solubility.

In relation to the saponification index, the analyses showed that the fatty acids of the samples have lower molecular weights, suggesting non-compliance with the results of the supplier reports for murumuru and bacuri. The lower the molecular weight of fatty acid (or short chain), the higher the saponification index. Thus, the saponification index may indicate changes in the molecular chain of oils or butters, possibly related to adulteration of the product. Aliphatic alcohols, tocopherols, sterols, phenols and pigments dissolved in fats and oils cannot be saponified.

The iodine index indicates the degree of unsaturation of esterified and free fatty acids and the presence of high carbon chains in a sample. Therefore, the greater the amount of unsaturated fatty acids, the higher these values. This parameter is indicative of the purity of the material, and hence the presence of adulterants.

The iodine indexes for murumuru and bacuri butters were lower than those described in their supplier reports and official compendia (6-60 gl\(_2\) 100 g\(^{-1}\)). Pesce carried out the physicochemical characterization of the murumuru and bacuri butters, obtaining iodine indexes of 12.40 and 57 gl\(_2\) 100 g\(^{-1}\), respectively, while Lima et al. reported iodine indexes of 13.12 and 33.09 gl\(_2\) 100 g\(^{-1}\), respectively. These results corroborate with those found in this current study. The higher the unsaturation of a fatty acid, the greater the ability to add iodine to molecule (involving the double bonding of unsaturated fatty acids and triglycerides), and hence, the higher the iodine index.

The iodine index for tucuma (2.78 meq kg\(^{-1}\)) was lower than the other butters, possibly due to higher saturated fatty acids, as highlighted by GC-MS. On the other hand, this method was not applied to ucuuba butter because unsaturated fatty acids were not identified in its composition by GC-MS. However, its supplier report provided an iodine index of 14 gl\(_2\) 100 g\(^{-1}\) for this butter, despite its fatty acid composition being 100% saturated.

The peroxide index indicates that the butters have not yet undergone the oxidation process, corroborating with the lack of change in relation to their sensory characteristics. The quality of these raw materials increase their value for use in pharmaceutical and cosmetic products. The peroxide index is an important indicator of the quality of oils and fats, as it highlights the degradation properties of the raw material, and hence the lipid matrix in drug delivery systems.

The results of the peroxide index obtained in this study for all butters were lower than those found in the supplier reports (10 – 15 meq kg\(^{-1}\)). Pereira et al. found a peroxide index of 2.14 ± 0.16 meq kg\(^{-1}\) for tucuma butter. The specifications from supplier reports cite values of up to 10 meq kg\(^{-1}\) as accepted criteria for cosmetics. However, the absence of specific
legislation to determine the quality of vegetable butters for this purpose allows a maximum acceptable peroxide index value of up to 15 meq kg$^{-1}$ for cold pressed oils$^{21}$.

A study by Cordeiro et al.$^{14}$ showed values for the peroxide index of 2.08 ± 0.10 meq kg$^{-1}$ when extracted by the Supercritical Fluid Extraction (SFE) technique and 1.00 ± 0.47 meq kg$^{-1}$ after extraction by Soxhlet, indicating that the extraction process may influence the peroxide index of a particular butter. These results for this parameter corroborate with those of the present study for ucuuba fat.

The determination of the fatty acid composition from the analyses of methyl esters containing 4 to 24 carbon atoms in a sample, may help to detect possible fraud and evaluate the chemical content of oils and fats from vegetable and animal sources. Fatty acid methyl esters were separated, identified and quantified by gas chromatography$^{18}$.

Pereira et al.$^{11}$ found similar results to those obtained in the present study for the composition of murumuru butter. Due to its chemical composition, being rich in unsaturated fatty acid, this butter showed potential properties for the development of nanoparticles$^{36}$ and biocosmetics$^{15}$. Bacuri butter presented palmitic and oleic acids as its major fatty acids, corroborating with the results of the supplier report. Pereira et al.$^{11}$ and dos Santos et al.$^{31}$ also reported these two fatty acids as major in studies of the chemical composition of this particular butter. It has since been used as an antioxidant agent in pharmaceutical products$^{37}$ and cosmetics$^{38}$.

Bacuri butter can also be used in the production of lipid nanoparticles in skin products. As it is rich in tripalmitin (PPP), a triglyceride derived from palmitic acid, it is responsible for high absorption and penetration through the skin$^{11}$. It also contains high concentrations of palmitoleic fatty acids compared to other oils, which gives it emollient and humectant properties, resulting in a formulation with high hydration capacity. It can therefore, be used as an alternative to beeswax in the production of lipsticks, for example, reducing the cost of the final product$^{10}$.

Pardauil et al.$^{28}$ reported that the fatty acid profile of tucuma oil from the kernel also showed a high content of saturated fatty acids (48% of lauric acid, 25% of myristic acid, and 13.50% of oleic acid), corroborating with our results. In general, the specifications of the butters were divergent to those issued by supplier, reinforcing the need confirmation of the results of the supplier reports of natural raw material as well as the qualification of the suppliers, ensuring the quality of the bioproducts, as pointed by Nunes et al.$^{39}$.

5 CONCLUSIONS

This study highlights the importance of quality control, standardization, and specific monographs in natural raw materials for pharmaceutical and cosmetic applications, mainly in the sensory and physicochemical analyses of Amazonian butters (murumuru, bacuri, tucuma, ucuuba), considering that some specifications differ when compared to supplier reports and official compendia. Inadequate transport, poor storage, bad handling, and possible adulteration can significantly contribute to the degradation of raw materials and reduce the shelf life of the bioproducts. Further studies will be required to qualify them for use as sustainable raw materials in the production of biocosmetics and crystalline liquid systems in sustained drug release.

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**Authors' contributions**

JMF, TSAS, AEFX – participated in the study design, performed experiments, contributed equally to this work to obtaining the results, and drafted the manuscript; WCSR, ACES, CVPC, FSA, RHVM – participated in the study design, performed experiments, contributed to obtaining the results, and drafted the manuscript; EGO, KMN – coordinated the work and critically reviewed the manuscript; All authors read and approved the final manuscript.