EDITORA ENTERPRISING

Incremental Innovation: Analytical Methods And Integrated Techniques Applied For Biotechnological Characterization Of Remaining Fruit Seeds In Northern Brazil

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Abstract

Brazil is a multi-diverse country, that is, it has great biological diversity, which has been gradually studied in higher education and research institutions in order to obtain greater knowledge and consequently provide more efficiently and effectively, products, processes for the market and even new methodologies. However, in some of these institutions, there is a certain difficulty, especially with regard to the first biotechnological studies related to the characterization of plant materials, some still unknown to researchers/students, which are necessary and require different methodologies that not always are possible to be applied in the same way as other institutions, such as those in the Midwest, South and Southeast. In this context, the objective was to define analytical methods and laboratory techniques to be integrated and applied sequentially, with incremental innovation, for biotechnological characterization of remaining seeds of fruits in the extreme north of Brazil, with a view to obtaining important knowledge, useful to scientists and researchers who envision the development of assets, processes and bioproducts of interest to society. Procedures were developed/adapted/adjusted to perform physical and physicochemical analyzes of mineral compounds, bioactives, pigments and verification of the main functional groups present in the remaining seed samples from a research carried out with the fruits of the Myrciaria dubia species. Based on the results obtained in the studies and analysis of M. dubia seed samples, it was verified that the analytical, classical and instrumental methods, and integrated techniques, when applied sequentially, provide scientists and researchers with important and unprecedented knowledge about the biotechnological characteristics of the remaining fruit seeds, as well as the development of assets that are beneficial to society.

Keywords: Biological diversity. Innovation. Methodology. *Myrciaria dubia.* Seeds.

INTRODUCTION

Brazil is a multi-diverse country, that is, it has a great biological diversity, which has been gradually studied in higher education and research institutions in order to

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obtain greater knowledge and consequently make available, with more efficiency and effectiveness, new methodologies, products and processes to the market.

However, it is known that not all institutions, in all regions of Brazil, have sufficient instruments and resources to satisfactorily meet the aforementioned points, especially those located in the North of Brazil, one of the great holders of biological diversity. In these institutions there is a certain difficulty, especially with regard to the first biotechnological studies related to the characterization of plant materials, some still unknown to the researchers/students of these institutions, which are necessary and require different methodologies, which are not always possible to be applied in the same way as other institutions, such as those in the Midwest, South and Southeast.

A possible solution to alleviate the aforementioned problem involves more modest improvements/adequacies/replacements, without forgetting, as well as keeping them at the same technological level on which they focus. It is incremental innovation, which generates continuous improvements and support in the various stages of the life cycle of a product or process and which, in this sense, generates incremental improvements in the performance or quality indicators where they are applied (AUDY, 2017).

Therefore, the present study intends to answer the following question: Do analytical methods and laboratory techniques selected and applied sequentially with incremental innovation allow the biotechnological characterization of remaining fruit seeds in Northern Brazil?

In this context, the objective was to study and define analytical methods and laboratory techniques to be integrated and applied sequentially, with incremental innovation, for biotechnological characterization of remaining seeds of fruits in the extreme north of Brazil, with a view to obtaining important knowledge and useful to scientists and researchers who envision the development of assets, processes and bioproducts of interest to society.

The seeds and peel of various fruits, remnants of the agro-industrial processing, have been studied in various fields of science in search of full potential exploitation, mainly in the form of bioproducts, such as products made from renewable resources, agriculture, forestry, and aquaculture, via biotechnological processes.

As an example, there is the fruit of *Myrciaria dubia* (Kunth) McVaugh, internationally known as camu-camu, which is present throughout the Amazon. Originating from a small shrub (3-8 m high), dispersed in the central region of the state of Pará to the Peruvian Amazon. It can be found in its natural state along streams and rivers or permanently flooded regions (Yuyama et al., 2011). (Yuyama et al., 2011).

Specifically in the northern Amazon, prospected by Chagas (2010) since 2010, at least 53% of the municipalities of Roraima have cacari trees. They have great economic and nutritional potential, especially given the high content of vitamin C, as found in fruits, up to 7,355 mg of ascorbic acid per 100 g^{-1} pulp (Chagas et al., 2015). However, in the literature, there are few studies on the technological potential of *M. dubia* peels and seeds. The seed has an equivalent weight ranging between 14 and 27% of the total fruit weight (Yuyama et al., 2011). Therefore, it is estimated up that seeds account for 270 kg of each metric ton of processed fruits, and a greater appreciation of this byproduct/waste is necessary.

MATERIALS AND METHODS

In the biotechnological characterization of remaining seeds of fruits in the North of Brazil, analytical, classical and instrumental methods and techniques integrated sequentially were studied, selected and applied, according to the resources, reagents, materials and instruments offered and available in educational and research partners, established in the North of Brazil. In these institutions, it was possible to develop/adapt/adjust procedures to perform physical and physicochemical analyzes of mineral compounds, bioactives, pigments and verification of the main functional groups present in the samples of seeds remaining from a research carried out with fruits of the species *Myrciaria dubia*.

The study was conducted in two stages: characterization of *M. dubia* (camu camu) seeds and identification of sources of compounds and bioproducts with potential for green biotechnology. The bioproducts were developed with different experiments/activities in three aspects: systematic and legal exploitation, location, and evaluation. The systematic and legal exploitation was carried out through observations of the legal measures of sustainability and environmental preservation, appropriate and necessary. The location of the raw material was carried out from the study of biodiversity and conventional and biotechnology techniques aimed at domestication, improvement, and evaluation of the native Amazonian fruit (Chagas, 2010).

As for evaluation of seeds (raw material), the fruits were collected from natural populations, as mapped by the research team, in eleven areas (63%) of occurrence (Chagas et al., 2015) and experimental areas belonging to the experimental field of Serra da Prata Embrapa Roraima, located in Mucajaí, state of Roraima, northern Amazon.

In addition, samples were collected from branches of flowering plants for herborization, which were submitted to authentication process in the Department of Botany, UFRR, Boa Vista-RR and deposited in the herbarium, in the form of dried specimens with six samples, obtaining the accession number 4922-4927. After collection, the fruits were properly packed in Styrofoam box with ice and transported to the Laboratory Center of Embrapa Roraima for the activities of identification of sources of compounds.

Initially, the fruit samples were subjected to biometric evaluation by quantifying the fruit mass (g), seed, peel, pulp, and juice. Finally, we evaluated the biometrics of seeds using a digital caliper to compare and validate the biometric characterization with the System for Seed Analysis (SAS) and to obtain the phenotypic information related to fresh and bioprocessed seed samples. The design was completely randomized with five replicates and 100 fruits per repetition.

For analysis of seeds in the SAS, the samples were processed in different ways, with five treatments: T1 - mature wet seeds, T2 - seeds pre dried in circulation air oven at 65 ° C ± 5 ° C, T3 – seeds pre dried and peeled from their protective film, that is, only the grains, T4 - semi-mature wet seeds, and T5 - semi-mature wet seeds peeled from the protective film.

The seed samples, separated according to each treatment, were placed into trays made of transparent film, type A4 - 210 x 297 mm with the dimensions of 19 cm x 26.5 cm x 1 cm for image analysis via SAS (System for Seed Analysis) (Sousa et al., 2017).

The preparation of samples for potential compound sources with identification for green biotechnology was performed using the simple Standard Operating Procedure (SOP). It consists of five steps: a) pre-treatment, b) inspection, c) preparation, d) repair and maintenance, and e) safety measures. From this procedure, we obtained the bioprocessed material as powder originating from seeds with and without film being part of this material. We kept for a year at an average temperature of ± 25º C. They were analyzed in specialized laboratories to obtain bioprospecting information on the physical and physico chemical characteristics, sources of formula (bioelements, trace elements, bioactives, and pigments), and main functional groups present in these bioproducts.

In addition, we used/adapted protocols and analysis methods in biotechnology laboratories for the analysis of protein, carbohydrate, and vitamin C from Univates (Univates, 2014), IAL (2008), and the Brazilian Pharmacopoeia (Brasil, 2010) for bioprospecting and comparative analysis of fresh and bioprocessed seeds.

The analysis of the metabolic intermediates and biomolecules was carried out from samples prepared and processed as powder, obtained via sieving, as Standard Operating Procedure (SOP) used in specialized laboratories of the Brazilian Agricultural Research Company in Roraima. Then, we obtained in triplicate, the pH (acidity), water content, and mineral constituents of the powders, based on dry matter, obtained via conventional methods (stove and oven) recommended by the Brazilian Pharmacopoeia (Brasil, 2010).

We also performed phytochemical analyses through phytochemical tests to detect the presence of classes of secondary metabolites (bioactive), specifically phenol, by the method described by Matos (1997). These tests were based on visual observation of color change or precipitate formation after addition of specific reagents.

Furthermore, we quantified the pigments of fresh seeds, because of the importance of this characteristic in identifying compounds with potential for green biotechnology as a function of the fruit harvest stage (mature, immature, semi-mature). Quantification of the content of photosynthetic pigments was carried out through indirect measurement of chlorophyll content in fresh seeds using chlorophyll meter SPAD-502 (Soil and Plant Analysis Development) (Minolta, 1989).

We also used infrared spectroscopy, which is a fast and simple instrumental technique for the identification of functional groups present in the molecules of bioprocessed seeds and their fixed oil extracts. Therefore, we used a spectrophotometer FT - IR Shimadzu, Prestige 21 model in the range 4000-400 cm-1, KBr absorption spectra pads in the infrared region, and the summary correlation tables of frequencies existing and consolidated in the literature to identify the main functional groups.

The data and information obtained from the steps and methodological phases defined and followed in this work were validated from their transformation into results, which were presented in a doctoral thesis defended in 2016[2].

They are composed of descriptive statistics in tables, graphs and descriptive measures, as well as were submitted as necessary to analysis of variance by the F test and Tukey test at 5% probability and regression using the SISVAR program (Ferreira, 2011).

RESULTS

The *M. dubia* seeds had an average mass of 1.39 g (Table 1), which was higher (0.52 g) than that found by Bardales et al. (2014) in the Peruvian Amazon. According to Tango, Carvalho and Soares et al. (2004), generally, the physical parameters of the fruits vary among species and genetic varieties, according to degree of ripeness, as well as shell thickness, diameter of the fruit, and respective weight (mass), in addition to the determination of the yield on pulp. However, differences may exist in the yield between fruits of the same species according to the variety, as well as differences in size and mass of pulp, peel, and seeds (Bardales et al., 2014).

Table 1. Mass (g) of fruit, peel, pulp, ripe juice and mature seeds collected in the fruiting period *M. dubia* the occurrence areas in the northern Amazon, Roraima State, Brazil**.**

Variables	n = (500)					
	Minimum	Maximum	Median	Standard	Sample	Trust level

[2]SOUSA, R. C.P. **Bioprospecção e desenvolvimento de produtos com potencial biotecnológico a apartir de sementes de *Myrciaria dubia* (H. B.K) Mc Vaugh da Amazônia Setentrional**, Boa Vista - RR, 2016. 135 f.:il: (Tese de Doutorado em Biodiversidade e Biotecnologia) - Rede de Biodiversidade e Biotecnologia da Amazônia Legal, Programa de Pós-Graduação em Biodiversidade e Biotecnologia, Universidade Federal de Roraima, 2015.

				deviation	Variance	(95,0%)
Whole fruit	8,37	11,53	10,02	1,58	2,51	3,94
Peel	2,24	2,57	2,39	0,17	0,03	0,41
Pulp	3,18	5,21	4,20	1,02	1,03	2,52
Juice	1,63	2,49	2,03	0,43	0,19	1,07
Seeds	1,26	1,60	1,39	0,18	0,03	0,45

Fonte: (Sousa, 2016)

Regarding LBP topology used in image extraction for the characteristics of *M. dubia* seeds, SAS describes the space itself and its properties as proposed by Valente (2000), in which, under certain conditions, there is no distinction between a drawing and a copy, even if poorly made; for example, a circle is topologically equivalent to an ellipse. If observed in the form of images - LBP topology, Figures 1 and 2 seeds were analyzed using SAS.

Thus, among the data obtained (Figures 1 and 2) in the treatments performed, we found that the fresh *M. dubia* seeds showed 1.72 cm mean diameter, with amplitudes of 1.50 cm - 1.99 cm and 0.49 cm long. The minimum average diameter was 1.18 cm, with amplitudes ranging from 1.01 to 1.34 cm.

There was a variation in seed weight (Table 1) above 37% when compared to the average values obtained by Bardales et al. (2014) in the Peruvian Amazon, another place of occurrence of the species. However, variability was approximately 12% in their yield (Table 2).

Table 2. Mass variation (%) in the peel, pulp, juice and mature seeds (M) and semi-mature (SM) in three *M. dubia* the occurrence of areas in the northern Amazon, Roraima State, Brazil.

Areas	Place of occurrence	Trat	Peel	Pulp	Juice	Seeds
1	City of Cantá (Lago do Preto)	1M	26,76	37,97	15,04	**20,23**
		1SM	31,31	37,35	6,71	**24,63**

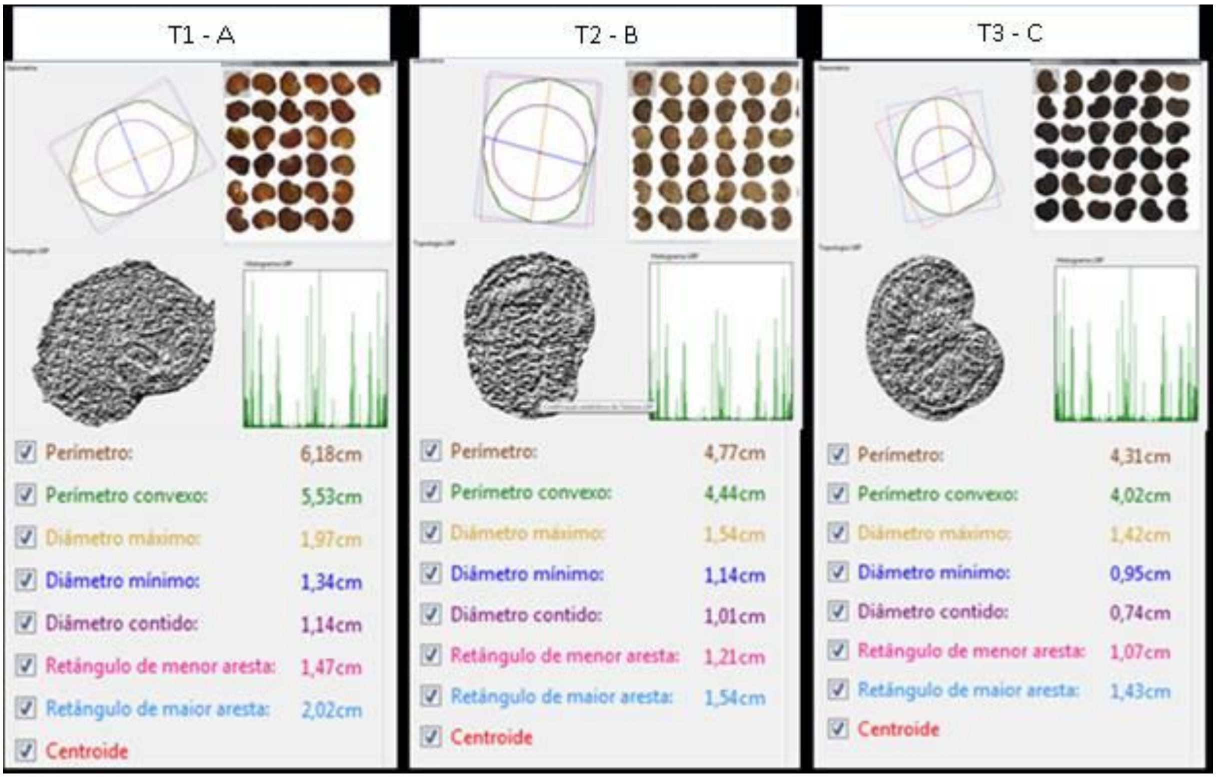
2	City of Caracaraí (Corredeiras do Bem-Querer)	2M	21,34	43,24	16,63	**18,79**
		2SM	24,80	42,87	6,40	**25,93**
3	City of Boa Vista (Lago da morena)	3M	23,38	41,49	13,81	**21,33**
		3SM	23,17	32,70	16,71	**27,42**
Median		-	25,13	39,27	12,55	**23,06**
Standard deviation (S)		-	3,53	4,05	4,77	**3,43**
Variance (V)		-	12,45	16,44	22,73	**11,79**

Fonte: (Sousa, 2016)

Thus, in this study we found and tested alternative methods to evaluate the above physical characteristics, among others. Figures 1 and 2 contain the results obtained via SAS. They list and present the results in the extraction of digital images on samples treated in different ways. They include geometry and numerical values of the perimeter, convex perimeter, maximum diameter, minimum diameter, and LBP and LBP histograms of fresh and bioprocessed *M. dubia* seeds.

In T1, T2, and T3, respectively, we present the inter-related, mature, wet seed (fresh) (A), pre-dried ripe seed (B), and predried ripe peeled seed (grains) (C) (Figure 1). The T4 and T5 treatments are the semi-mature wet seeds (A) and semi-mature peeled seeds (grains) (B), respectively, both fresh (Figure 2).

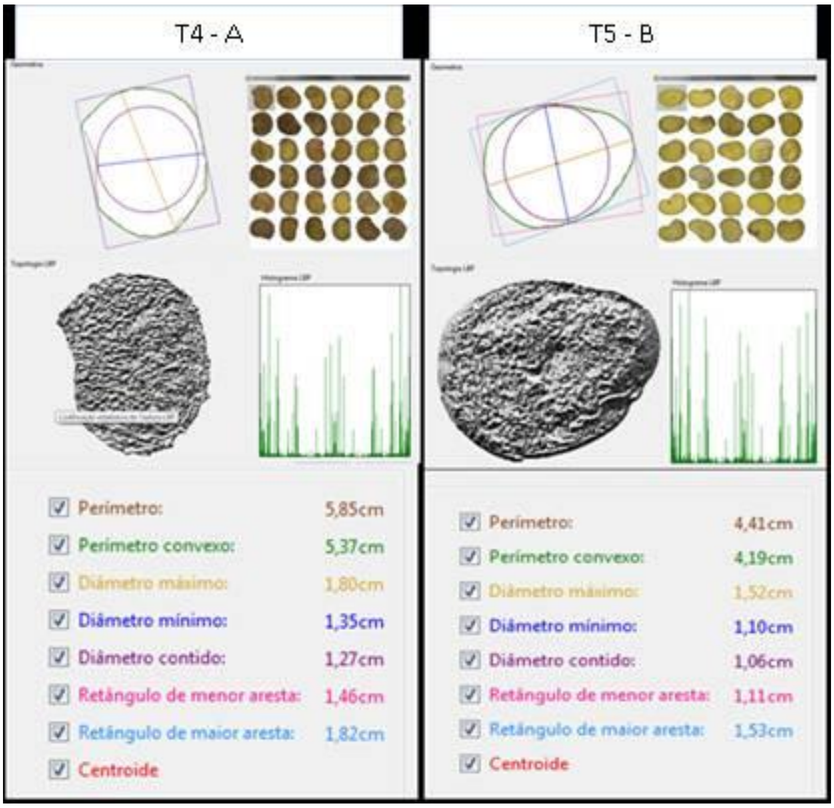
Fig. 1 - Geometry, topology and histogram LBP extracted in the selection of images of wet ripe *M. dubia* seeds (A); pre-dried ripe (B); and pre-dried ripe peeled from the protective film (grains) (C).



Fonte: (Sousa, 2016)

According to Villachica et al. (1996), the seed has roughened surface covered by knitted white fibrils, similar to the images obtained in the topology, except for the color of the fibrils. The embryo cotyledon is comprised of a green mass and can be clearly observed in Figure 2B. In addition, its format can also be seen, as the image topology, even when the seed is bioprocessed (Figure 1C).

Fig. 2 - Geometry, topology and LBP histogram extracted by the image selection wet seeds of semi-mature *M. dubia* (A) and peeled semi-mature seeds (grains) (B).

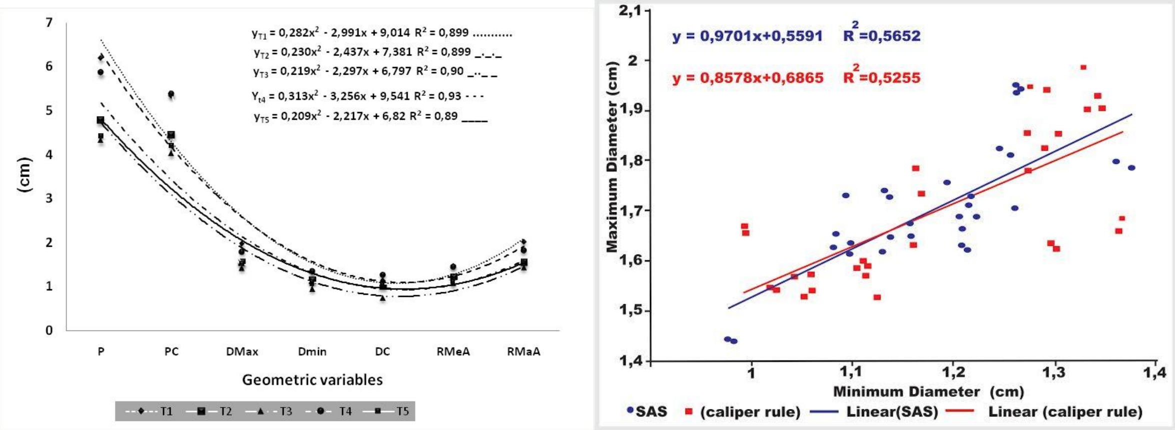


Fonte: (Sousa, 2016)

Figures 1 and 2 show images (LBP topology) of the *M. dubia* seeds (*M. dubia*) which have no endosperm, regardless of degree of ripeness. In the statistical evaluation concerning the methods used, SAS and caliper, linear regression was obtained in SAS R = 0.752, with estimated standard error = 0.080, and for Caliper R = 0.725, estimated standard error = 0.105. These results showed no significant difference between them (Figure 3B). Therefore, this is a satisfactory and possible indication of the use of SAS as an alternative tool to replace the manual method for physical characterizations of *M. dubia* seeds.

The figure 3 shows the values of the geometric dimensions measured in centimeters (cm) for the seven variables selected from the SAS and related in Figures 1 and 2, which show a comparative analysis of the five treatments established in this study and also the statistical statement obtained in a comparative analysis with SAS, with automated method (A) and caliper/manual method (B); specifically, the first treatment is shown in Figure 3B.

Fig. 3 - Comparative analysis of the treatments applied *M. dubia* seeds related to geometrical dimensions in centimeters (cm) of seven variables selected in the SAS (A) and benchmarking between automated method (SAS) and caliper / manual method (B).



Legend: (P - Perimeter, PC - convex perimeter; DMax - Maximum diameter; DMin - Minimum diameter, DC - convex diameter; RMEA - Rectangle lower edge; RMAA - Rectangle larger edge).

Fonte: (Sousa, 2016)

In Figure 3A, we can observe that both treatments differ in the geometric dimensions, which is an expected behavior because of the pre-processing performed and the difference in the degree of seed maturation. The general trend is that there is loss of yield, depending on the type of bioprocessing. However, the proposal was established as acceptable for use in the plant material.

Paradoxically, for the displayed phenotypic variations (Figure 3A and 3B), most of the components of the seeds do not differ qualitatively from constituents found in other plant parts, only quantitatively. The *M. dubia* fruits have important sources of different bioactive compounds and good source of minerals such as sodium, potassium, calcium, zinc, magnesium, manganese, and copper (Schwertz, et al., 2012).

In the process of identification of promising compound sources for green biotechnology, the dehydration process has been suggested by some researchers (Fracassetti et al., 2013), as an alternative for cacari ingredients in the form of powder, which may be used to preserve the nutritional value, vitamin C, and bioactive compounds, among others to be used in various food products. In this work, we obtained powder bioproducts from seeds with and without film (SBCP and SBSP), which yielded de husked seeds (Table 3) after preservation for a year at an average temperature of 25º C.

Table 3. Income obtained in filmless M. dubia seeds of bioprocessing, kept for a year at an average temperature of 25 ° C in the northern Amazon, Roraima State, Brazil.

Repetitions	Am. Uniform (%)	Am. Retained (%)	Am. Lost (%)	Time (min:seg)
Median	97,9	1,88	0,22	43:11
Mediana	97,85	1,80	0,23	43:20
Standard Deviation (S)	0,26907	1,8618	0,22405	00:30

Fonte: (Sousa, 2016)

The results (Table 3) and the yield of the raw material of bioprocessed *M. dubia* seeds are satisfactory, approximately almost 100%, which demonstrates that the applied drying and conservation were effective, as recommended by Simões (2007).

The physical and physico chemical characteristics and sources of compounds identified in the bioprospecting analysis of fresh and bioprocessed *M. dubia* seeds are shown in Table 4, and the mean values were obtained from representative *M. dubia* samples in the areas of occurrence in the northern Amazon.

We found in studies by Maeda and Andrade (2003) and Zanatta et al. (2005) that the pH of M. dubia fruits was between 2.4 and 3.2. The seeds analyzed (Table 4) showed higher pH, between 3.7 and 4.1. Both results are satisfactory, present acidic state, desirable technological characteristic in the delimitation of the time and the type of heat treatment for industrial processing, as in Maeda et al. (2006), which is a limiting factor for the growth of pathogenic bacteria and spoilage.

Table 4. Average values related to physical, physical-chemical and metabolic intermediates, bioelements, trace elements and bioactive compounds in bioproducts (SBCP and SBSP) seeds of *M. dubia* in the northern Amazon, Roraima State, Brazil.

Determinations	Treatments					
	SBCP*			**SBSP****		
	MPS*** 60±5ºC	MS**** (60±5) a (110ºC) Min.	MS**** (60±5) a (110ºC) Máx.	MPS 60±5ºC	MS (60±5) a (110ºC) Min.	MS (60±5) a (110ºC) Máx.
Hydrogen potential (pH)	3,7	Nd	Nd	4,1	Nd	Nd
Density (g/cm^3)	0,78	Nd	Nd	0,86	Nd	Nd
Lost moisture a 60±5ºC (%)	4,75	Nd	Nd	5,34	Nd	Nd
Moisture lost between 65 and 110ºC (%)	3,85	Nd	Nd	4,24	Nd	Nd
Total moisture 110ºC ±5ºC (%)	8,6	Nd	Nd	9,6	nd	Nd
Protein (%)	5,81	Nd	Nd	7,59	nd	Nd
Total lipids (ethereal extract)	0,82	Nd	Nd	3,13	nd	Nd
Total carbohydrates (sugars) (%)	83,73	Nd	Nd	76,66	nd	Nd
Total solids (Dry matter) (%)	91,40	95,96	100,00	90,42	95,52	100,00
Total mineral residue (%)	0,54	0,57	0,59	2,36	2,49	2,61
Mineral insoluble residue (%)	0,04	0,04	0,04	0,05	0,05	0,06
Soluble mineral residue (%)	0,50	0,52	0,55	2,31	2,44	2,55

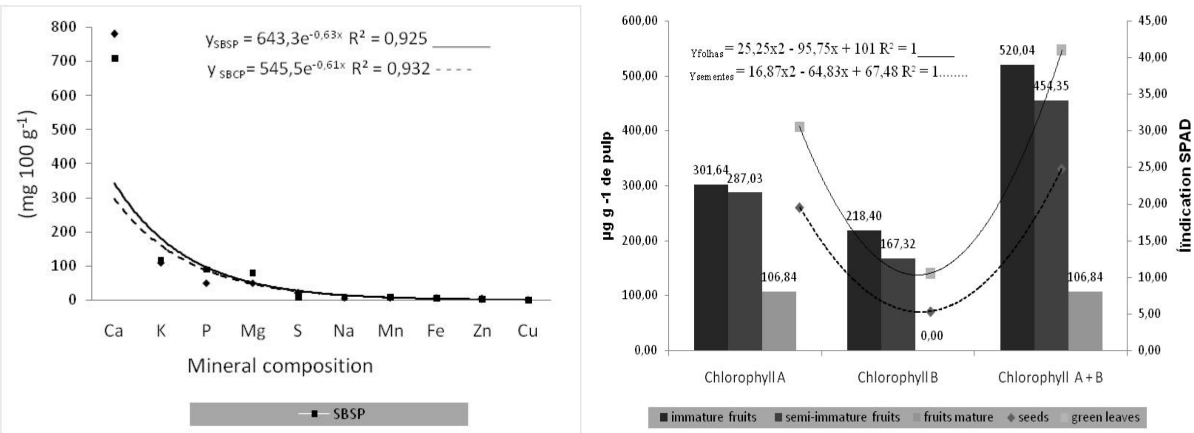
....................................**Bioelements**....................................					**Bioactive compounds**
Primary	Carbon (C) (%)	Nitrogen (N) (%)	Phosphorus (P) (%)	Sulphur (S) (%)	Total polyphenol (PT) (mg 100 g^{-1})
SBCP	53,04	1,01	0,05	0,02	5000

SBSP	52,00	1,32	0,09	0,01		4000
Secondary	Sodium(Na) (mg 100 g1)	Potassium (K) (%)	Calcium (Ca) (%)	Magnesium (Mg) (%)		Vitamin C (C_6H_8O) (mg100 g^{-1})
SBCP	7,80	0,13	0,78	0,05		16,42
SBSP	7,90	0,87	0,71	0,08		14,08
Trace elements (mg 100 g1)	Manganese (Mn)	Copper (Cu)	Iron (Fe)	Zinc (Zn)	Boron (B)	-
SBCP	7,50	1,00	7,50	2,80	1,00	-
SBSP	9,40	1,10	6,30	3,10	1,00	-

SBCP*= Seeds bio processed bio unpeeled; SBSP**= Seed peel bio processed without; MPS***= Almost dry vegetable matter; MS****= Dry vegetable matter; nd= not determined.
Fonte: (Sousa, 2016)

In Figure 4, we can comparatively see the mineral elements and pigments found in the seeds bioprocessed (SBCP) and without film (SBSP), and in Table 4, we can see by classes the bioelements, trace elements, and also one of the major bioactive compounds found in fruits, in the bioprocessed seeds also identified in cacari, presenting a considerable amount of phenolic compounds.

Fig. 4 - Mineral composition (A) in SBCP and SBSP and pigment content in seeds in nature and leaves correlated to the levels found in the fruits of *M. dubia* at different stages (B).



Fonte: (Sousa, 2016)

Regarding the fixed mineral residue, Table 4, its determination provides an indication of the amount of mineral elements in the sample (MAPA, 2016). The SBCP and SBSP for cacari showed average values of 0.54% and 2.36%, respectively, of fixed mineral residue, with a significant difference between them, and we found the following minerals listed in descending order: Ca> K> P> Mg > S> in> Mn> Fe> Zn> Cu (Figure 4A), which was close in SBCP and higher in SBSP as those obtained in the acerola seed flour (0.44g.100g-1) by Aguiar et al. (2010).

Density is an important measure related to physical properties, being widely used, according to Caesar et al. (2004), to distinguish pure material from crude material (or metal alloy), as the density of non-pure materials (mixtures) is a function of the composition, as well as in the identification and quality control of a given industrial product, also related to the concentration of solutions (extracts), which was purpose of this study. Moreover, as the amounts or masses to be measured in a certain tool vary depending on the density of each food or preparation, the obtained measurement values can help when purchasing equipment Almeida, Nunes and Andrade (2007).

We also obtained via Infrared Spectroscopy (IR), information about the functional groups present in the structure of substances of byproducts produced from *M. dubia* seeds, their powders and oil extracted. We obtained two infrared spectra, shown in Figure 5. We could observe qualitatively that the green-oil spectra (Fig. 5A) and pink powder (Fig. 5B) do not show significant differences. These results were expected because the oil is extracted directly from the powder of *M. dubia* seeds. Therefore, that there was no environmental interference in the process of extraction of the products.

The total water content obtained (Table 4) for the two bioproducts showed satisfactory results for the condition. They were lower than the maximum moisture value (15%) allowed by current legislation in Brazil (Brasil, 2005), corroborating Faroni et al. (2007), who mention that moisture content (water) below the maximum allowed value normally ensure the preservation of the quality of flour during commercial storage, similar to the results in this study. In addition, the recommendations of the Brazilian Pharmacopoeia (Brasil, 2010) establish a 8% to 14% residual moisture for plant drugs.

Therefore, according to Vasconcelos et al. (2011), these data are significant factors, as they will influence the conservation process, which combined with proper storage, maintain the quality of plant species, and the stability and preservation of their therapeutic properties. In this sense, the seeds were studied in relation to the chemical composition of their reserves and such interest is useful not only for the nutritional content, but also in the manufacture of industrial products (Buckeridge and Tiné, 2004; Corte, 2006), among many other purposes.

Moreover, the study of the chemical composition is of practical interest for seed technology, because the power of the seed storage potential is influenced by the content of the present compounds (Carvalho and Nakagawa, 2000; Corte, 2006). Thus, Table 4 also show the results related to metabolic intermediates (biomolecules), in the study of bioproducts of cacari seeds, kept for a year at an average temperature of 25º C.

The *M. dubia* seeds even when bioprocessed and preserved for a period of one (1) year showed good protein sources, of 5.81 and 7.59%, respectively for SBCP and SBSP (Table 4), with higher values than those obtained by Chagas et al. (2015) in the fruits at different stages of maturation, averaging 1.24% of crude protein. In addition, our values were higher than the results obtained by Vieira et al. (2008) in the mango residue (3.87%) and Boari Lima et al. (2008) (1.17%) in jabuticaba (*Myrciaria cauliflora* Berg) seeds. Only for SBSP the obtained protein (7.59%) are similar to those obtained for maize flour or maize flour (7 and 8%) and those obtained by Lousada Jr. et al. (2006), approximately 10.54% for acerola seed flour and the byproducts of some fruits such as pineapple (8.35%) and guava (8.47%).

Regarding the levels of lipids and carbohydrates in both SBCP and SBSP seeds, we had better results (Table 4) than those obtained by Chagas et al. (2015) in cacari fruit, averaging 0.25% and 3.34%, respectively. Our values were superior only in SBSP (3.13%) for the lipid content observed by Borges, Bonilha and Cordeiro (2006) for jackfruit seed flour (1.13%), and both (SBCP and SBSP) by Boari Lima et al. (2008) with jabuticaba seeds (0.58%). On the other hand, the average content of lipids (3.92%) was lower than that found by Aguiar et al. (2010) in acerola seeds, but higher in carbohydrates (83.3 and 76.66%) (57.24%), which is a significant amount, as well as compared to recognized and consumed food, cited in RFN (2016), such as oats (68%), wheat (70%), corn, rye and barley (73%), and also similar to the rice value (77%) of SBSP (76.66%).

Consequently, SBCP and SBSP of *M. dubia* also showed high total solids content (91.40 and 90.42%) similar to that obtained by Aguiar et al. (2010) in acerola seed flour (90.60%) and Vieira et al. (2008) in mango by-product (92.23%). According to Lousada Junior et al. (2006) and Aguiar et al. (2010), the high dry matter content (total solids) found allows the product to be stored for long periods provided it is suitably packaged, since it is influenced by the drying exposure time and the storage conditions.

In relation to mineral composition in SBCP and SBSP of *M. dubia* analyzing the results obtained (Figure 4A and Table 4), we found that they did not differ significantly from each other, and that among others listed bioelements, cacari can be considered an excellent source of calcium (Ca). This stood out the most in both treatments applied, with 780 and 710 mg 100 g^{-1}, respectively, which is higher than many types of food related in the Table Food Composition (2006), such as cow's milk with 130 mg 100 ml^{-1} and mozzarella with 467 mg $100g^{-1}$.

Samples of SBCP and SBSP of cacari also showed higher mean values (10.5 mg 100 g^{-1}) of iron (Fe), approximately 31% when compared to the average levels found by Buzzo et al. (2012), 5.4 mg 100 g^{-1} and 4.7 mg $100g^{-1}$ Fe, respectively, for wheat and corn flour. In addition, we also highlight the contents of sodium (Na), 7.8 and 7.9 mg $100g^{-1}$, and manganese (Mn), 7.5 and 9.4 mg $100g^{-1}$ (Table 4).

In relation to the bioactive compounds also in Table 4, the average content of total polyphenols, obtained for SBCP and SBSP of cacari was 5000 mg 100 g^{-1}, values above obtained by Jauregui et al. (2009) for peel, 2293.57 mg of gallic acid /100g^{-1}, and Sotero et al. (2009) and Fracassetti et al. (2013), for *M. dubia* seeds, 2969.20 and 4007.95 mg of gallic acid /100g^{-1}. In addition, it was five times greater than the value obtained in the pulp (1260.73 mg gallic acid/100g^{-1}) by Barreto et al. (2013).

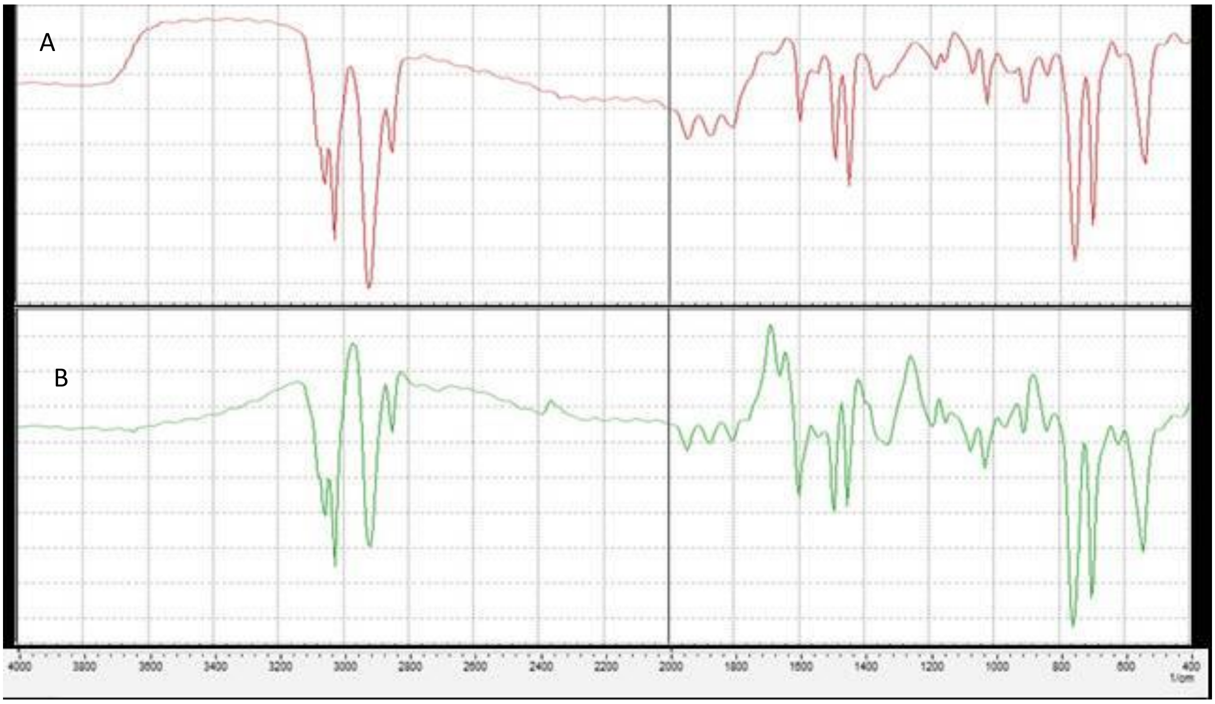
For vitamin C, we found that SBCP and SBSP do not differ (Table 4). However, both did not show a significant content of this vitamin, as found in the pulp and peel of *M. dubia* fruits by Chagas et al. (2015), up to 7,355 mg of ascorbic acid per 100 g^{-1} pulp, but with higher average (15.25 mg 100g^{-1}) than other seeds, such as linseed, 0.50 mg 100g^{-1}.

In relation to the pigment values obtained in fresh cacari seeds in Figure 4B, when recording the amount of chlorophyll (Chl) A, B, and total (A + B) in fresh seeds extracted from *M. dubia* (cacari) fruits considered as Mature, we noted that the highest concentration of these pigments is correlated to the average values obtained by Chagas et al. (2015) for semi-mature fruits. Regression analysis applied to the data of Chl A and B and the equation of the polynomial type (Figure 4B) allowed the visualization of a different and meaningful behavior in cacari leaves related to chlorophyll results in immature fruit.

The *M. dubia* seeds showed Chl A values between 200 and 300 ug g^{-1}, which os close to the values obtained by Chagas et al. (2015) tending to immature and semi-immature fruits. For Chl B, we obtained values from 0 to 100 ug g^{-1}, half of the value for the semi-mature fruit. For Chl total (A + B), we obtained values between 300 and 400 ug g^{-1}, slightly below those obtained for the semi-mature fruit. Thus, we can see that *M. dubia* seeds have different maturation stages (Figure 4B), which may influence the composition of minerals to be obtained in the processing, if the ideal period of maturation of both fruits and seeds is not observed.

The obtained spectra (Figure 5) allow the determination of the presence and structure of the organic material without the molecular formula as in the guidelines of Ribeiro and Souza (2007), in addition to further information about the main functional groups present in the structure of substances of powder and oil bioproducts.

Fig. 5 - Spectrums of the powder (A) and oil (B) extracted from the seeds *M. dubia* spectroscopy in the infrared region (IR).



Wave number (cm^{-1})

Fonte: (Sousa, 2016)

In the infrared spectrum (Figure 5), we could distinguish some important regions: the first between 3000-2800 cm^{-1} in which we can observe the absorption band characteristics of symmetric and asymmetric vibration of the methyl and methylene groups. In the region of 1600-1450 cm^{-1} of the spectrum, often there is an overlap of various absorption bands which can be shifted to lower wavelength values, which is characteristic of the stretching of the C = C aromatic groups.

The absorption situated between 1700 and 1600 cm^{-1} is a characteristic of the stretching of the carbonyl (C = O) with axial deformation in the vibration of the C = O bond which appears in the region between 1300 and 1000 cm^{-1}, suggesting the presence of carboxylic acids and esters linked to aromatic groups. In the region of 1000-700 cm^{-1}, which supposedly verifies the presence of olefins, still indicates aromatic substitution of the ring in the region of 700-400 cm^{-1}.

We also observed, when comparing with other spectra, the absence of a strong band in the range of 1820-1650 cm^{-1}, the presence of a band in the range of 3650-3200 cm^{-1}, a band in the range of 3100-2800 cm^{-1}, weak bands in the range of 2300-1900 cm^{-1}, and a band in the range of 1560 to 1350 cm^{-1}. In this case, the existence of hydrocarbon functions (HC), carboxylic acid, amide, esters, NO_2, and phenols is possible in the molecular structure of the substance in question. The results of this analysis are consistent with those obtained in Table 4, with significant values that suggest the presence of phenolic and carbonylic groups in bioproducts.

The *Myrciaria dubia* seed proved to be a raw material with high potential for use as an enriching agent of food. Bioprospecting studies are preliminary and facilitated the modeling scenarios for Practical Applications of Biotechnology (PAB) and the development of new bioproducts. The bioprocessed seeds present compounds with potential agrifood, if their purposes are clearly observed.

CONCLUSIONS

The analytical, conventional and advanced technological methods, and integrated techniques, applied sequentially, an incremental innovation, in exclusive teaching and research institutions, in the North of Brazil, will enable scientists and researchers to have important and unprecedented biotechnological knowledge about the remaining seeds. of fruits and their potential for the development of processes and bioproducts of interest to society.

They are new methodological ways of obtaining chemical and biological knowledge and new technologies, validated from their application in the characterization of seeds, in natura and bioprocessed, remnants of M. dubia fruits from the extreme north of Brazil.

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Incremental Innovation: Analytical Methods And Integrated Techniques Applied For Biotechnological Characterization Of Remaining Fruit Seeds In Northern Brazil

Resumo

O Brasil é um país multidiverso, ou seja, possuidor de grande diversidade biológica, as quais vem sendo estudadas gradativamente nas Instituições de ensino superior e de pesquisa com intuito de obterem maiores conhecimentos e consequentemente disponibilizarem com mais eficiência e eficácia, produtos, processos para o mercado e até novas metodologias. Entretanto, em algumas destas Instituições, há uma certa dificuldade, especialmente, no que diz respeito aos primeiros estudos biotecnológicos relacionados a caracterização de materiais vegetais, alguns, ainda, desconhecidos dos investigadores/estudantes, os quais são necessárias e requerem diferentes metodologias que nem sempre são possíveis de serem aplicadas nos mesmos moldes de outras instituições, como por exemplo, as do centro-oeste, sul e sudeste. Neste contexto, objetivou-se definir métodos analíticos e técnicas de laboratório a serem integrados e aplicados de forma sequencial, com inovação incremental, para caracterização biotecnológica de sementes remanescentes de frutos no extremo Norte do Brasil, com vistas a obtenção de conhecimentos importantes, úteis a cientistas e pesquisadores que vislumbram o desenvolvimento de ativos, processos e bioprodutos de interesse à sociedade. Foram realizados o desenvolvimento/adaptação/ajustes de procedimentos para realização de análises física e físico-química de compostos minerais, bioativos, pigmentos e verificação dos principais grupos funcionais presentes nas amostras de sementes remanescentes de uma pesquisa realizada com os frutos da espécie *Myrciaria dubia*. Verificou-se a partir dos resultados obtidos nos estudos e análises das amostras de sementes de *M. dubia* que os métodos analíticos, clássicos e instrumentais, e técnicas integrados, quando aplicados de forma sequencial, possibilitam a cientistas e pesquisadores conhecimentos importantes e inéditos sobre as características biotecnológicas das sementes remanescentes de frutos, bem como, o desenvolvimento de ativos que sejam proveitosos à sociedade.

Palavras chaves: Diversidade biológica.Inovação. Metodologia. *Myrciaria dubia.* Sementes.