



Metabolomic study of *Virola surinamensis* (Rol.) Warb raw seed butter from different Amazonian regions

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Abstract

Introduction: *Virola surinamensis*, known in the Brazilian Amazonian region as “ucuuba”, produces seeds with high lipid content. The butter extracted from ucuuba seeds has a high moisturize potential, skin repairing properties and soft texture, which is interesting for cosmetic application.

Methods: *Virola surinamensis* seeds from three different Amazonian regions were collected and studied: Pará's Northeast (Pará/Brazil), Marajó Island (Pará/Brazil) and RESEX Médio Juruá (Amazon as/Brazil). Fatty acid and triacylglycerides compositions were analyzed by gas chromatography with flame ionization detector (GC-FID). Secondary metabolite profiles were analyzed by ultra-performance liquid chromatography coupled with electrospray ionization and quadrupole time-of-flight mass spectrometer (UPLC-ESI-QToF-MS), in both negative and positive ionization modes.

Results: Results revealed that capric and lauric acids contents of RESEX Médio Juruá region butter were different from Pará's Northeast and Marajó island regions. LaMM and MMM triacylglycerols were the majoritarian triacylglycerides and the distribution of RESEX Médio Juruá region was also distinct. Different metabolites profiles were observed for the samples. Primary and secondary metabolites differences between *V. surinamensis* seed butter from three different Amazonian regions suggests that the environment influences the metabolome.

Conclusion: Advanced analytical techniques and statistical analysis have proved to be essential for the metabolomic studies and for better understanding of the global chemical composition of different vegetable samples.

Keywords: *Virola surinamensis*, Amazonian regions, metabolomics, primary metabolites, secondary metabolites, phytochemical characterization



Introduction

Ucuuba is a *tupi* denomination that means “tree that produces fatty”. This designation is usually applied in the Brazilian Amazon for most species of the botanical genus *Virola*.¹

Virola surinamensis (Rol.) Warb, known as ucuuba, is considered as a typically Amazonian species and its habitat is the lowlands and *igapós*. The population thrives in flooded areas, river banks, *igarapés* and *furos*, or areas that might be reached by river flooding.² Thriving species in this environment are açaí, murumuru, patawa and buriti palms, and ucuuba, samaúba, munguba and rubber trees. It is considered as an important biological, social and economic resource for Amazon, as a key species for biological maintenance of flooded areas, due to its environmental interrelations.³

In the 18th and 19th centuries, butter extracted from ucuuba seeds was initially used as a lighting fuel by

Amazonian riverside communities.⁴ However, it was in the 1970s and 1980s that the tree was intensely explored for national wood production.

Until 1998, ucuuba was the third most exported and commercialized species, representing almost 50% of riverside communities' family income.⁵ This factor led ucuuba to be in the international endangered species list of IUCN.⁶ Despite the legal measures in Brazil, in the 1990s and the beginning of 2000s, ucuuba was still the third most exported tree species for the international market of tropical woods.⁷

Currently, the species is once more in the spotlight, due to the non-timber potential. Butter extracted from the seeds has high moisturizing potential and, at the same time, a soft texture, rapid absorption and velvet dry touch. These characteristics make the ucuuba butter an interesting ingredient for cosmetic formulations.

RESEX Médio Juruá is in the margins of Juruá River,



in the municipality of Carauari, south-west of Amazonas state. On the other hand, Pará's Northeast is in heart of Pará state, while Marajó is a fluvio-maritime coastal island, bathed concomitantly by the Atlantic Ocean, the Amazon River and the Pará River.

Although all the regions are situated in the Amazon Biome, their geographic location and the surroundings interfere directly the climatic (solar radiation, temperature and precipitation) and edaphic factors (soil structure and composition). These factors vary in function of location, which determine the environmental diversity and affect vegetal metabolism.^{8,9}

Metabolomics is the OMICs technology purpose of which is to give a general understanding through a global view of metabolites present in a biological system. Metabolites present in a complex system, as a plant extract or raw vegetable oil, might comprehend different and numerous chemical classes. Therefore, advanced analytical techniques, such as chromatography and mass spectrometry, are crucial support metabolomic studies and to obtain significant information about the metabolome. In addition, statistical analysis of the complex data obtained is also a powerful tool to the metabolomics field.^{10,11}

The objective of this work was to study the metabolomic differences of ucuuba (*Virola surinamensis*) raw seed butter from three distinct Amazonian regions in order to characterize the chemical differences between samples of the same species provided from different environments. Advanced analytical techniques (GC-FID and UPLC-ESI-QToF-MS) were used to provide information about primary and secondary metabolites present in the samples. Statistical analysis was also applied to the markers generated by mass spectrometry analysis.

Materials and Methods

Seed Collection

Virola surinamensis seeds of identified individuals were collected from the 3 Amazonian regions, during early April 2016, directly from the trees in Pará's Northeast (Pará/Brazil), Marajó Island (Pará/Brazil) and RESEX Médio Juruá (Amazonas/Brazil) (Figure 1). Collecting nets were used to assist the collection of *V. surinamensis* seeds, to ensure exact origin.

Collected *V. surinamensis* seeds were dried in solar kiln, called "drying houses", until 8-10% humidity. Dried seeds were transported to raw seed butter extraction.

Raw Butter Extraction

After collection and drying, seeds were transported to the extraction process, which happened during late April 2016. Butter was extracted from the seeds through cold pressing, using an expeller press, followed by a filtering process, using a press-filter equipment. *V. surinamensis* raw seed butter was cooled down and packed.



Figure 1. Amazonian Regions – Seeds Collection.

Fatty Acid Composition and Triacylglycerides Distribution

AOCS official methods were reproduced for the *V. surinamensis* raw seed butter, to determine fatty acid composition and triacylglycerides distribution.¹²

Secondary Metabolites Analysis

A Waters XEVO G2-S ultra-performance liquid chromatography coupled with electrospray ionization and quadrupole time-of-flight mass spectrometer (UPLC-ESI-QToF-MS) system was utilized for analysis of the raw butter.

The samples were prepared diluting raw seed butter in tetrahydrofuran, in the concentration 15 mL⁻¹, until total solubilization.

For chromatography separation, a reverse-phase analytical column, Acquity UPLC® BEH C8, 1.7 µm particle size, 2.1 x 100 mm, was used at 60°C and flow rate of 0.600 mL min⁻¹.

Electrospray ionization was used as ionization source, in both positive and negative ionization modes. Data acquisition was performed by MSE scanning, using Waters® Masslynx software version 4.1 for instrumental control.

Statistical Analysis

Statistical analysis was performed in the statistical programming language R v3.5.1. Principal component analysis and K-means clustering analysis were used to access the differences between the samples from different regions. Before that, pre-treatment and pre-processing of the data was performed using software Waters® MarkerLynx software.

Results and Discussion

Fatty Acid Composition

Lipids represent 96%-97% of *V. surinamensis* raw seed butter. Lauric (C12:0) and myristic (C14:0) are the main fatty acids in ucuuba raw seed butter, representing a sum

of approximately 85% of total fatty acid content (Table 1). Capric (C10:0), myristoleic (C14:1), palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids are also present in concentrations higher than 1% of total fatty acid content. Other fatty acids have concentrations lower than 1%.

Myristic and lauric acids were reported as the main fatty acids in *V. surinamensis* raw seed butter.¹³ Ucuuba is a rare source of myristic acid in vegetable butters and oils, as this fatty acid represent up to 60% of total fatty acid content.

RESEX Médio Juruá raw seed butter presented considerably lower concentration of myristic acid and higher concentration of lauric acid, when compared to Pará's Northeast and Marajó Island samples.

Capric and myristoleic acids content in RESEX Médio Juruá raw seed butter have also showed significant difference. Although these fatty acids are minoritarian, they are important to differentiate *V. surinamensis* RESEX Médio Juruá raw seed butter.

Other fatty acid contents were similar to raw seed butter samples from all the three Amazonian regions studied.

Triacylglyceride distribution

Figure 2 shows the triacylglyceride distributions of *Virola surinamensis* raw seed butter from different Amazonian regions. LaMM (Lauric-Myristic-Myristic) and Myristic-Myristic-Myristic are the main tryacylglycerides present in the samples.

Triacylglyceride distributions of *V. surinamensis* raw seed butter from Pará's Northeast and Marajó Island were similar. However, RESEX Médio Juruá butter has a higher content of LaMM and lower MMM. This result was convergent with the fatty acid composition, as RESEX Médio Juruá seed butter had a higher lauric acid content and lower myristic acid.

Secondary Metabolites Analysis

Secondary metabolites represent 3% to 4% of *Virola*

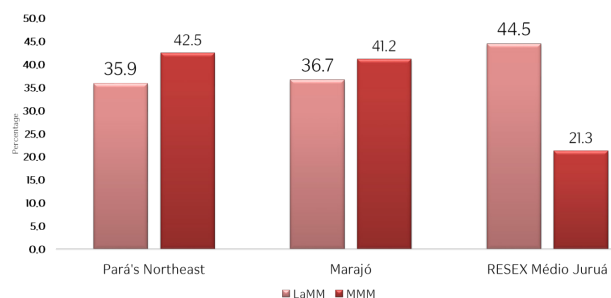


Figure 2. Triacylglyceride Distribution of *Virola surinamensis* Raw Butter from Different Amazonian Regions.

surinamensis raw seed butter. Compared to other raw vegetable butters and oils, where secondary metabolites represent about 0.5% to 1%, ucuuba raw seed butter has a high unsaponifiable content.

A variety of phytochemical classes were reported in *V. surinamensis* leaves and seed, as lignans, tannins, γ -lactones, propiophenones, butanolides and flavonoids.¹⁴⁻¹⁷

Ultra-performance liquid chromatography coupled with Electrospray Ionization and quadrupole time-of-flight mass spectrometry (UPLC-ESI-QToF-MS) performed in both negative and positive ionization modes provide a profile of metabolites present in the *V. surinamensis* raw seed butter. In this case, negative ionization mode was more relevant to evaluate the secondary metabolites profile.

Figure 3 shows the comparison between the metabolite profile of the samples studied in negative ionization mode. All the three samples evaluated showed a rich metabolite profile. However, it is possible to notice some differences in the profile, qualitative and quantitatively, especially in the RESEX Médio Juruá sample.

Statistical multivariate analysis of data generated by UPLC-ESI-QToF-MS analysis (negative ionization mode) was performed. K-means clustering analysis is shown in

Table 1. Fatty Acid Composition of *Virola surinamensis* Raw Butter from Different Amazonian Regions

FattyAcid	Amazonian Region		
	RESEX Médio Juruá	Marajó Island	Pará's Northeast
Caprilic (C8:0)	0.26 \pm 0.04	0.19 \pm 0.04	0.19 \pm 0.07
Capric (C10:0)	1.68 \pm 0.22	0.76 \pm 0.11	0.67 \pm 0.15
Lauric (C12:0)	22.65 \pm 1.61	15.64 \pm 1.59	14.64 \pm 3.71
Myristic (C14:0)	62.88 \pm 1.84	71.89 \pm 1.69	73.06 \pm 3.19
Myristoleic (C14:1)	1.45 \pm 0.39	0.74 \pm 0.19	0.65 \pm 0.26
Palmitic (C16:0)	4.27 \pm 0.38	4.64 \pm 0.39	4.89 \pm 0.53
Palmitoleic (C16:1)	0.54 \pm 0.12	0.33 \pm 0.09	0.36 \pm 0.12
Stearic (C18:0)	0.93 \pm 0.27	0.70 \pm 0.21	0.88 \pm 0.45
Oleic (C18:1)	4.25 \pm 0.28	3.87 \pm 0.45	3.77 \pm 0.31
Linoleic (C18:2)	0.61 \pm 0.06	0.64 \pm 0.1	0.46 \pm 0.05
Linolenic (C18:3)	0.03 \pm 0.01	0.05 \pm 0.02	0.03 \pm 0.01
Arachidic (C20:0)	0.06 \pm 0.02	0.06 \pm 0.03	0.08 \pm 0.04
Eicosenoic (C20:1)	0.34 \pm 0.1	0.30 \pm 0.06	0.28 \pm 0.08

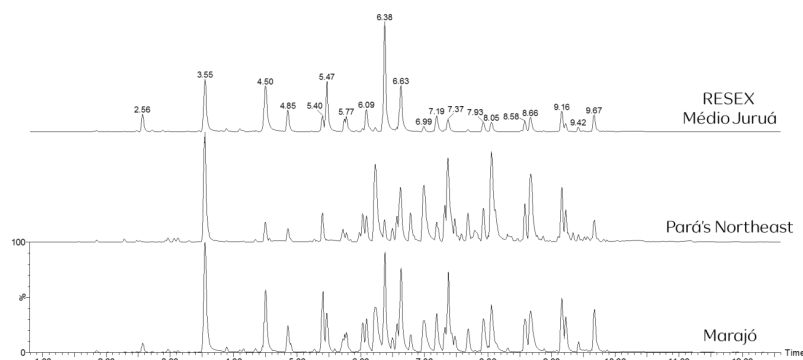


Figure 3. UPLC-ESI-Q-ToF Metabolic Profile of *Virola surinamensis* Raw Butter from Different Regions (Negative Ionization Mode).

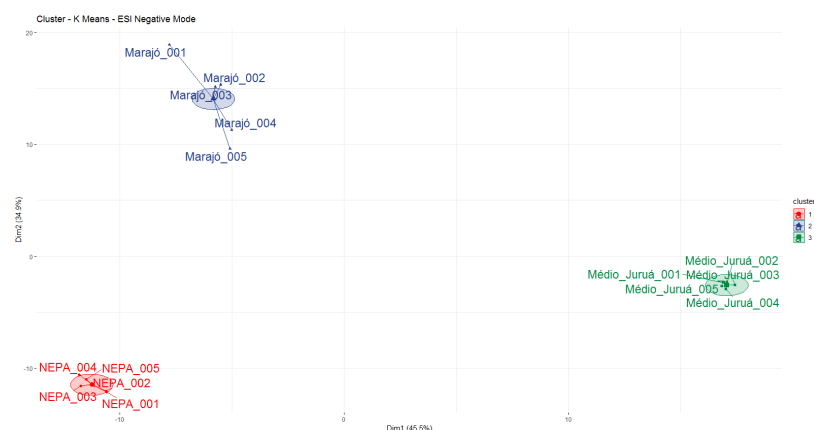


Figure 4. K-means Analysis – Markers obtained by UPLC-ESI-Q-ToF analysis (negative ionization mode) – Green = RESEX MédioJuruá; Blue = Marajó Island; Red = Pará's Northeast.

Figure 4, where different colors represent the three groups divided statistically (Dim1 + Dim2 = 80.40%). The analysis shows that the samples from the three Amazonian regions correspond to the different groups, which are positioned in distinct quadrants. Therefore, considering the secondary metabolite profile, the samples are statistically different.

RESEX MédioJuruá raw seed butter (green) is the more distant from the Marajó Island (blue) and Pará's Northeast (red) samples, which indicates that it is the more distinct sample amongst all.

Significant metabolic differences in *V. surinamensis* raw seed butter were found in primary and secondary metabolites profiles, through metabolomics tools. These results support the proposal that, although RESEX Médio Juruá, Pará's Northeast region and Marajó Island region are all located in the Amazon Biome, different edapho-climatic conditions influence the environmental diversity and plant metabolism.

Numerous factors have been reported as to influence the secondary and primary metabolites content in plants. Secondary metabolites, as chemical interface with the environment, may be greatly affected by external conditions. Climatic (solar radiation, temperature) and edaphic (soil structure and macro and micronutrients

composition) factors, harvest time (seasonality, circadian rhythm and plant development), geographic location, genetic variations are the main factors studied.^{9,18}

Conclusions

According to the results, *V. surinamensis* raw seed butter from three different Amazonian regions (Pará's Northeast, Marajó Island and RESEX Médio Juruá) has distinct primary and secondary metabolite profiles. RESEX Médio Juruá butter presented different fatty acids composition and tryacylglycerides distribution, compared to the other regions.

Secondary metabolite analyzed by UPLC-ESI-QToF-MS (negative ionization mode) showed different chromatographic profiles. Statistical analysis of generated markers showed significant difference between the three raw butter samples.

These results suggest that the environment factors influence the plant metabolism and, consequently, phytochemical composition of seeds and the butter extracted. Advanced analytical techniques, statistical analysis and metabolomics tools were essential for the metabolomic study of the different samples.

Competing Interests

None.

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