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PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE E BIOTECNOLOGIA
DA REDE BIONORTE



**BIOPROSPECÇÃO DE FRUTAS CULTIVADAS NA AMAZÔNIA COM
POTENCIAL DE COMPOSTOS BIOATIVOS, CAPACIDADE
ANTIOXIDANTE E ESTUDOS MICROBIOLÓGICOS**

ISMAEL MONTERO FERNÁNDEZ

Boa Vista-RR

JUNHO/2019

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Orientador: Dr. Edvan Alves Chagas.

Coorientadores: Dr. Antonio Alves de Melo Filho.

Dr. Ricardo Carvalho dos Santos.

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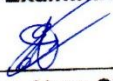
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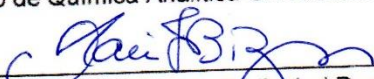
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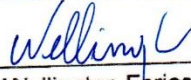
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A meus familiares e amigos.

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O homem sábio deve ser equilibrado em tudo, como o próprio universo, cujo uno nunca destoa do verso. Quando o homem-ego pretende fazer mais do que o homem-eu permite, o desequilíbrio é infalível e o desequilíbrio é a infelicidade do homem. O homem deve em tudo ser universalizado agindo de dentro para fora. Lao-Tse.

FERNÁNDEZ, Ismael Montero. **Bioprospecção de frutas cultivadas na Amazônia com potencial de compostos bioativos, capacidade antioxidante e estudos biológicos**. 2019. 232f. Tese (Doutorado em Biodiversidade e Biotecnologia da Rede Bionorte)-Universidade Federal de Roraima, Boa Vista, 2019.

RESUMO

A Amazônia possui uma extensa e rica área no território brasileiro. Inúmeras pesquisas vêm sendo realizadas ao longo dos anos com objetivo de explorar sua grandeza, potencial e biodiversidade, assim como, o descobrimento de novos bioprodutos com aplicações como produtos naturais, cosméticos, fitoterápicos, dentre outros. Assim, objetiva-se estudar o potencial biotecnológico de nove frutas amazônicas, a saber: abiu, acerola, araçá amarelo, bacuparí, biribá, camu-camu, fruta-do-conde, graviola e taperebá. Neste sentido, foram estudadas as três partes de cada fruta por separado como polpa, casca e semente. Quanto às polpas, o maior valor energético foi encontrado para as polpas da graviola e bacuparí com $76,83 \pm 0,02$ Kcal 100 g^{-1} e de $53,15 \pm 0,02$ Kcal 100 g^{-1} , respectivamente. Entre os minerais nas polpas, destaca-se a elevada concentração de potássio, especialmente, na graviola com concentração de $541,16 \pm 0,24$ mg 100 g^{-1} e para o biribá com concentração de $468,21 \pm 0,13$ mg 100 g^{-1} . Entre os micronutrientes destaca-se a elevada concentração de manganês na polpa de abiu com $6,61 \pm 0,11$ mg 100 g^{-1} destacando a presença de cobalto em concentrações de traços em algumas das polpas estudadas. Quanto à análise das peles e cascas das frutas, o potássio se destacou, também, como macronutriente, cuja concentração foi de $521,04$ mg 100 g^{-1} na graviola, seguida da concentração de magnésio na pele do biribá com concentração de $64,21$ mg 100 g^{-1} . Quanto à análise da pele do araçá amarelo, este apresentou alto valor energético ($276,29$ Kcal 100 g^{-1}). Entre as propriedades físicoquímicas estudadas os valores de pH oscilaram entre (2,1-4,7) e a relação sólidos solúvel /Acidez titulável foi maior para a pele do taperebá com $3,35 \pm 0,1$. Altamente energéticas são as sementes devido a sua elevada concentração de ácidos graxos. O potássio é também o elemento majoritário nas sementes com concentração de $554,23$ mg 100 g^{-1} para a graviola, destacando entre os micronutrientes uma concentração significativa de zinco na semente do abiu. Destacam nas sementes maiores concentrações de ácidos graxos insaturados que de saturados, destacando a concentração de ácido oleico na semente do bacuparí com 47,4%. Foi avaliada neste trabalho a atividade antioxidante e compostos fenólicos totais, destacando a elevada concentração de compostos fenólicos na pele do camu-camu com concentração de $1241,1$ mg 100 g^{-1} de ácido gálico, sendo esta fruta também que apresentou maior concentração de vitamina C, $2521,51$ mg 100 g^{-1} e carotenoides totais na sua casca com $0,67$ mg 100 g^{-1} . A concentração de açúcares totais foram maiores nas polpas que para as outras partes das frutas. Os óleos e extratos foram testados para avaliar a atividade antimicrobiana e antiacetilcolinesterase (AChE), apresentando maiores percentuais de inibição para a levedura *Candida albicans* na pele do taperebá com 94,46% de inibição. A inibição de AChE foi especialmente elevada para o extrato da pele do biribá, com 86,39%, seguido da semente do taperebá com 62,17%. Foram aplicados métodos de análise multivariada para estabelecer correlações e agrupações entre os diferentes dados obtidos.

Palavras-chave: Minerais; Alimento Funcional; Ácido Ascórbico; Acetilcolinesterase.

FERNÁNDEZ, Ismael Montero. **Bioprospecting of fruits crops in the Amazon with potential of bioactive compounds, antioxidant capacity and microbiological studies.** 2019. 232f. Tese (Doutorado em Biodiversidade e Biotecnologia da Rede Bionorte)-Universidade Federal de Roraima, Boa Vista, 2019.

ABSTRACT

The Amazon has an extensive and rich area in the Brazilian territory. Numerous researches have been carried out over the years to explore its greatness, potential and biodiversity, as well as the discovery of new bioproducts with applications such as natural products, cosmetics, herbal medicines, among others. Thus, the objective is to study the biotechnological potential of nine Amazonian fruits, namely: *abiu*, *acerola*, *araçá*, *bacuparí*, *biribá*, *camu-camu*, *fruta-do-conde*, *graviola* and *taperebá*. In this sense, the three parts of each fruit were studied separately as pulps, barks and seeds. Pulps, the highest energy value was found for *graviola* and *bacupari* pulps with 76.83 ± 0.02 Kcal 100 g^{-1} and 53.15 ± 0.02 Kcal 100 g^{-1} , respectively. Among the minerals in the pulps, the high concentration of potassium, especially in *graviola* pulps with concentration of $541,16 \pm 0,24$ mg 100 g^{-1} and for the *biribá* pulps with concentration of $468,21 \pm 0,13$ mg 100g^{-1} . Among the micronutrients, the high concentration of manganese in the *abiu* pulp with 6.61 ± 0.11 mg 100 g^{-1} . It's remarkable, highlighting the presence of cobalt in trace concentrations in some of the studied pulps. Regarding fruit barks and seeds analysis, potassium was also highlighted as a macronutrient, whose concentration was 521.04 mg 100 g^{-1} in *graviola*, followed by the concentration of magnesium in the *biribá* barks with a concentration of 64.21 mg 100 g^{-1} . As for the analysis of the of *araçá* barks, this presented high energy value (276.29 Kcal 100 g^{-1}). Among the physicochemical properties studied, pH values in ranged from (2.1-4.7) and soluble solids/ titratable acidity ratio was higher for the *taperebá* barks with 3.35 ± 0.1 . Highly energetic are the seeds because of their high concentration of fatty acids. Potassium is also the major element in seeds with a concentration of 554.23 mg 100 g^{-1} for *graviola* seeds, with a significant concentration of zinc in the *abiu* seed among the micronutrients. In the seeds, higher concentrations of unsaturated fatty acids than saturated, with the concentration of oleic acid in the *bacupari* seed stand out with 47.4%. It was evaluated the antioxidant activity and total phenolic compounds, highlighting the high concentration of phenolic compounds in the *camu-camu* barks with a concentration of 1241.1 mg 100 g^{-1} of GAE, which fruit also presented higher concentration of vitamin C, 2521.51 mg 100 g^{-1} and total carotenoids in their barks with 0.67 mg 100 g^{-1} . The concentration of total sugars were higher in pulps than in other parts of the fruit. The oils and extracts were tested to evaluate the antimicrobial activity and antiacetylcholinesterase (AChE), presenting higher inhibition rates for yeast *Candida albicans* on the *taperebá* barks with inhibition of 94.46%. Inhibition of AChE was especially high for the extract of *biribá* barks, with 86.39%, followed by the *taperebá* seed with 62.17%. Multivariate

analysis methods were applied to establish correlations and groupings between the different data obtained

KEYWORDS: Minerals; Funtional Food; Ascorbic Acid; Acetylcholinesterase.

FERNÁNDEZ, Ismael Montero. **Bioprospección de frutas cultivadas en el Amazonas, com potencial de compuestos bioactivos, capacidade antioxidante y estudios microbiológicos**. 2019. 232f. Tesis (Doctorado en Biodiversidade e Biotecnologia da Rede Bionorte)-Universiade Federal de Roraima, Boa Vista, 2019.

RESUMEN

El Amazonas tiene un área extensa y rica en el territorio brasileño. Se han realizado numerosas investigaciones a lo largo de los años para explorar su grandeza, potencial y biodiversidad, así como el descubrimiento de nuevos bioproductos con aplicaciones como productos naturales, cosméticos y plantas medicinales, entre otros. Por lo tanto, el objetivo de este trabajo es estudiar el potencial biotecnológico de nueve frutas amazónicas, a saber: *abiu*, *acerola*, *araçá*, *bacuparí*, *biribá*, *camu-camu*, *fruta-do-conde*, *graviola* y *taperebá*. En este sentido, las tres partes de cada fruta fueron estudiadas por separado como pulpa, corteza y semilla. En cuanto a las pulpas, el valor energético más alto se encontró para las pulpas de *graviola* y *bacupari* con 76.83 ± 0.02 Kcal 100 g^{-1} y 53.15 ± 0.02 Kcal 100 g^{-1} , respectivamente. Entre los minerales en las pulpas, la alta concentración de potasio, especialmente para *graviola* con una concentración de $541,16 \pm 0,24$ mg 100 g^{-1} y para el *biribá* con una concentración de $468,21 \pm 0,13$ mg 100 g^{-1} . Entre los micronutrientes, destaca la alta concentración de manganeso en la pulpa de *abiu* con 6.61 ± 0.11 mg 100 g^{-1} , destacando la presencia de cobalto en concentraciones de trazas en algunas de las pulpas estudiadas. Con respecto al análisis de piel y semilla de frutas, el potasio también se destacó como macronutriente, cuya concentración fue de 521.04 mg 100 g^{-1} para *graviola*, seguida de la concentración de magnesio en la piel del *biribá* con una concentración de 64.21 mg 100 g^{-1} . En cuanto al análisis de la piel de *araçá*, este presentó un alto valor energético ($276,29$ Kcal 100 g^{-1}). Entre las propiedades fisicoquímicas estudiadas, los valores de pH oscilaron entre (2.1-4.7) y la relación de sólidos solubles / acidez titulable fue mayor para la piel de *taperebá* con 3.35 ± 0.1 . Las semillas son altamente energéticas debido a su alta concentración de ácidos grasos. El potasio es también el elemento principal en semillas con una concentración de 554.23 mg 100 g^{-1} para *graviola*, con una concentración significativa de zinc para la semilla de *abiu* entre los micronutrientes. En las semillas, se destacan las concentraciones de ácidos grasos insaturados más altas que los ácidos grasos saturados, siendo la concentración de ácido oleico en la semilla de *bacupari* de 47,4%. Se evaluó la actividad antioxidante y los compuestos fenólicos totales, destacándose la alta concentración de compuestos fenólicos en la piel de *camu-camu* con una concentración de 1241.1 mg 100 g^{-1} de ácido gálico, cuya fruta también presentó una mayor concentración de vitamina. C, 2521.51 mg 100 g^{-1} y carotenoides totales en su cáscara con 0.67 mg 100 g^{-1} . La concentración de azúcares totales fue mayor en pulpas que en otras partes de la fruta. Los aceites y extractos se analizaron para evaluar la actividad antimicrobiana y la acetilcolinesterasa (AChE), presentando tasas de inhibición más altas para la levadura *Candida albicans* en la piel de *taperebá* con un 94,46% de inhibición. La inhibición de la AChE fue especialmente alta para el extracto de piel de *biribá*, con un 86,39%, seguido de la semilla de *taperebá* con un 62,17%. Se aplicaron métodos de análisis multivariado para establecer correlaciones y agrupaciones entre los diferentes datos obtenidos.

Palavras-chave: Minerales; Alimento Funcional; Ácido Ascórbico; Acetilcolinesterasa.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

AChE	Acetilcolinesterase
AES	Espectroscopia de emissão atômica
Al	Alumínio
AT	Acidez Titulável
B	Boro
<i>B. cereus</i>	<i>Bacillus cereus</i>
C 14:0	Ácido Mirístico
C 16:0	Ácido Palmítico
C 16:1	Ácido Palmitoleico
C 18:0	Ácido Esteráico
C 18:1	Ácido oleico
C 18:2	Ácido linoleico
C 18:3	Ácido linolênico
C 20:0	Ácido Araquinoico
<i>C. albicans</i>	<i>Candida albicans</i>
Ca	Cálcio
CFU	Colony Forming Unit
Co	Cobalto
CP1	Componente Principal 1
CP2	Componente Principal 2
Cu	Cobre
DMSO	Dimetilsulfóxido
DPPH	1,1-diphenyl-2-picrylhydrazyl
<i>E. coli</i>	<i>Escherichia coli</i>
EGA	Equivalent of Gallic Acid
FAAS	Flame Atomic Absorption Spectroscopy (Espectroscopia de Absorção Atômica em Chama)
FAO	Food and Agriculture Organization of the United Nation
Fe	Ferro

FRAP	Ferric Reducing Antioxidant Power
g	Gramas
HCA	Hierachical Component Analysis (Análise de Componentes Hierárquicos)
K	Potássio
Kcal	Quilocalorias
L	Litro
LOD	Limite de detecção
LOQ	Limite de Quantificação
MFA	Monosaturated Fatty Acids (Ácidos Graxos Monoinsaturados)
mg	Miligramas
Mg	Magnésio
mmol	Milimol
Mn	Manganês
Na	Sódio
nm	Nanômetros
NPPGCT	Núcleo de Pesquisa e Pós-graduação em Ciência e Tecnologia
NUPAGRI	Núcleo de Pesquisa em Agricultura
°Brix	Graus Brix
°C	Graus Celsius
P	Fósforo
PCA	Principal Component Analysis (Análise de Componentes Principais)
pH	Potencial de Hidrogênio
PUFAs	Polyunsaturated Fatty Acids (Ácidos Graxos Poliinsaturados)
r^2	Coeficiente de correlação linear
S	Enxofre
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>
SFA	Satured Fatty Acids (Ácidos Graxos Saturados)
SS	Sólidos solúveis

UFA	Unsaturated Fatty Acids (Ácidos Graxos Insaturados)
UFMG	Universidade Federal de Minas Gerais
UFRR	Universidade Federal de Roraima
UV-visível	Ultravioleta visível
WHO	World Health Organization (Organização Mundial de Saúde)
Zn	Zinco
λ	Comprimento de onda
μL	Microlitros
ω -3	Ômega 3 (ácido linoléico)
ω -6	Ômega 6 (ácido linolênico)
ω 6/ ω 3	Relação ácido linoléico /ácido linolênico
ω -9	Ômega 9 (Ácido oleico)

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1 INTRODUÇÃO

A incomparável diversidade brasileira animal, vegetal e microbiana pressupõe uma imensa variedade de substâncias químicas, decorrente, inclusive das interações plantas/microrganismos e animais/microrganismos, expressas basicamente em linguagem química. Assim diversos interesses sobre o usufruto da biodiversidade brasileira pode leva-la à destruição. Desta forma, medidas que inviabilizem esse resultado negativo, possibilitando o seu acesso levariam, portanto, em justo crescimento, aos limites da sustentabilidade necessária à sobrevivência do planeta (VIEIRA et al., 2009)

O Brasil possui a maior biodiversidade amazônica, com mais de 40.000 espécies de plantas, sendo 75% destas do tipo endêmicas. Para Carvalho (2011), o expressivo número de espécies vegetais, destacando-se frutíferas nativas, coloca ao Brasil em segundo lugar como grande centro de origem de espécies frutíferas tropicais, vindo logo após do sudeste asiático, poucas delas, até então, atingiram participação expressiva no agronegócio de frutas, logra despertar interesse em seu consumo *in natura*. Por outro lado, a Europa é o maior consumidor de frutas brasileira, chegando a consumir até o 70% das exportações (RIBEIRO et al., 2010). O consumo de frutas tropicais, é geralmente consumido *in natura*, dado que suas características sensoriais como cor, textura e propriedades nutricionais podem ser mais bem aproveitadas nestas condições (INFANTE et al., 2013).

Essa biodiversidade denota uma fonte inestimável de substâncias biologicamente ativas e sua preservação é de fundamental importância, em virtude da riqueza biológica e do amplo potencial em originar novos fármacos. Esta vem despertando a cobiça de laboratórios de pesquisa em empresas farmacêuticas (NEWMAN; CRAGG, 2007), procurando novos produtos que contenham concentrações naturais de frutas em suas formulações surgindo uma alternativa interessante na produção de alimentos com propriedades funcionais e nutricionais, abrindo novas vias de mercado, com produtos mais atrativos (VIANA et al., 2012).

As plantas e seus constituintes são fontes de medicamentos para várias doenças, muitas delas são utilizadas popularmente sem comprovação científica disponível (FOGLIO et al., 2006). Entre as propriedades dos óleos e extratos de plantas medicinais na indústria, destacam suas aplicações antimicrobianas, sendo outro destaque o potencial de inibição da enzima acetilcolinesterase que pode atuar

em doenças neurodegenerativas, como as doenças de o Alzheimer e o Parkinson, e pode ser encontrada em vitaminas lipossolúveis, principalmente α -tocoferol, encontrado na vitamina E, já que a doença de Alzheimer é a patologia neurodegenerativa associada à idade, para a qual, até pouco tempo, não havia tratamento eficaz. Atualmente, há uma preocupação por parte da população em garantir uma melhor qualidade de vida. Isso inclui o consumo de mais alimentos saudáveis visando à prevenção de doenças crônicas não transmissíveis, como o câncer, diabetes e doenças respiratórias crônicas, sendo que, de acordo com Achutti e Azambuja (2004), estas são responsáveis por 60% das mortes de incapacidade em todo o mundo, podendo chegar a 73% de todas as mortes em 2020.

Por outro lado, há cada vez mais uma maior preocupação pelos resíduos gerados na indústria de processamento de frutas. De acordo com Godim et al., (2005), o Brasil produz 140 milhões de toneladas de alimentos por ano, sendo um dos principais países de exportação de produtos agrícolas a nível mundial, mais ao mesmo tempo é o país que tem milhões de excluídos sem acesso aos alimentos de qualidade e quantidade adequadas, sendo esses resíduos uma importante fonte de compostos químicos com potencial biotecnológico.

Entre os ecossistemas que existem na região amazônica, sendo estas, as florestas de terras firmes, inundadas, várzeas, igapós e savanas, albergando cerca de 1,5 milhões de espécies vegetais catalogadas (IBAMA, 2019), sendo destacadas as frutas a seguir: abiu (*Pouteria caimito*), acerola (*Malpighia emarginata*), araçá (*Psidium cattleianum*), bacupari (*Rheedia gardneriana*), biribá (*Rollinia mucosa*) camu-camu (*Myrciaria dúbia Krunth*), graviola (*Annona muricata*) fruta-do-conde (*Annona squamosa*) e taperebá (*Spondias mombin* L.).

Assim, esta tese é estruturada em seis capítulos com formato de artigos, a saber:

Capítulo I - *Bromatological and Mineralogical Study in Fruits pulps cultivated in the Northern Amazon.*

Capítulo II - *Use of Amazon Fruits Barks as Source of Nutrients.*

Capítulo III - *Chemical Characterization o Amazon fruits seeds and its nutritional contribution as foods functional and Biotechnological interest.*

Capítulo IV - *Evaluation of Total Phenolic Compounds and Antioxidant Activity in Amazon Fruits.*

Capítulo V - *Characterization of Bioactive Compounds in Northern Amazon Fruits.*

Capítulo VI - *Antimicrobial Activity and Acetylcholinesterase Inhibition of Oils and Amazon Fruits Extracts.*

2 REFERENCIAL TEÓRICO

2.1 ESPÉCIES VEGETAIS EM ESTUDO

2.1.1 Abiu (*Pouteria caimito* (Ruiz & Pavón) Radlk.) (Sapotaceae)

O abiu (*Pouteria caimito*), (Figura 1) pertence à família das Sapotaceae, do gênero *pouteria*, sendo uma planta com folhas que apresentam 14,8 cm em média de comprimento e 4,7 cm de largura, produzindo inflorescência em fascículos auxiliares ou caule, com flores amarelo-esverdeadas e pequenas, unissexuais e hermafroditas, apresentando tronco irregular chegando até 30 cm de diâmetro (CARVALHO; MÜLLER, 2005).

De acordo com Lorenzi et al. (2006), o abiu é uma fruta de clima tropical ou subtropical sendo mais adaptado ao clima quente úmido. Exige pouca fertilidade da terra exceto quando a planta é ainda nova. Apesar de adaptar-se as diferentes tipos de solo do Brasil o melhor crescimento, desenvolvimento, frutificação e produção tem sido possível em locais com solos argilosos, rico em matéria orgânica bem drenada.

Figura 1- Abiu.



Fonte: de Melo Filho et al. (2018).

Trata-se de uma fruta com características sensoriais únicas pela elevada concentração de nutrientes que apresenta. Dodadio, Moro e Servidone (2002) definem o abiu como um fruto bastante atrativo, com polpa doce e de grande

aceitação popular. Seu polpa apresenta compostos com atividade antioxidante e concentração de compostos fenólicos 172,75 mg 100 g⁻¹ de polpa medidos em função da concentração de ácido gálico (VIRGOLIN; SEIXAS; JANZANTTI, 2017).

Por outro lado, é importante ressaltar que o óleo extraído a partir de suas sementes é rico em ácidos graxos, com potencial biotecnológico como os ácidos graxos insaturados como ácido oleico 43,1% e ácido linolênico 8,6% e entre os ácidos graxos saturados, destaca o ácido palmítico com 27,3% (MELO FILHO et al., 2018).

2.1.2 Acerola (*Malpighia emarginata* D.C.) (Myrtaceae)

A acerola (*Malpighia emarginata* D.C.) (Figura 2) é o fruto da aceroleira, de tamanho e peso variado. O epicarpio uma película fina, o mesocarpo é a polpa e o endocarpo está constituído por três caroços unidos com textura pergaminácea, que dão ao fruto o aspecto trilobado, tendo cada caroço no seu interior a semente com comprimento entre 3-5 mm, de forma ovoide e com dois cotilédones (ALMEIDA et al., 2002). É uma planta frutífera originada das Antilhas, norte da América do Sul e América Central, que vem apresentando boa adaptação em diversos países sendo, sobretudo, cultivada no Brasil, Porto Rico, Cuba e Estados Unidos (CARVALHO, 2000).

Figura 2 - Acerola.



Fonte: Autor.

Esta fruta apresenta uma grande importância nutricional devido ao seu elevado teor de vitamina C, rica também em compostos antioxidantes (MERCALI et al., 2014) e minerais como potássio, cálcio e ferro. Além de apresentar propriedades antioxidantes, a acerola apresenta também importantes propriedades

antimutagênicas e anticancerígenas, que autores como Lima et al., (2005) relacionam essas propriedades com seu elevado valor em ácido ascórbico cujos valores oscilam entre 1247,10 até 1845,79 mg 100 g⁻¹. Aguilar et al., (2010) estudam a composição de minerais na polpa de acerola encontrando entre os macronutrientes concentrações de potássio de 146,0 mg 100 g⁻¹ e magnésio com concentrações de 18,0 mg 100 g⁻¹. Enquanto, aos microminerais ela é rica em ferro com concentrações de 0,20 mg 100 g⁻¹.

Em termos de produção desta fruta, de acordo com Sousa (2010), o Brasil é o país que mais acerola produz, exporta e ao mesmo tempo consome do mundo, já que a aceroilera possui floração durante o ano todo, ocorrendo sua frutificação entre 22 e 25 dias. Por outro lado, Astn (2001) afirma que as acerolas processadas geram aproximadamente, 18 mil toneladas de sucos e polpas por ano, concentrando-se esta produção na região Nordeste, sendo processadas no Brasil cerca de 34,40 mil toneladas de acerolas por ano, equivalente a 7,16% do total de frutas processadas pelas empresas.

2.1.3 Araçá amarelo (*Psidium catteaianum*) (Myrtaceae)

O araçá (*Psidium cattleianum*), (Myrtaceae) (Figura 3) é uma frutífera não cultivada, com ocorrência preferentemente em cerrados, campos e savanas de quase todo o território brasileiro (FERREIRA et al., 2011).

Para Mattos (1989) é encontrado em estado nativo no Brasil desde Minas Gerais até o Rio Grande do Sul, e dado que seus frutos são de cor amarela ou vermelha, surgem as duas divisões da espécie em araçá amarelo ou araçá vermelho (LORENZI, 1992). Apresenta duas espécies comestíveis, *Psidium catteyanum* Sabine e *Psidium guineense* Swartz (CISNEIROS et al., 2003), sendo um arbusto não cultivado, com ocorrência frequente nos cerrados, campos, savanas e cerradões de praticamente em todo o território brasileiro. Pertencente à família das Myrtaceae, alcançando os 1,5 metros de altura com o caule de casca lisa, folhas simples com renovações e margem levemente onduladas (BEZERRA et al., 2006; FERREIRA et al., 2011). Seus frutos são bagas globosas, coroadas pelas sépalas persistentes, com polpa suculenta de cor esbranquiçada, com muitas sementes, de sabor muito agradável, ocorrendo sua maturação entre janeiro e março (LORENZI, 2006).

Figura 3 - Araçá amarelo.



Fonte: Autor.

Os araçazeiros são frutas muito interessantes do ponto de vista comercial, devido a apresentar um sabor exótico, amplamente aceitável pelos consumidores e com altos valores de vitamina C. (FRANZON, et al., 2009). Sua composição química variável em função das chuvas, altitude, clima e características dos solos onde a fruta é colhida (CALDEIRA et al., 2004). A concentração de ácido ascórbico encontra-se entre quatro a sete vezes mais ácidas que os cítricos convencionais (WILLE, 2004). Dada essa alta acidez é apta para a fabricação de compotas, geleias, doces em calda etc (MANICA, 2000). Além do potencial antioxidante que apresentam os frutos do araçá, Gordon et al. (2011).

2.1.4 Bacupari (*Rheedia gardneriana* Tr. & Planch) (Clusiaceae)

A espécie *Rheedia gardneriana* Tr. & Planch. (Clusiaceae) (Figura 4) conhecida popularmente como bacupari ou bacuripari. É uma planta de origem amazônica que se desenvolve também no Rio Grande do Sul, de porte arbóreo, apresenta altura entre 5 e 7 metros com tronco de 15 a 25 cm de diâmetro com folhas simples, coriáceas e glabras, mostrando frutos que têm sabor agridoce (LORENZI, 2009). Suas flores são hermafroditas, com florescimento de julho a setembro e frutificação de setembro a dezembro (ALMEIDA et al., 1998). Segundo Barroso et al. (2002), o bacupari também é conhecido como bacoparé, é uma fruta que pertence à família das Clusiaceae, encontrando-se no estado silvestre em igapós e capoeiras, sendo uma família representada por 21 gêneros e 182 espécies

como são o abricó (*Mammea americana* L.). De todas as espécies, destacam o abricó (*Mammea americana* L.) e o bacuri (*Platonia insignis* Mart) (BARROSO et al., 2002).

Figura 4 – Bacupari.



Fonte: Fruto do Bacupari Autor (2018). Árvore de bacupari. <https://www.vinaec.com.br>

De acordo com Sobreira et al. (2009), o bacupari apresenta um elevado potencial para exploração econômica pela larga aceitação de seus frutos tanto para consumo *in natura* como na forma processada, podendo, a médio e longo prazo, estabelecer-se como uma nova opção para o mercado interno e externo de frutas exóticas, além de apresentar potencial analgésico e antibacteriano com aplicações na indústria química (ALMEIDA et al., 2008), sendo encontradas propriedades antibacterianas para *Pseudomonas* spp., *Streptococcus* spp. e *Clavibacter* spp. e propriedades analgésicas de compostos extraídos das folhas (CRUZ et al., 2006; SANTOS et al., 1999). Segundo trabalhos desenvolvidos por Guimarães et al. (2004) também é fonte de matéria prima para o tratamento de inflamações, dores, infecções urinárias assim como atividade vasodilatadora.

2.1.5 Biribá (*Rollinia mucosa* Jacq. Baill) (Annonaceae)

O biribá (*Rollinia mucosa* Jacq. Baill) (Figura 5) pertence à família das Annonaceae, sendo uma frutífera nativa da região Amazônica e da Mata Atlântica (FONSECA et al., 2012; CAVALCANTE, 1996) presente na América Central e do Sul e desenvolve-se em áreas de clima quente e úmido (SANTOS et al., 2005). O fruto apresenta coloração amarela, globosa, composta por diversas partes hexagonais,

muito unidas dando um aspecto caraterístico, sendo sua polpa com um aspecto caraterístico que pode variar desde esbranquiçada a creme, com sementes de cor escura, sendo seus frutos de aroma agradável, podendo pesar até 1,3 kg (LORENZI, 1992).

Figura 5 –Biribá.



Fonte: Autor.

De acordo com Costa e Muller (1995), os frutos tem grande aceitação popular, sendo consumidos *in natura*, apresentando a planta grande diversidade nos nomes de seus frutos como biribá de Pará, fruta da condessa, biribá de Pernambuco, pinha, anona e jaca de pobre.

Esta fruta, ao igual que outras da família das Annonaceae, apresentam numerosas substâncias bioativas presentes nas diferentes partes da fruta, seja nas folhas, raízes, frutos ou sementes, destacando grande quantidade de alcaloides que possuem propriedades inseticidas (KRINSKI; MASSAROLI; MACHADO, 2014) e a presença de acetogeninas nesta planta com efeito antitumoral (CHÁVEZ et al., 1998).

Nas sementes desta fruta, Rivera e Álvarez (2018) testaram o extrato etanólico obtido a partir das sementes, encontrando bioatividade como controlador das pragas de *Corythucha gossypii* devido a suas propriedades inseticidas. Por outro lado, nas folhas de biribá, foram encontradas lignanas furofuránicas como magnolina, epiudesmina, epiyangamabina e yangambina (VILLEGAS et al., 2001).

2.1.6 Camu-camu (*Myrciaria dúbia* (Krunth) Mc Vaugh) (Myrtaceae)

O camu-camu (*Myrciaria dúbia* (Krunth) Mc Vaugh) (Myrtaceae) (Figura 6) é uma fruta amazônica, da família das *Myrtaceae*, também conhecido como araçá d'água, caçari ou sarão. É uma espécie distribuída em várzeas e lagos da região Amazônica (MAEDA et al., 2007), sendo encontrado no baixo, meio e alto Rio Amazonas, na parte oriental da Cordilheira dos Andes e países amazônicos como Colômbia, Venezuela, Guiana Inglesa, Bolívia, Perú e Brasil (ANDRADE, 1995).

A planta mede de 4 a 8 m de altura, apresentando ramificação que começa na base, ramas cilíndricas lisas de cor marrom claro ou vermelho, sendo a flor de cor branca, subséssil com quatro pétalas, sendo os frutos globulosos de superfície lisa e brilhante, de cor vermelho escura até preta púrpura ao amadurecer, os quais medem entre dois até quatro centímetros de diâmetro (PINEDO et al., 2002).

Figura 6 - Camu-camu.



Fonte: Autor

A principal característica desta fruta amazônica é a elevada concentração em vitamina C, sendo um recurso para ser explorado como alimento funcional devido a sua elevada capacidade antioxidante (CORREA et al., 2011). A concentração de vitamina C, podendo chegar a 6000 mg 100 g⁻¹ Rufino et al., (2010),

A elevada concentração da vitamina C favorece a formação do colágeno, responsável presente em ossos, dentes e tecidos conjuntivos entre outros (AKTER et al., 2011).

Além da vitamina C, apresenta outros nutrientes como os β -carotenos (ZANATTA et al., 2007) e minerais como alta concentração de potássio em relação

com os outros macronutrientes, sendo determinados por Ribeiro et al., (2016) concentrações de K de 1939 mg 100 g⁻¹ e entre os micronutrientes, destaca o ferro (Fe) com concentrações de 17,7 mg 100 g⁻¹, sendo as concentrações de minerais mais elevadas em ambientes secos que em ambientes alagados.

Por outro lado, o camu-camu é uma importante fonte de aminoácidos e outros compostos bioativos com capacidade antioxidante como os flavonoides, sendo encontradas concentrações de 20,1 ± 4,4 mg 100 g⁻¹, mas essas concentrações depende do grau de maturidade do fruto (AKTER et al., 2011).

Dadas as propriedades desta fruta, tanto o Japão como a União Europeia, são grandes mercados consumidores de camu-camu (AKTER et al., 2011), sendo a polpa transformada em diversos compostos como bebidas gaseificadas, vinagre, recheios de pão, aperitivos, sorvetes, doces e medicamentos. No Brasil o fruto é pouco conhecido (YUYAMA, 2011) e seu consumo ainda é muito restrito, com exceção da região norte, onde é comercializado em forma de polpa o suco (RODRIGUES et al., 2001).

2.1.7 Fruta-do-conde (*Annona squamosa* L.) (Annonaceae).

A fruta-do-conde (*Annona squamosa* L.) (Figura 7) também conhecida como pinha ou ata, pertence a família das Annonaceae, sendo uma família distribuída entre as áreas tropicais da América, África e Ásia, abrigando cerca de 2500 espécies em 140 generos. Na África existem aproximadamente 450 espécies, cerca de 900 espécies, encontram-se entre os Neotrópicos e quase 1200 nas áreas tropicais da Ásia e Austrália (PINTO et al., 2005). Dentro da família das Annonaceae, a fruta-do-conde, de acordo com Bonfim et al. (2014), é uma das representativas dessa família, já que apresenta grande importância econômica no Brasil.

De acordo com Lemos, Pereira e Cavalcanti (1989), é considerada como uma árvore de baixo porte, com 4 a 6 metros de altura e muito ramificada. As folhas são decíduas, de lâminas oblongo-elípticas, de ápice obtuso ou acuminado, medindo de 4,5 a 15,6 cm de comprimento por 2,1 a 6,2 cm de largura, sendo de coloração verde-brilhante na parte superior e verde-azulado na parte inferior.

O fruto é um sincarpo arredondado, ovóide, esférico ou cordiforme, tendo de 5 a 13 cm de diâmetro, cobertos externamente de saliências achatadas em forma de tubérculo regularmente exposto, sendo sua espécie verde-escura coberta no início

do desenvolvimento do fruto por um pó esbranquiçado, existindo também frutos amarelos e roxos (MANICA et al., 2003).

Figura 7 - Fruta-do-conde



Fonte: Autor

As sementes desta fruta apresentam grande potencial biotecnológico, devido à apresentar quantidades significativas de ácidos graxos insaturados como o ácido oleico (47.4%) e ácido linoleico (22.9%) (RANA, 2014).

Entre os usos medicinais desta fruta, estão suas propriedades inseticidas nas folhas, raízes e sementes e possuem metabolitos secundários tipo acetogeninas, asimicina, butalacina, bulatacinona e escuamocina e outras propriedades medicinais como as atribuições que tem o chá das folhas em processos de depressão e doença da medula espinal (CORDEIRO; PINTO; RAMOS, 2000). Por outro lado, existem compostos nas suas sementes envolvidas contra células tumorais do câncer hepático (H22) capaz de induzir a apoptose nas mitocôndrias (YANG et al., 2015).

2.1.8 Graviola (*Annona muricata* L.) (Annonaceae)

A graviola (*Annona muricata* L.) (Annonaceae) (Figura 8) pertence a família das Annonaceae, sendo uma importante fruta na região do Nordeste Brasileiros em

Estados como Pará e região Norte, sendo uma fruta aproveitada para a fabricação de sucos, sorvetes, compotas, geleias e doces. A referida família está formada por 130 gêneros e 2300 espécies (LEBOEUF et al., 1980; MISHRA et al., 2013).

Tanto *Annona muricata* L. como outras espécies da mesma família são utilizadas pelo ser humano como fonte de alimento para o tratamento de doenças como artritis, diarreias, febres, malária, reumatismo entre outras (ADEWOLE; CAXTON-MARTINS, 2006).

Luna (2007) afirma que a família apresenta uma ampla biodiversidade de compostos químicos como aromáticos, ácidos orgânicos, ácidos fenólicos, taninos, flavonoides, compostos benzênicos, catequinas, proantocianidina, óleos essenciais, esteroides, terpenos, alcaloides, acetogeninas, carboidratos, lipídeos, proteínas, lactonas, vitaminas e carotenos.

Figura 8 – Graviola.



Fonte: Autor.

As folhas são obovado-oblongas, medindo de 8-15 cm de comprimento e a altura da árvore até 8 metros. Suas flores são solitárias, com cálice de sépalas triangulares e pétalas externas grossas de cor amarelada (BRANDÃO, 2003).

2.1.9 Taperebá (*Spondias. mombin* L.) (Anacardiaceae)

O taperebá (*Spondias. mombin* L.) (Figura 9) é uma planta originária na América tropical, sendo muito encontrada na região Amazônica, com frutos que podem ser consumidos *in natura* e também em forma de sucos, sorvetes, picolés, cremes e mousses (AZEVEDO et al., 2004). De acordo com Carvalho et al. (2011), na Amazônia, este fruto possui diferentes nomes, entre eles Taperebá, e na região do nordeste como Cajá, em relação com o nome dado pelos indígenas (tapiriba e cajá).

Pertence ao gênero *Spondias*, da família Anacardiaceae, englobando diversas espécies frutíferas de interesse econômico, destacando-se o cajá ou taperebá (*S.mombim* L.), o umbu (*S. tuberosa* Arr. Câmara), o umbu-cajá (*Spondias* sp.) e a umbuguela (*Spondias* sp.), as quais são nativas do Brasil e a ciriguela (*S. purpúrea* L.), a cajarana (*S. dulcis* Park.) ou cajamanga (*S. cytherea*.) consideradas exóticas (SACRAMENTO; SOUZA, 2009).

Figura 9 – Taperebá.



Fonte: Autor

Quanto às propriedades nutricionais desta fruta, destaca-se seu elevado teor de carotenoides, vitamina C e taninos, que atuam como antioxidantes dando ao taperebá certo valor comercial (MATTIETTO; LOPES; MENESES, 2010). Por outro lado, devido que ao taperebá apresentar baixos valores de pH e elevados concentração de açúcares, permitem o desenvolvimento de microrganismos deteriorantes como bolores, leveduras e bactérias que podem causar no alimento alteração das suas propriedades sensoriais e composição química (MAIA et al.,

2007). De acordo com Sacramento e Souza (2009), o fruto do taperebazeiro é classificado como drupa e núculeos, perfumados, com mesocarpo carnosos, amarelo, de sabor azedo, contendo carotenoides, açúcares, vitamina A e C.

Enquanto às propriedades medicinais associadas com esta planta, temos a função antidiarreica e antidesintérica da casca e as folhas servem para combater certos herpes já que apresentam compostos com atividade antiviral (ALVES; FILGUEIRAS; MOURA, 2000).

Os produtos realizados a partir desta fruta, apresentam boa aceitação no mercado, o que confirma seu potencial agrossocioeconômico de exploração desta espécie, tanto *in natura* como processada (MELO et al., 2010).

2.2 COMPOSTOS BIOATIVOS E SUAS FONTES NATURAIS

De acordo com Rufino (2011), o Brasil, devido a sua localização geográfica, dimensão territorial e clima tropical, possui uma das maiores reservas de espécies nativas do mundo com importantes centros de diversidade genética, mas muitas dessas espécies são pouco estudadas.

Existem estudos onde justificam que o aumento de consumo em frutas e legumes ricos em compostos bioativos, leva a redução do risco de desenvolvimento de doenças crônicas e degenerativas, como é o caso de cardiopatias, diabetes tipo II e câncer (ULLOA; SUAREZ, 2004). Dado que o consumo de frutas tropicais está aumentando, tanto no mercado nacional como internacional, e seu reconhecimento das propriedades terapêuticas e nutricionais, sendo interessante o estudo de seus compostos bioativos (REIS et al., 2015).

É importante realizar a caracterização e quantificação dos compostos bioativos presentes em vegetais para o conhecimento do valor nutricional para assim poder agregar um valor adicional ao produto (BOMFIM et al., 2017). Entre os compostos bioativos destacam os minerais, ácidos graxos, compostos com atividade antioxidante e vitaminas.

2.2.1 Minerais

Os minerais são considerados um grupo de nutrientes essenciais, eles não apresentam função energética para o organismo, mais os humanos não conseguem sintetizá-los e devem ser incorporados mediante a dieta (KESSENICH, 2008). Eles são substâncias encontradas em alimentos de origem vegetal e animal em

quantidades pequenas em comparação com as outras biomoléculas como carboidratos, proteínas e lipídeos, sendo as frutas e hortaliças ricas em minerais (BRASIL, 2008). Eles são substâncias inorgânicas que ajudam a regular as funções do corpo (ROACH, 2009). Estão classificados em macrominerais (Ca, Mg, P, K, Na e S) por estar presentes em concentrações maiores e em microminerais por se encontrar em concentrações de traços e ultra traços, sendo estes (B, Cl, Cu, Fe, Mn, Mo e Zn) requeridos pelas plantas em concentrações baixas, estando envolvidos em processos de crescimento e desenvolvimento (DECHEN; NACHTIGALL, 2006). Recentemente, tem despertado interesse o estudo dos micronutrientes, por acreditar-se que muitos problemas de saúde estão relacionados com a deficiência destes micronutrientes (SMOLIN; GROSVENOR, 2007).

Por outro lado, estão envolvidos em outras funções específicas no organismo, já que participam da composição de líquidos corporais e da formação da medula óssea, regulação metabólica, manutenção do equilíbrio ácido-básico, nas funções nervosa e muscular, da pressão osmótica, facilitação da transferência de compostos pelas membranas celulares, composição de tecidos, além de efeitos sinérgicos entre eles (MANGANARO, 2008). Atuam também como eletrólitos envolvidos no equilíbrio de fluidos, acidez gástrica e equilíbrio ácido-base além de desenvolver funções metabólicas importantes como transporte ativo, regulação da pressão arterial, potencial de membrana e transmissão nervosa (SULAIMAN et al., 2011; FREELAND-GRAVES; TROTTER, 2003).

A necessidade de minerais varia entre o 4-15% do peso corporal, correspondendo o 50% ao cálcio, 25% ao fósforo e 25% corresponde a outros minerais como o magnésio, sódio, potássio, cobre (CÁCERES et al., 2006). Por isso as frutas são consideradas como importante fonte nutricional na dieta humana, sendo elas um aporte importante de elementos essenciais para o organismo como vitaminas A e C, minerais, fibras e vários fitoquímicos (HARDISSON et al., 2001; SMOLIN; GROSVENOR, 2007).

2.2.1.1 Potássio (K)

O potássio desenvolve um importante papel no organismo humano, já que está presente na manutenção do equilíbrio hidroelettrico nas células e juntamente com o sódio, eles ocorrem em concentrações específicas tanto dentro como fora da

célula e para manter esse equilíbrio a concentração de potássio deve ser elevada dentro da célula e a concentração de sódio elevada fora da célula (CUPPARI; BAZANELLI, 2010). De acordo com Elcinto (2000), o potássio é encontrado em concentrações elevadas em frutas e verduras frescas, especialmente na casca e talo das plantas comestíveis. Desenvolve um papel importante na qualidade dos frutos agrícolas, já que está implicado no aumento do tamanho dos frutos, na espessura da casca, no índice de acidez da polpa, no desenvolvimento da raiz, controla a turgidez dos tecidos, abertura e fechamentos dos estomas, transporte de metabolitos primários como os carboidratos e amidos e síntese de proteínas, por tanto, trata-se de um elemento indispensável para obter uma boa produtividade (SOARES et al., 2005).

Por outro lado, a ingestão de potássio é importante porque está envolvido com a redução da pressão arterial, já que um aumento na concentração de potássio no corpo, produz uma vasodilatação dependente do endotélico pela hiperpolarização da célula endotelial consequente à estimulação do sódio-potássio APTase e da abertura dos canais do potássio, sendo as necessidades consideradas para adultos entre 1600 – 2000 mg dia⁻¹ (MAHAN; STUMP, 2005).

O potássio também está envolvido no metabolismo energético e na secreção de insulina. Na regulação do pH do organismo, o potássio desenvolve um importante papel, já que uma dieta rica em potássio, proveniente principalmente de frutas e hortaliças, afeta favoravelmente o metabolismo ácido-base já que nas frutas, o potássio está associado a íons orgânicos que ajudam na formação de bicarbonatos e têm um importante papel na neutralização dos ácidos orgânicos gerados pelo organismo (CUPPARI; BAZANELLI, 2010).

Quanto às recomendações do potássio, dado que ele fica ligado com outros ions a ingestão adequada do potássio é de 4,7 g dia⁻¹, para adultos e jovens, de acordo com as recomendações do *Recommended Dietary Allowances* (RDA) (CUPPARI; BAZANELLI, 2010).

2.2.1.2 Sódio (Na)

O sódio é outro elemento importante para o organismo, envolvido em processos de regulação da pressão arterial, volume sanguíneo, equilíbrio ácido-base, transmissão do impulso nervoso, função muscular e, ao mesmo, tempo função

celular normal, mas atualmente, o consumo médio de sódio no mundo é maior que as concentrações requeridas para o bom funcionamento do corpo (ABURTO et al., 2013). Ele está associado na forma de cloreto de sódio nos alimentos, estando relacionado seriamente com os problemas de hipertensão sendo as recomendações deste elemento na população adulta de acordo com a Organização Mundial da Saúde (OMS) de 5 gramas de cloreto de sódio ou 2000 mg de sódio por dia em população adulta (WHO, 2012).

Uma das mais importantes funções do sódio no organismo é na sua participação na bomba de sódio-potássio, já que o sódio assim como o potássio, são elementos que estão em concentrações específicas dentro e fora da célula, já que o sódio está em concentrações elevadas no meio extracelular e passa ao meio intracelular mediante o processo de difusão e com o potássio acontece ao contrário, porém para manter a concentração ideal dos dois íons a bomba sódio-potássio bombeia sódio para fora da célula e potássio para dentro dela, sendo um transporte realizado contra a gradiente de concentração destes dois elementos, realizado com a energia obtida do clivagem de ATP (BAZANELLI; CUPPARI, 2009).

A ingesta deste elemento não só está presente nos alimentos sólidos que consumimos, também está presente nos líquidos, já que a concentração média nas águas puras possui aproximadamente um 1% de sódio (JEREB, 2016).

2.2.1.3 Cálcio (Ca)

O cálcio é o elemento predominante no corpo humano, sendo essencial para a mineralização de ossos e dentes e para a regulação de eventos intracelulares em diversos tecidos, sendo distribuído no corpo da forma seguinte: 1% no sangue, tecido extracelular e tecidos moles, sendo que 50% desse, encontram-se na forma ionizada, 40% ligado a proteínas não difundíveis como albumina e os 10% restantes sob a forma de complexos com íons fosfato e citrato (de FRANÇA; MARTINI, 2014).

O cálcio, uma vez que é ingerido, é ionizado e absorvido pelo intestino delgado onde vai interagir com outros componentes da dieta formando complexo cuja solubilidade depende de diferentes fatores que afetam a taxa de absorção (KRUGER et al., 2006).

É um elemento principal na fração inorgânica dos ossos, sendo responsável pela sua dureza. Por outro lado, o cálcio apresenta função neuromuscular

participando das sinapses químicas e envolvidas nos processos de coagulação sanguínea, onde sua presença é fundamental para que todo o processo ocorra uma vez que é estimulada a liberação de tromboplastina das plaquetas do sangue e age como cofator para a conversão da protrombina em trombina, a qual auxilia na polimerização do fibrinogênio em fibrina. Outros processos onde o cálcio está envolvido são nos processos de permeabilidade das membranas, secreção de hormônios e digestão, apresenta efeito hipotensor mediante via suplementação reduzindo a vasoconstrição (de FRANÇA; MARTINI, 2014).

Nas frutas, apresentam uma interessante propriedade relacionada com a firmeza delas, já que elevados valores de cálcio, estão associados com a qualidade da fruta, por conta do que acontece com o nitrogênio e potássio que apresentam efeito negativo quanto a sua firmeza (JOHNSTON; HEWETT; HERTOY, 2002; POOVAIAH et al., 1988).

As recomendações de cálcio de acordo com o *Recommended Dietary Allowances* (RDA) variam conforme a idade e estado fisiológico, sendo superior no final da infância e adolescência donde as necessidades de cálcio são superiores. Na idade adulta, a formação e reabsorção de cálcio estão estáveis e mantida em torno de 1000 mg dia⁻¹ para ambos sexos (de FRANÇA E MARTINI, 2014).

2.2.1.4 Magnésio (Mg)

O magnésio está dentro dos macronutrientes, estando presente em diversos minerais como cereais, verduras, frutas, legumes, nozes e tubérculos como a batata (VOLPE, 2013), sendo no corpo humano o quarto cátion mais abundante depois do cálcio, potássio e sódio, participando em uma ampla variedade de reações bioquímicas (WOLFE; CITTADINI, 2003).

Este elemento está envolvido em numerosas reações metabólicas, atuando como cofator em mais de 300 reações metabólicas, implicado em importantes reações como o metabolismo da glicose, na homeostase insulínica e glicêmica assim como na síntese de ATP, proteínas e ácidos nucleicos (VOLPE, 2013). Por outro lado, o magnésio é interessante no metabolismo ósseo, sendo necessário para manter o metabolismo, estando metabolicamente relacionado com o cálcio, podendo atuar como sinergista e ao mesmo tempo como antagonista (MONTEIRO; VANNUCCHI, 2010).

No corpo humano a quantidade total do magnésio é de 25 g estando presente entre 60 – 65% nos ossos e nos músculos na forma de fosfatos e carbonato. O restante está localizado no tecido mole (27% no músculo) e interior das células, sendo o segundo cátion mais importante no médio intracelular. Por outro lado, o 1 % deste elemento, é encontrado no plasma (MONTEIRO; VANNUCCHI, 2010).

Em quanto à biodisponibilidade do magnésio, cerca dos 45% de magnésio ingerido é absorvido pela dieta no intestino delgado, cólon e em menor proporção pelo estômago, sendo o restante excretado pelas fezes (MONTEIRO; VANNUCCHI, 2010). Por outro lado, outros compostos podem atuar como inibidores da sua absorção como é o caso dos ftalatos, oxalatos, fosfatos, fibras alimentares e algumas proteínas que podem alterar o processo, chegando inclusive a reduzir o processo de absorção quando a ingestão proteica é inferior a 30 g dia⁻¹ (NAITHANI, 2014). Esse efeito inibitorio dos ftalatos, radica na influencia dos ftalatos radical de formar complexos solúveis e resistentes (BENAVIDES et al., 2011).

As recomendações deste elemento de acordo com *Recommended Dietary Allowances* (RDA) são: crianças com idade até 6 meses 30 mg dia⁻¹, entre 7 e 12 meses 75 mg dia⁻¹, idade entre 1-3 anos as recomendações são de 80 mg dia⁻¹, entre 4 - 8 anos 130 mg dia⁻¹, de 9-13 anos as recomendações diárias são de 240 mg dia⁻¹. Posteriormente, na adolescência (14-18 anos), as recomendações para homens e mulheres mudam necessitando os homem uma maior concentração de magnésio de 410 mg dia⁻¹ e para mulheres de 360 mg dia⁻¹. Para jovens entre 19 e 30 anos, o homen precisa de 400 mg dia⁻¹ e mulheres de 310 mg dia⁻¹. Na idade adulta, a partir dos 31 anos, os níveis recomendados de magnésio são de 420 mg dia⁻¹ para homens e de 320 mg dia⁻¹ para mulheres.

2.2.1.5 Fósforo (P)

O fósforo é outro dos macroconstituintes presentes em frutas e verduras estando implicado no organismo em diversas funções como atuar no metabolismo energético do ATP, implicado no metabolismo dos carboidratos e presente ao mesmo tempo na síntese de açúcares fosfatados, ácidos nucleicos e coenzimas (EPSTEIN; BLOOM, 2006). No corpo, o fósforo que na maior parte não está mineralizado nos tecidos, se encontra na forma de fosfato inorgânico, a forma livre,

ou orgânico, a forma covalentemente ligada a açúcares, proteínas e outros componentes celulares (MONTEIRO; VANNUCCHI, 2010).

As fontes vegetais de absorção de fósforo para o organismo são os fosfolípidos, ácidos nucleicos, sendo um elemento encontrado nas sementes de vegetais em concentrações maiores que nos caules e folhas (LIMA, 2000).

No corpo, as concentrações de fósforo encontradas são de 600-900 g, correspondente entre 0,8 a 1,1% do peso corporal total do indivíduo adulto, estando desse total, 85% junto ao cálcio na estrutura mineral de ossos e dentes e o restante junto com os tecidos moles no líquido extracelular (MONTEIRO; VANNUCCHI, 2010).

Um parâmetro interessante em relação ao fósforo é que no metabolismo de absorção, eles estão relacionados na proporção cálcio: fósforo (2:1) para que a taxa de absorção seja ótima (ANDRIGUETTO et al., 1990).

As recomendações de fósforo diárias, de acordo com o Dietary Reference Intakes (2009), são os seguintes: crianças entre 0- 6 meses necessitam 100 mg dia⁻¹, adultos a partir de 19 anos as recomendações de fósforo são de 700 mg dia⁻¹ e crianças entre 7 - 12 meses os níveis requeridos de fósforo aumentam até 275 mg dia⁻¹. Entre 1-3 anos as necessidades de fósforo são de 380 mg dia⁻¹, entre 4-8 anos as necessidades são de 405 mg dia⁻¹, de 9 -18 anos as concentrações de fósforo recomendadas são de 1,250 mg dia⁻¹ e para Adultos a partir de 19 anos as recomendações de fósforo são de 700 mg dia⁻¹.

2.2.1.6 Enxofre (S)

O enxofre (S) é outro dos elementos considerados como macronutriente sendo expresso na forma de S elementar o como trióxido de enxofre SO₃, sendo considerado como essencial para a vida, formando parte da estrutura de biomoléculas como são as proteínas e encontrando-se no corpo humano cerca de 140 g de enxofre (LISBOA, 2015). Ao mesmo tempo ele está presente em outras biomoléculas como são os carbodidratos lipídeos assim como em metabolitos secundários (RAAB; FELDMANN, 2019).

2.2.1.7 Ferro (Fe)

O Fe é um micronutriente essencial para as células vivas, participando de importantes reações metabólicas como componente do ciclo de Krebs. O Fe é insolúvel no meio ambiente, encontrando-se nos estados de oxidação ferroso (Fe^{2+}) e férrico (Fe^{3+}), sendo que em soluções aquosas o ferro no estado ferroso é rapidamente oxidado para a forma férrica sendo insolúvel em pH fisiológico. Para que seja mantido em solução e seja utilizado pelo organismo, é fundamental estar associado a agentes quelantes (FISBERG et al., 2017).

A disponibilidade do ferro depende da composição e da forma como é consumido na dieta (FANTISI et al., 2008). No organismo, o conteúdo de ferro corporal corresponde entre 3 e 5 gramas, distribuindo-se basicamente em duas categorias: a dos compostos essenciais ou funcionais que correspondem a cerca de 80 % desse ferro como hemoglobina, mioglobina, citocromo-oxidase a,b,c, transferases, catalases e outras enzimas, que fazem parte desse grupo, sendo o outro 20 % aquele encontrado sob a forma de depósito, estocado nos hepatócitos e nas células do sistema retículo-endotelial (SRE), na forma de ferritina e hemossiderina (FISBERG et al., 2017).

As recomendações de Fe de acordo com o *Dietary Reference Intake* (DRI, 2001), são de 0,27 mg dia⁻¹, para crianças entre 0 - 6 meses, para crianças entre 7 meses até 1 ano de 11 mg dia⁻¹, para pessoas entre 1 - 3 anos as recomendações de Fe são de 3 mg dia⁻¹ e para idade entre 4 - 8 anos as recomendações de Fe são de 10 mg dia⁻¹. Para idade entre 9-13 anos as recomendações de Fe para homens são de 8 mg dia⁻¹ e para mulheres também de 8,0 mg dia⁻¹, já na idade entre 14 -18 anos, as recomendações de Fe são de 11 mg dia⁻¹ para homens e 15 mg dia⁻¹ para mulheres. Na idade adulta para os homens a recomendação diária é de 8 mg dia⁻¹ e para as mulheres entre 19 - 50 anos as concentrações de Fe recomendadas são de 18 mg dia⁻¹ e a partir de 50 anos, as concentrações requeridas são de 8 mg dia⁻¹.

2.2.1.8 Zinco (Zn)

O Zn é um elemento necessário para o funcionamento de todas as células do organismo, sendo essencial para o bom funcionamento do sistema imunológico, controle da diabetes, melhora nos níveis de estresse, acne e cicatrização entre

outros (FREITAS; SILVA; DA SILVA, 2016). Encontra-se distribuído no corpo em pequenas concentrações entre 1,5 a 2,5 gramas (HAMBIDGE et al., 2008).

O Zn apresenta diferentes funções fisiológicas como a mobilização hepática da vitamina A, na maturação sexual, fertilidade e reprodução, função fagocitária, imunitária celular e humoral, no paladar e apetite (MANGANARO, 2008). Por outro lado, o zinco, exerce um grande papel em reações enzimáticas, atuando em mais de 300 metaloenzimas, assim como seu papel antioxidante participando no metabolismo da enzima superóxido dismutase como componente estrutural (COMINETTI, 2009). Essa enzima tem a propriedade de reduzir a toxicidade de espécies reativas de oxigênio, transformando uma espécie altamente reativa (radical ión superóxido) em uma forma menos danosa para as células (peróxido de hidrogênio) (KOURY; DONANGELO, 2003).

Enquanto à ingestão do Zn no organismo, tem que ser considerado o termo de biodisponibilidade, que indica a quantidade do zinco consumida que é absorvida pelo organismo para satisfazer as necessidades fisiológicas, sendo que, para este elemento, a biodisponibilidade no processo intestinal ou sanguínea (PEREIRA; HESSEL, 2009).

Uma importante função do Zn e sua vinculação com o sistema nervoso, estando presente em circuitos neurais, relacionando-se com algumas sinapses glutamatérgica atuando como receptor pós-sináptico (PERSON; NARDI; FÉRES, 2004). Por outro lado, o zinco está envolvido em processos de carcinogêneses, relacionando-se que a suplementação de zinco exerce efeitos benéficos ao paciente com câncer (FERNANDES; MAFRA, 2005).

Quanto à toxicidade do Zn, ela está presente quando este supera os limites estabelecidos, não sendo comum com a exceção dos indivíduos que consomem alimentos marinhos em grandes quantidades, com valores superiores aos 45 mg dia⁻¹, produzindo na fase aguda náuseas, vômitos, dor abdominal, gosto metálico e cefaleia, e na crônica, deficiência de cobre e anemia (FAO/WHO, 2001; MANGANARO, 2008).

Por outro lado, existe certo sinergismo entre o Zn, Fe e Cu, dado que absorção de Zn pode ser afetada pela suplementação do Fe, em quanto à ingestão em excesso de Zn pode reduzir à absorção de cobre (PINHEIRO, PORTO; MENEZES, 2005).

As recomendações de Zn de acordo com *Recommended Dietary Allowances* (DRA), 2001 aumentam à medida que a pessoa envelhece até estabilizar a partir dos 19 anos. Uma criança entre 7 meses até 3 anos precisa de concentrações de 2,5 mg dia⁻¹, entre 4-8 anos precisaria concentrações de 4 mg dia⁻¹, entre 9-13 anos precisaria de concentrações de 7 mg dia⁻¹, de 14 a 18 concentrações de 7,3 mg dia⁻¹, os homens a partir de 19 anos e durante toda a vida precisam 9,4 mg dia⁻¹ e para as mulheres, concentrações de 11 mg dia⁻¹.

2.2.1.9 Manganês (Mn)

O Mn é um micronutriente encontrado na natureza como óxido de manganês (MnO₂), conhecido como pirolusita, também na forma hidratada, a manganita (Mn₂O₃H₂O) e como hausmanita (Mn₃O₄), sendo o terceiro metal de transição mais abundante na crosta terrestre (COSTA; FIGUEIREDO, 2001). Em frutas, a concentração de manganês de acordo com WHO (1999), os valores de este elemento oscilam entre 0,20-10,38 mg kg⁻¹.

É considerado um microelemento essencial para as plantas, sendo de acordo com Malavolta (2006), o segundo micronutriente mais exigido pelas culturas, aparecendo depois do Fe. Nas plantas, ele apresenta propriedades semelhantes com outros íons metálicos como Ca, Mg, Fe e Zn sendo que esses metais têm a inibir a absorção e transporte do Mn²⁺, mediante competição entre eles, especialmente o Fe (FRANCO, 2007) já que na dieta, o excesso de manganês pode provocar a redução na absorção do ferro provocando anemia, além de afetar ao sistema nervoso central (ROELS et al., 1997).

Um aspecto interessante em relação a sua toxicidade é que a diferença de outros elementos tóxicos como é o caso do mercúrio e chumbo, já que o Mn é um oligoelemento essencial participando em numerosas reações metabólicas, envolvido em reações de reposta imune e regulação e síntese de ATP, assim como cofator em metaloenzimas como Mn-superóxido dismutase, arginase, fosfoenol-piruvato descarboxilase e glutamina sintetase (BURTON; GUILLARTE, 2009). Essa enzima superóxido de dismutase (MnSOD) integra o sistema de defesa de radicais livres (CORREIA, 2001). Altas doses de ingestão do Mn alcançam o cérebro através da inalação, seguindo da ingestão, sendo a maioria de efeitos tóxicos deste elemento a ingestão crônica desse mineral (WHO, 1999). Por outro lado, tem um importante

papel na mineralização óssea, no metabolismo energético e proteico assim como, na proteção celular dos radicais livres e na formação de glicosamina (WHO, 1999).

As concentrações de Mn no organismo de acordo com *Recommended Dietary Allowances* RDA (2001), variam em função da idade, sendo necessárias concentrações de 3 $\mu\text{g dia}^{-1}$, para crianças entre 0-6 meses de idade, para crianças entre 7-12 meses concentrações de 0,6 mg dia^{-1} , para crianças entre 1-3 anos são necessárias doses de Mn de 1,2 mg dia^{-1} e para crianças de 4-8 anos as doses aumentam até 1,5 mg dia^{-1} . Enquanto meninos entre 9-13 anos as doses recomendadas de manganês são de 1,9 mg dia^{-1} , e entre 14 e 18 anos de 2,2 mg dia^{-1} . No caso das meninas entre 9-18 anos as concentrações requeridas são de 1,6 mg dia^{-1} . Na idade adulta, as concentrações de manganês para homens são de 2,3 mg dia^{-1} e para as mulheres de 1,8 mg dia^{-1} .

2.2.1.10 Cobre (Cu)

O Cu é outro dos nutrientes essenciais não sintetizado pelo organismo, sendo indispensável para a vida, estando presente em quantidades de traços em leite e produtos lácteos e em pequenas quantidades em óleos, vegetais, frutas e cereais, leguminosas, nozes e frutos do mar, sendo os níveis deste micronutriente em frutas de 0,02 – 0,66 $\text{mg Cu } 100 \text{ g}^{-1}$ (AMANCIO, 2017).

É um elemento que apresenta atividade enzimática óxido reductase: lisis-6-oxidase, catecol oxidase, superóxido de dismutase I e ceruloplasmina, participando ao mesmo tempo em manter o sistema imune, envolvido na expressão genética e ao mesmo tempo atua como componente alostérico (AMANCIO, 2017; LUGO, 2017).

O Cu ao mesmo tempo, está relacionado com o metabolismo do ferro, já que, de acordo com Linder (1996), na ceruloplasmina que é uma enzima extracelular, estão presentes seis átomos de cobre por molécula apresentando duas funções: eliminar os radicais oxigênicos e por outro lado, atuar também como uma ferroxidase oxidando o ferro ferroso, oxidação necessária para a ligação do Fe à transferrina, envolvida nos processos de transferência do ferro a partir dos depósitos locais de síntese de hemoglobina.

Também está envolvido em processos de sínteses no tecido conjuntivo, na síntese da melanina e envolvido nos processos de neurotransmissão onde a

monoamina-oxidase promove a degradação da serotonina, norepinefrina, tiramina e dopamina (TURNLUND, 1999).

A deficiência de cobre está relacionada com as doenças de Wilson e de Menkes e ao mesmo tempo pela baixa ingestão alimentar e aumento na excreção manifestando-se clinicamente como anemia hipocrômica, anormalidades ósseas e anormalidades no metabolismo do colesterol e glicose (AMANCIO, 2017).

De acordo com *Recommended Dietary Allowances* (RDD), (2011) as recomendações de Cu são as seguintes: crianças entre 0-6 meses 200 $\mu\text{g dia}^{-1}$, entre 7-12 meses 220 mg dia^{-1} . Adolescentes entre 9-13 anos necessitam concentrações diárias de cobre de 540 $\mu\text{g dia}^{-1}$ e entre 14-18 anos a dose de cobre requerida é de 685 $\mu\text{g dia}^{-1}$. Em adultos a partir de 19 anos e durante toda a vida, as concentrações de cobre requeridas são de 700 $\mu\text{g dia}^{-1}$ por pessoa.

Quanto à toxicidade do cobre, de acordo com Mendes (2007), ele passa a ser tóxico quando chega a concentrações elevadas. O Cu quando permanece no solo, afeta fortemente aos minerais e matéria orgânica, podendo se acumular em plantas e animais. No organismo, o excesso de cobre pode ser prejudicial já que tem afinidade pelos grupos tiol (SH) presentes nas proteínas e enzimas causando doenças como a epilepsia, melanomas, artrite, reumatoide e doenças psiquiátricas (AZEVEDO, 2003).

2.2.1.11 Boro (B)

O B é um elemento essencial para o homem, sendo sua principal via de ingestão as frutas, vegetais, água e outros produtos de origem animal (VIELMA et al., 2017). Ele é absorvido pelas plantas a partir do solo em sua fração solúvel como ácido bórico (H_3BO_3) chegando às raízes via fluxo de massa (MATTIELLO et al., 2009), sendo um micronutriente requerido em quantidades pequenas para as plantas mas vital para o crescimento normal das mesmas, dado que está envolvido na síntese da parede celular e manutenção da integridade da membrana plasmática (BROWN et al., 2002).

Quanto às funções fisiológicas benéficas para o organismo, Penland (1994) destaca sua importância na função das membranas e eletrofisiologia cerebral e implicado ao mesmo tempo no desenvolvimento cognitivo, porém, outros autores como Armstrong et al., (2002) afirmam que o B tem influência no metabolismo de

minerais interagindo com outros nutrientes, e, ao mesmo tempo, envolvido no metabolismo ósseo.

Sobre as recomendações de B no organismo, ainda não estão estabelecidos os níveis recomendados a ser ingeridos pelos seres humanos, apenas sua importância fisiológica no crescimento ósseo, prevenção de artrite e regulação hormonal, sendo estabelecida por Nielsen (2014), uma ingestão diária de al menos 3 mg dia⁻¹. De acordo com os valores estabelecidos pelo *Recommended Dietary Allowances* RDA (2001), as recomendações de B aumentam com a idade, sendo estabelecidos para crianças entre 1-3 anos valores recomendados de 3 mg dia⁻¹ de B, para crianças entre 4-8 anos de 6 mg dia⁻¹ de B e entre 9-13 anos de 11 mg dia⁻¹ de B. Na adolescência, as recomendações são de 17 mg dia⁻¹ de B e para adultos entre 19-50 anos de idade de 20 mg dia⁻¹ de B.

2.2.1.12 Cobalto (Co)

O Co é um microelemento encontrado nos alimentos em concentrações de traços, sendo considerado um elemento essencial já que sua deficiência pode causar anemia perniciosa, estando presente na vitamina B12 também conhecida como cobalamina, uma vitamina incapaz de ser sintetizada pelo organismo, sendo a recomendação diária de ingestão de vitamina B12 de 2,4 µg da qual corresponde ao 0,1 µg de Co de acordo com a *Agency for Toxic Substances and Disease Registry* (2001). Esta vitamina é a principal fonte de reserva deste elemento no organismo (PRASAD, 2004). Dado que o Co tem um papel fundamental para evitar a anemia mais ele ajuda a aumentar a produção de eritrócitos e ajudar ao mesmo tempo a melhorar a absorção de ferro no organismo sendo os níveis recomendados de ingestão deste elemento de 0,16 a 1,0 mg de Co por quilograma de massa corporal (*Agency for Toxic Substances and Disease Registry*, 2001; GOODHART; SHILS, 1973). Por outro lado, a FAO/WHO (2003) estabelecem que a ingestão média de Co no organismo é de 0,58 mg kg⁻¹ em função do peso corporal do indivíduo. Se o consumo deste elemento é baseado em frutas, a concentração deste elemento será de 0,58 mg kg⁻¹. No solo, é um metal envolvido na absorção do nitrogênio, via simbiótica, sendo essencial para a fixação simbiótica e ao mesmo tempo para o crescimento do *Rhizobium* (LOWE; EVANS, 1962; AHMED; EVANS, 1960).

Por outro lado, o Co dado que é um metal pesado, pode causar danos ao organismo já que pode ser bioacumulado no corpo (BAHEMUKA; MUBOFU, 1991)

apresentando problemas de toxicidade como é a cardiomiopatia um mecanismo que envolve a inibição de desidrogenase mitocondrial com consequente falência da respiração celular, também vinculado a problemas nervosos e engrossamento do sangue (SEGHIZZI et al., 1994; US National Library of medicine, 2015). Dados os problemas de toxicidade que pode apresentar o Co, é importante seu controle nos alimentos para prevenir doenças que sua ingestão em excesso pode apresentar.

2.2.2 Ácidos graxos

Os lipídeos são compostos constituídos por um grupo numeroso de substâncias caracterizadas todas elas principalmente por ser insolúveis em água. Eles são classificados em lipídeos simples que são aqueles que não produzem ácidos graxos após o processo de hidrólises ou em lipídeos compostos que são aqueles formados por unidades de glicerol e ácidos graxos que podem (ou não) estar ligados à aminiálcoois ou lipídeos mais simples. Entre os lipídeos compostos os mais abundantes são os triglicéridos que apresentam função de reserva energética. Outros lipídeos compostos são os fosfoglicerídeos ou os esfingolipídeos, constituintes das membranas celulares. Por outro lado, entre os lipídeos simples temos os esteróis, sendo o colesterol o mais importante, os derivados de ácidos graxos com função metabólica e as vitaminas lipossolúveis A, D, K e E (MOREIRA et al., 2002; GONZÁLEZ, 2006).

Os ácidos graxos são considerados os lípidos mais importantes já que desenvolvem importantes funções fisiológicas no corpo, como é sua função energética, aportando 9 kcal g⁻¹ e também, formam parte das estruturas dos fosfolípidos, nas membranas celulares. Em lactantes, os ácidos graxos aportam mais do 50 % das necessidades energéticas diárias e temos alguns ácidos graxos que são considerados essenciais, sendo requeridos para sintetizar ácidos graxos de cadeia maior como os eicosanoides e docosanoides como podem ser os leucotrienos, prostanglandinas, tromboxanos, prostanciclinas, protectinas e resolvins, e ao mesmo tempo tem ácidos graxos implicados como mensageiros na regulação da expressão génica (WEYLANDT et al., 2012).

Quanto à biosíntese de ácidos graxos a partir das plantas, geralmente apresentam entre 12 e 24 átomos de carbono, sempre em número par, já que são sintetizados mediante a condensação de unidades de acetato, constituídas por 2

átomos de carbono, atuando a enzima malonil-CoA como substrato para a reação de carboxilação, sendo a montagem dos carbonos no substrato malonil-CoA um ciclo em quatro passos onde cada passo pelo ciclo aumenta dois átomos de carbono provenientes do acetil-CoA que possui dois carbonos em sua molécula liberando ao mesmo tempo uma molécula de CO_2 e utiliza como agente redutor NADPH (LEHNINGER; NELSON; COX, 2006).

Os humanos são capazes de sintetizar mediante o processo da glicose aminoácidos os ácidos graxos saturados e monoinsaturados através de diferentes reações catalisadas por enzimas mediante alongamento de dois átomos de carbono e por outro lado, dessaturação que consiste na criação de novas duplas ligações, porém, não possuem as enzimas dessaturases responsável pela adição da dupla ligação antes do carbono 9 a partir da extremidade metil, por isso o ser humano não pode sintetizar esses ácidos graxos. Por conseguinte, os mamíferos só podem introduzir dupla ligação só na posição 9 do ácido graxo saturado, mais não pode introduzir a dupla ligação nos carbonos próximos na posição 1 (BLASBALG et al., 2011).

2.2.2.1 Classificação

Os ácidos graxos são constituídos por cadeias de hidrocarbonetos que contém um grupo carboxila e um grupo metila, estando os carbonos ligados mediante uma ou duplas ligações, sendo o número de átomos de carbono na cadeia, o tipo de ligação que determina os diferentes tipos de ácidos graxos (PATTERSON et al., 2011).

2.2.2.1.1 Ácidos graxos saturados (AGS)

Os ácidos graxos simples são aqueles que apresentam ligações simples entre os carbonos que constituem as cadeias. Os AGS saturados podem ser divididos em dois grandes grupos: aqueles de cadeia média que são os que apresentam entre 8-12 átomos de carbono na cadeia, os de cadeia longa que são aqueles que apresentam acima de 14 átomos de carbono. Esses ácidos graxos de cadeia média são transferidos para a circulação sanguínea. Nesse transporte, são ligados à proteína albumina e diretamente passam ao fígado onde são metabolizados e causam o aumento dos níveis do colesterol sérico (PATTERSON et al., 2011).

Na Tabela 1, são apresentados os ácidos graxos saturados assim como suas principais características químicas e físicas.

Tabela 1 - Clasificação e ponto de fusão dos AGS.

Nomenclatura	Nome comum	Nome sistemático	Fórmula	Ponto de fusão (°C)
C4:0	Butírico	Ácido butanoico	$\text{CH}_3(\text{CH}_2)_2\text{COOH}$	-5,3
C6:0	Caproico	Ácido Hexanóico	$\text{CH}_3(\text{CH}_2)_4\text{COOH}$	-3,2
C8:0	Caprílico	Ácido ctanoico caprílico	$\text{CH}_3(\text{CH}_2)_6\text{COOH}$	16,50
C10:0	Cáprico	Ácido decanóico	$\text{CH}_3(\text{CH}_2)_8\text{COOH}$	31,60
C12:0	Láurico	Ácido dodecanóico	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	44,8
C14:0	Mirístico	Ácido tetradecanóico	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	54,4
C16:0	Palmítico	Ácido hexadecanóico	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	62,9
C18:0	Esteráico	Ácido Octadecanoico	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	70,1
C20:0	Araquídico	Ácido Eicosanoico	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$	76,1
C22:0	Behénico	Ácido docosanóico	$\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$	80,0
C24:0	Lignocénico	Ácido tetracosanóico	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$	84,2

Fonte: Valenzuela e Valenzuela

2.2.2.1.2 Ácidos graxos insaturados (AGI).

Estes ácidos podem ser do tipo mono-, di- o poli-insaturados e possuem duplas ligações entre os carbonos. Dentro dos AGI podem ser feitas duas classificações: por um lado temos os ácidos graxos monoinsaturados (AGMI) que

são aqueles que apresentam uma única saturação e os ácidos graxos poli-insaturados (AGPI), que são aqueles que apresentam duas ou mais insaturações.

A sua vez, os AGPI podem ser divididos em duas categorias de acordo ao tamanho da cadeia carbônica. Aqueles que possuem átomos de carbono maior ou igual a 16 são denominados ácidos graxos poli-insaturados de cadeia longa (AGPI-CL) e aqueles que apresentam unidades carbônicas iguais ou superiores a 20, são denominados ácidos graxos poli-insaturados de cadeia muito mais longa (Perini et al., 2010).

Na Tabela 2, é apresentada a classificação dos principais AGI junto com seus pontos de fusão.

Tabela 2 - Classificação e ponto de fusão dos AGI.

Nomenclatura	Nome comum	Nome sistemático	Fórmula	Ponto de fusão (°C)
C16:1	Palmitoleico	<i>cis</i> -9-hexadecanoico	C ₁₅ H ₂₉ COOH	0,0
C18:1	Oléico	<i>cis</i> -9-octadecanoico	C ₁₇ H ₃₃ COOH	16,30
C18:2	Linoleico	9,12-octadecadienoico	C ₁₇ H ₃₁ COOH	-5,00
C18:3	Linolênico	9,12,15-octadecatrienoico	C ₁₇ H ₂₉ COOH	-1,00
C20:4	Araquidônico	5,8,11,14-eicosatetraenoico	C ₁₉ H ₃₁ COOH	49.5

Fonte: Valenzuela e Valenzuela (2013) adaptada.

Dentro dos AGI é importante falar da sua conformação dado que os AGI que acontecem na natureza estão presentes na conformação *cis*, e sua alteração para a conformação *trans*, pode afetar vários processos fisiológicos (PADOVESI et al., 2002). Nos óleos vegetais, são encontrados quando acontece a hidrogenação parcial dos mesmos, processo realizado mediante o aquecimento do óleo na presença de níquel ou outra espécie metálica, processo feito para aumentar a estabilidade do óleo e diminuir sua viscosidade para aumentar a variedade de

aplicações nos processos industriais, sendo as mudanças que acontecem além de transformar as ligações *cis* em ligações *trans*, migração de duplas ligações ao longo da cadeia acil formando diferentes isômeros de posição e por outro lado, a formação de ácidos graxos saturados (LICHTENSTEIN et al., 2001).

Dado que os ácidos graxos *trans*, apresentam o mesmo peso molecular e tamanho que seu isômero *cis*, só mostram diferenças no ponto de fusão (VALENZUELA; MORGADO, 1999; MANCINI-FILHO, 2001).

O problema que apresentam os ácidos graxos *trans* é sua rápida absorção sendo incorporados rapidamente na maioria dos tecidos e competem com o metabolismo dos ácidos graxos essenciais, alterando a agregação plaquetária e a função vascular, provocando, quando são incorporados nas membranas lipídicas sofram processos de absorção e ao mesmo tempo podem alterar as propriedades físicas das membranas (VALENZUELA; MORGADO, 1999; IBRAHIM; GRAFOO-RUNISSA, 2001).

2.2.2.2 Importância biotecnológica dos ácidos graxos vegetais.

Nas Tabelas 3 e 4, são apresentados a composição percentual média de ácidos graxos em óleos e gorduras animais e vegetais de usso comum para poder ser comparados com os valores obtidos para as diferentes frutas amazônicas neste trabalho.

Tabela 3 - Composição de ácidos graxos saturados em óleos e gorduras comuns.

Composição média de ácidos graxos (%)								
Saturados								
Gordura ou óleo	C 4:0	C 6:0	C 8:0	C 10:0	C 12:0	C 14:0	C 16:0	C 18:0
Gorduras animais								
Manteiga	3-4	1-2	0-1	2-3	2-5	8-15	25-29	9-12
Banha						1-2	25-30	12-18
Sebo de boi						2-5	24-34	15-30
Óleos vegetais								
Oliva						0-1	5-15	1-4
Amendoim							7-12	2-6
Milho						1-2	7-11	3-4

Semente de algodão					1-2	18-25	1-2
Soja					1-2	6-10	2-4
Linhaça						4-7	2-4
Coco	0-1	5-7	7-9	40-50	15-20	9-12	2-4
Óleos marinhos							
Fígado de bacalhau					5-7	8-10	0-1

Fonte: Solomons e Fryhle (2006)

Tabela 4 - Composição de ácidos graxos insaturados em óleos e gorduras comuns

Composição média de ácidos graxos (%)				
Insaturados				
Gordura ou óleo	C 16:1	C 18:1	C 18:2	C 18:3
Gorduras animais				
Manteiga	4-6	18-33	2-4	
Banha	4-6	48-50	6-12	0-1
Sebo de boi		35-45	1-3	0-1
Óleos vegetais				
Oliva		67-84	8-12	
Amendoim		30-60	20-38	
Milho	1-2	25-35	50-60	
Semente de algodão	1-3	17-38	45-55	
Soja		20-30	50-58	5-10
Linhaça		14-30	14-25	45-60
Coco		6-9	0-1	
Óleos marinhos				
Fígado de bacalhau	18-22	27-33	27-32	

Fonte: Solomons e Fryhle (2006).

Os AGI são os majoritários em vegetais, já que eles são considerados como gorduras boas, desempenhando importantes funções para o organismo. Dentro de eles destacam os ácidos ômega 3 e ômega 6, considerados como os mais importantes, já que são essenciais pois o organismo não consegue sintetizá-los e

tem a propriedade de se transformar em substâncias biologicamente mais ativas com funções específicas como é o equilíbrio homeostático e componente estrutural das membranas celulares e do tecido cerebral e nervoso (SALDANHA; GONZALES, 2012; PATTERSON et al., 2011). Outro valor potencial atribuído aos ácidos oleico e linolênico é sua importância na prevenção de doenças cardiovasculares, sendo capaz de diminuir os níveis séricos de triglicéridos que contribuem para a redução da inflamação dos vasos sanguíneos (CUNNANE et al., 2009). Um aumento na dieta das concentrações de ácido linolênico reduz a taxa total de colesterol no sangue (VISENTAINER et al., 2003).

Dado que existe uma transformação enzimática, dos ácidos graxos linoleico e linolênico em ácidos graxos altamente poliinsaturados das séries $\omega 6$ e $\omega 3$ é necessário que exista um equilíbrio entre ambos ácidos na dieta. Não existe um critério definido sobre qual deve ser essa relação, pois depende dos hábitos culturais de cada país, mais de acordo com a *Food and Agricultural Organization* (FAO), a relação deve ser de 5:10:1 (SIMOPOULOS, A.P; LEAF, A.P; SALEM, N., 2000). Por outro lado, existem outras organizações que em lugar de estabelecer razão entre esses ácidos graxos, estabelecem recomendações deles, como é o caso da *Food and Nutrition Board*, que estabelece níveis de ingestão adequada de ácido oleico de 17 g dia⁻¹ para homens e de 12 g dia⁻¹ para mulheres. Em quanto ao ácido linolênico estabelece valores de 1.6 g dia⁻¹ para homens e de 1.1 g dia⁻¹ para mulheres (NATIONAL ACADEMIC PRESS, 2002).

O ácido araquidônico apresenta um papel muito importante nos sistemas biológicos, já que é liberado dos fosfolípidos pela ação da enzima fosfolipase A2 e ao mesmo tempo serve como precursor de eicosanoides pró-inflamatórios que incluem prostaglandinas das duas séries (PGE2 e PGD2) assim como leucotrienos das duas séries (LTA₄, LTB₄, LTC₄, LTD₄ e LTE₄) assim como lipoxinas 1 (SCHWAB; SERTHAN, 2006) Esse ácido está presente fundamentalmente em carnes cuja ingestão estimada está entre 50-500 mg dia⁻¹ (RISTIC-MEDIC et al., 2013).

2.2.3. Compostos fenólicos.

Numerosos compostos bioativos apresentam atividade antioxidante, sendo associados à saúde humana contra doenças degenerativas crônicas. Entre os

compostos bioativos, estão os compostos fenólicos que atuam como antioxidantes e quando são incorporados na alimentação, reduzem o risco de patologias como arteriosclerose e o câncer (LAKO et al., 2007).

Os compostos fenólicos são metabólitos secundários das plantas, apresentando na sua estrutura química um anel aromático com um ou mais grupos hidroxila, podendo variar de uma simples molécula fenólica a um complexo de alto peso molecular, sendo enquadrados dentro de diversas categorias em função do número de anéis aromáticos e aos elementos estruturais que se ligam a esses anéis, sendo os principais fenólicos os compostos simples como os ácidos hidroxibenzoicos, ácidos hidroxicinâmicos, ácidos fenilacéticos, flavonoides, estilbenos, taninos condensados, lignanas e ligninas (IGNAT; VOLF; POPA, 2011).

De acordo com Melo et al. (2008), as frutas são as principais fontes dietéticas de compostos fenólicos apresentando variações quantitativas e qualitativas na composição desses constituintes em função de fatores intrínsecos como cultivar, variedade, estágio de maturação e extrínsecos tais como: condições climáticas e edáficas, dependendo a eficácia da ação antioxidante da concentração destes fitoquímicos no alimento.

Os compostos fenólicos estão ligados aos alimentos funcionais, sendo estes definidos como compostos que deveriam ter uma série de características e especificações como oferecer vários benefícios à saúde, além de apresentar certo valor nutritivo que seja inerente a sua composição química podendo desempenhar um papel potencialmente benéfico na redução de doenças degenerativas (TAIPINA et al., 2002), sendo classificados quanto a origem vegetal ou animal, assim como a fonte de procedência (SOUZA et al., 2003). De acordo com Moraes e Colla (2002), os alimentos funcionais apresentam as seguintes características: devem ser alimentos convencionais e serem consumidos na dieta normal, ao mesmo tempo compostos por componentes naturais, na maioria das vezes em elevada concentração, devem ter efeitos positivos além do valor básico nutritivo.

2.2.4 Vitamina C

As vitaminas são as “aminas da vida” sendo o grupo de micronutrientes que estão presentes em pequenas quantidades nos alimentos e têm funções fisiológicas no corpo humano, e as quantidades necessárias variam em função de: sexo, idade,

massa, altura, necessidades energéticas, período gestacional entre outras (SILVA, 2005).

As vitaminas são classificadas em quatro grupos dependendo das funções metabólicas: estabilizadores de membrana, doadores e receptores de hidrogênio e elétrons, hormônios e coenzimas (MAHAN; STUMP, 2005).

De todas as vitaminas hidrossolúveis, tem importância neste trabalho a vitamina C, descoberta em 1930 por Albert Sezent-Györgyi, sendo um micronutriente essencial para o organismo na prevenção do escorbuto (SVIRBELY; GYÖRGYI, 1932). Devido a seu potencial antioxidante que ajuda ao organismo a combater doenças no funcionamento do aparato digestivo, doenças cutâneas e afeções no sistema nervoso o também o ácido fólico envolvido na regeneração de tecidos (KOPRUSZINSKI et al., 2015). Entre outras funções da vitamina C estão no fortalecimento do sistema imune e prevenção de fluxo de infecções (SORICE et al., 2014) sendo esta vitamina encontrada em concentrações elevadas em frutas como acerola, goiaba, caju, morango e laranja (BECHTHOLD et al., 2015).

2.2.5 Carotenoides

Os carotenoides são pigmentos sintetizados apenas pelas plantas, algas e bactérias fotossintetizantes, responsável pelas cores amarelo, laranja e vermelho das plantas, existindo na natureza aproximadamente 600 carotenoides, dos quais 30-40 estão presentes na alimentação, destacando entre eles o β -caroteno, α -caroteno, β -criptoxantina, luteína, zeaxantina e licopeno (KHACHIK et al., 1997). O nome científico deste grupo de moléculas é derivado da cenoura reconhecido por Wackenroder em 1831 como a primeira fonte de carotenos (GOODWIN, 1952). Existem os carotenoides fitoeno e fitoflueno que são incolores (SIES; STAHL, 2004). De acordo com Ishida e Chapman (2009) os carotenoides são o grupo de compostos bioativos mais estudados em alimentos tanto suas propriedades assim como seu uso para o desenvolvimento de produtos alimentícios enriquecidos devido a suas propriedades como corantes naturais.

Devido que eles são moléculas muito atrativas, eles apresentam diversas aplicações melhorando a aparência do alimento e cada vez mais utilizados no mercado, posto que apesar de ser mais caros, são mais seguros e apresentam

boas propriedades funcionais. Também tem aplicações na aquicultura e avicultura, assim como suas aplicações na indústria farmacêutica, nutracêutica e cosmecêutica, destacando entre elas os distúrbios de fotossensibilidade, doenças cardiovasculares, diabetes, distúrbios de visão, distúrbios neurológicos e doenças imunológicas (MESQUITA; TEIXEIRA; SERVULO, 2017). Outra importante propriedade funcional dos carotenoides, é sua unção antioxidante, desempenhando um papel importante na prevenção de doenças associadas ao processo de estresse oxidativo e retardo do processo do envelhecimento (RIBEIRO; SERAVALLI, 2004).

Quanto à disponibilidade dos carotenoides, eles estão no alimento sempre associados a proteínas e outras biomoléculas como são os polissacarídeos, sendo que, para acontecer a absorção eles devem ser liberados do alimento da origem processo que acontece durante a cocção, mastigação e no próprio estomago resultado entre o 11-50% da biodisponibilidade (BOREL et al., 2005). Porém, os carotenoides que apresentam atividade pró-vitamina A são cclados na mucosa intestinal a retinol e posteriormente convertidos a éster de retinil onde são convertidos no lúmen intestinal a retinol e lá são associados à proteína celular ligante de retinol (ROSS, 2003).

2.3. ATIVIDADE ANTIMICROBIANA DE ÓLEOS E EXTRATOS VEGETAIS

Atualmente, na área da microbiologia, estão sendo realizadas pesquisas em busca de compostos com resistência farmacológica frente aos antibacterianos que atuam como antifúngicos (GIOLO; SVIDZINSKI, 2010). As propriedades antibacterianas são reconhecidas empiricamente há séculos, mais estão sendo comprovadas, cientificamente, apenas recentemente, estando estas substâncias principalmente, presentes em extratos e óleos essenciais produzidos pelas plantas como consequência do metabolismo secundário (HELFAND; COWEN, 1990).

As leveduras do gênero *Candida* são encontradas em diferentes ecossistemas como solo, alimentos e água, formando parte da microbiota dos seres humanos e animais, cuja função é degradar carboidratos e proteínas que irão servir como fonte de carbono e nitrogênio para seu desenvolvimento, porém essas leveduras podem se desenvolver em condições de anaerobiose (GIOLO; SVIDZINSKI, 2010). Essa levedura ocasiona uma infecção oportunista superficial ou sistemática denominada candidíase (GIORDANI; SANTIN; CLEFF, 2015). Calderoni e Fonzi (2001) destacam que os principais fatores de virulência nas leveduras são:

sua capacidade de expressão de enzimas extracelulares, fosfolipases e proteinases, capazes de degradar os tecidos do hospedeiro, produção de substâncias tóxicas que produzem lesões celulares, capacidade de adesão a células e tecidos, formação de biofilmes sobre células e superfície inanimadas, produção do tubo germinativo por algumas espécies de *Candida* spp. Neto e Moraes (2003) apontam que há um grande número de extratos vegetais com ação anti-*Candida*.

Por outro lado, outro grupo de microorganismos de interesse são as bactérias. como o *Staphylococcus aureus* (*S. aureus*), uma bactéria Gram positiva encontrada nas fossas nasais ou na pele de neonatos, crianças e adultos podendo-se expandir para as mucosas podendo se instalar no tecido e provocar lesões locais (ROBERT; CHAMBERS, 2005). Outra das bactérias Gram positiva encontrada em alimentos é o *Bacillus cereus* (*B. cereus*), sendo responsável de contaminações de origem alimentar, durante o manuseio, processamento, estocagem e distribuição de alimentos, podendo se manifestar sob a forma de duas síndromes, uma emética similar a doença causada pela enterotoxina produzida por *Staphylococcus aureus* (*S. aureus*) e outra diarreica semelhante à causada pela enterotoxina de *Clostridium fringens* (AZEREDO, 1998).

Entre as bactérias Gram-negativas mais importantes na contaminação de alimentos cuja origem é mediante contaminação fecal estão a *Escherichia coli* (*E. coli*) causando sérios problemas à saúde, assim como, reduzir a vida útil dos alimentos provocando grandes perdas econômicas (SOUZA et al., 2003). Uma alternativa para a conservação de alimentos, diminuindo a concentração de aditivos sintéticos, é a utilização de plantas aromáticas, que possuem ações flavorizantes, com ação antibacteriana (MOREIRA et al., 2010).

Outra bactéria Gram negativa de interesse para a saúde é a *Salmonella typhimurium* (*S. typhimurium*) que causa a febre tifoidea, caracterizada pela febre prolongada, cefaleia, mialgia, artralgia, e mal estar generalizado assim como diarreias ou constipação, resultando em complicações como perfuração intestinal podendo provocar hemorragia e confusão mental progressiva (CHANH et al., 2004). Essa bactéria também provoca gastroenterite, sendo seu controle de importância mundial já que é uma barreira ao comércio internacional de alimentos (BUTAYE et al., 2003). Para reduzir doenças e danos econômicos causados por microorganismos patogênicos, são usados os produtos naturais como compostos antimicrobianos

para controlar a presença de bactérias patogênicas e estender assim a vida dos alimentos processados (NEDOROSTOVA et al., 2009).

2.4 ATIVIDADE ANTIACETILCOLINESTERASE DE ÓLEOS E EXTRATOS VEGETAIS

A doença do Alzheimer foi descoberta no ano 1907 pelo neurologista Alois Alzheimer afetando cerca de 35,6 milhões de pessoas atualmente, sendo a maioria idosos. É uma doença neurodegenerativa e irreversível ocasionando diferentes distúrbios cognitivos como a perda da linguagem, a memória e habilidade de cuidar de si próprio, apresentando quatro fases, das quais ainda não é conhecida a causa específica desta doença mais existem estudos que podem estar envolvidos fatores genéticos assim como agentes etiológicos a toxicidade, o alumínio, espécies reativas de oxigênio e assim como certos aminoácidos neurotóxicos (PETRONILHO; PINTO; VILLAR, 2011). Segundo Abraz (2019), no Brasil existe cerca de 1,2 milhões de casos de Alzheimer, estimando a Organização Mundial da Saúde que dentro de aproximadamente 20 anos, as doenças mentais e neurológicas constituirão a segunda causa de morte no mundo, sendo que a previsão em 2050 de pessoas acometidas com essa doença pode chegar a 13,8 milhões (ALZHEIMER'S ASSOCIATION, 2013).

O sistema colinérgico é um dos principais responsável pela disfunção cognitiva e da memória no envelhecimento normal, estando envolvidos na transmissão colinérgica componentes como a neurotransmissora acetilcolina, a acetilcolinesterase, acetiltransferase e receptores como o receptor muscarínico e nicotínico, assim cada vez estão sendo realizados estudos envolvendo novos fármacos para melhorar a cognição e memória de pacientes com doença de Alzheimer (PAUL et al., 2015).

Nesse contexto, é determinante destacar a importância dos óleos e extratos vegetais como alvo de moléculas bioativas para inibir a enzima Acetilcolinesterase (AChE), apresentando grande relevância para o desenvolvimento de novos fármacos ou pesticidas (ARAÚJO; SANTOS; GONSALVES, 2016). Esses fármacos denominados inibidores da AChE são denominados anticolinesterásicos, sendo terapêuticamente utilizados nos casos de: reverter o bloqueio neuromuscular promovido por microrrelaxantes adespolarizantes, no tratamento da doença do Alzheimer (STANDAERT et al., 2006; INOUE; OLIVEIRA, 2004).

Na Amazônia, existem diversas pesquisas na procura de substâncias inibidoras da AChE como é no caso da família das *Lauraceae*, plantas de grande importância na produção de óleo e grande potencial comercial (ALCÁNTARA et al., 2010). Também estudos realizados por Barbosa Filho et al. (2006) apresentam 309 plantas pertencentes a 92 famílias botânicas, que foram testadas frente à inibição da AChE apresentando resultados significativos.

3 OBJETIVOS

3.1 OBJETIVOS GERAIS

Realizar uma bioprospeção de frutas nativas e cultivadas na Amazônia setentrional (abiu, camu-camu, bacupari, taperebá, fruta-do-conde, graviola, acerola, biribá e araçá) para gerar informações sobre diferentes partes da fruta (polpa, pele e semente) desde o ponto de vista nutricional, aporte de compostos bioativos e atividade antimicrobiana e antiacetilcolinesterase.

3.2 OBJETIVOS ESPECÍFICOS

- Caracterizar mineralologicamente os diferentes frutos, assim como, sua composição bromatológica em base a quantidade de carboidratos, cinzas, umidade e proteínas em cada uma;
- Caracterizar os principais ácidos graxos nos óleos e extratos hexânicos a partir das sementes;
- Avaliar as propriedades físico-químicas na casca das diferentes frutas (pH, °Brix, acidez titulável e sólidos solúveis;
- Quantificar a vitamina C, açúcares redutores e não redutores assim como carotenoides totais;
- Avaliar diferentes extratos os compostos fenólicos totais e atividade antioxidante mediante o método do DPPH e redução do ferro;
- Aplicar extratos das diferentes frutas para avaliar seu potencial de inibição microbiológica quanto aos fungos (leveduras), e bactérias (Gram-negativas e Gram-positivas), assim como, a inibição da enzima acetilcolinesterase;

4 JUSTIFICATIVA

Existem poucas informações sobre as propriedades químicas de frutas nativas e cultivadas na Amazônia, dada a pouca exploração e estudos sobre as propriedades destas frutas amazônicas, desperta o interesse nesse trabalho, caracterizar quimicamente as diferentes frutas, já que são de grande interesse biotecnológico, como importante fonte de metabólitos de interesse biotecnológico.

Além disso, é preciso avaliar o potencial microbiológico dos diferentes extratos, a fim de avaliar a capacidade de inibir fungos, bactérias e a inibição da enzima acetilcolinesterasa *in vitro*.

Os dados obtidos neste trabalho podem ser considerados para elaborar alimentos derivados destas frutas com interesse funcional ou aplicações na indústria de cosmética e farmacêutica.

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6 LISTA DE PUBLICAÇÕES

CAPÍTULO I

BROMATOLOGICAL AND MINERALOGICAL STUDY IN FRUITS PULPS CULTIVATED IN THE NORTHERN AMAZON¹

ABSTRACT

In this work, nine fruits cultivated in the northern Amazon were studied: *abiu*, *acerola*, *araçá*, *bacupari*, *biribá*, *camu-camu*, *fruta-do-conde*, *graviola* and *taperebá*, with the objective of carrying out a bromatological and nutritional study of the pulps of fruits studied. Of all of them, are the pulps of *graviola* (76.83 ± 0.02 Kcal 100 g⁻¹), *bacupari* (53.15 ± 0.02 Kcal 100 g⁻¹) and *fruta-do-conde* (46.66 ± 0.02 Kcal 100 g⁻¹). Among the macronutrients, the high concentration of potassium stands out, especially in the *graviola* (541.16 ± 0.24 mg.100 g⁻¹) and the *biribá* (468.21 ± 0.13 mg 100 g⁻¹). Among the micronutrients, iron concentrations are representative for *araçá* pulp (3.04 ± 0.02 mg 100 g⁻¹), *abiu* is rich in zinc (3.71 ± 0.02 mg 100 g⁻¹) and manganese (6.61 ± 0.11 mg 100 g⁻¹). The presence of cobalt at the level of traces in some of the pulps studied stands out. The Pearson correlation coefficient was evaluated, as well as the statistical treatment by multivariate analysis PCA to establish the correlation between the variables studied.

Keyword: *Amazonian fruit, funtional food, PCA, Person.*

1 INTRODUCTION

The Amazon region along with the sweet water biome, presents the largest biodiversity on the planet with more than 5000 species (Hubbell, He, Condit, Borda-de-água, Kellner & Steege, 2008). The fruits of this biodiversity, native and exotic, present an expressive potential of bioactive compounds, which can be a source of bioproducts for the development of humanity (Mariutti, Rodrigues, Chiste, Fernandes & Mercadete, 2014).

¹ Submit Revista Brasileira de Engenharia Agrícola e Ambiental.

The Amazonian fruits arouse great study interest due to their great biodiversity, which, according to Drewnoski & Fulgoni (2008) and Darmon, Briend & Drewnowski, (2004) present outstanding results in quality and attractive attributes such as appearance in large sizes, different shapes, colors, textures and different flavors.

In the human diet, fruits are considered the main sources of necessary minerals, playing a vital role in the peculiar development and good health of the human body (Hardisson, Rubio, Baez, Martín, Álvarez & Díaz, 2001), because they participate in many biochemical reactions, being divided according to Krause & Mahan (2005), in macronutrients (minerals required for humans in amounts greater than or equal to 100 mg day⁻¹ as calcium, magnesium, potassium, phosphorus, sulfur, chlorine and sodium) and micronutrients (minerals required for humans in amounts less than 100 mg day⁻¹, such as copper, iron, zinc, manganese, selenium, molybdenum and fluorine).

On the other hand, besides minerals, Arts & Hollman (2005) affirm that the beneficial effects in the fruits, are also due to the high content of antioxidants, micronutrients including vitamin C, carotenoids and polyphenolic compounds.

Many tropical fruits are not well known, which can be associated to the lack of knowledge of the production and conservation system, as well as other aspects related to the quality of these fruits (Leterme, Buldgen, Estrada & Londoño, 2006).

Thus, many of the pulps fruits cultivated, Amazonian or introduced in the region, do not present information on the nutritional and mineralogical composition. For this reason, the objective of this work is to analyze the composition of minerals and nutrients in the pulp of nine fruits grown in the Northern Amazon (Figure 1) and the correlation between existing data using the Pearson test as well as to use multivariate analysis methods such as Principal Component Analysis (PCA) and Hierarchical Component Groupings (HCA).

Figure 1- Fruits in the Northern Amazon under study. (Pictures by I. F. Montero).



Fruta-do-conde
(*A. squamosa*)



Biribá
(*R. mucosa*)



Graviola
(*A. muricata*)



Bacuparí
(*R. gardneriana*)



Abiu
(*P. caimito*)



Acerola
(*M. emarginata*)



Taperebá
(*S. mombin*)



Camu-camu
(*M. dubia*)



Araçá
(*P. cattleianum*)

2 MATERIALS AND METHODS

2.1 PREPARATION OF SAMPLES

The samples of the different fruits studied were collected in randomized points of the State of Roraima (Brazil) to guarantee the representativeness of the sample. Each of the fruits was collected in the corresponding production period and were collected in the ripening stage suitable for consumption. From all the samples collected at the different sampling points, a single composite sample was prepared for each of the fruits where they were taken to the Environmental Chemistry Laboratory of the Federal University of Roraima, where those that presented an optimum conservation status were selected washed with 1% sodium hypochlorite solution and again with distilled water.

Subsequently, a representative sample of each fruit was selected according to the following criteria: *acerola*, *camu-camu* and *taperebá* was selected 1 kg of fresh fruit: *abiu*, *araçá* and *bacuparí* was selected 2 kg of fruit for *biribá*, *fruta-do-conde* and *graviola* were selected 10 units according with NTON 17002-02 (2002). The pulps of the different fruits were separated from the different parts of the fruits and were placed in Ultrafreezer at -80 °C and then lyophilized in lyophilizer LIOTOP model L 101 for 48 hours until complete drying of the material and subsequently ground in LABOR model SP31 punch mill and stored material in airtight bags in the absence of light until the moment of performing the different analyzes.

Table 1- Names and families of fruits cultivated in the Northern Amazon under study

Scientific name	Family	Name in Brazil
<i>Pouteria caimito</i>	Sapotaceae	<i>Abiu</i>
<i>Malpighia emarginata</i>	Malpighiaceae	<i>Acerola</i>
<i>Psidium cattleianum</i>	Myrtaceae	<i>Araçá</i>
<i>Rheedia gardneriana</i>	Clusiaceae	<i>Bacuparí</i>
<i>Rollinia mucosa</i>	Annonaceae	<i>Biribá</i>
<i>Myrciaria dubia</i>	Myrtaceae	<i>Camu-camu</i>
<i>Annona squamosa</i>	Annonaceae	<i>Fruta-do-conde</i>

<i>Annona muricata</i>	Annonaceae	<i>Graviola</i>
<i>Spondias mombin</i> L.	Anacardiaceae	<i>Taperebá</i>

2.2 NUTRITIONAL ANALYSIS

The physical parameters evaluated to determine the nutritional composition were the percentage of moisture and ash. The other nutritional parameters evaluated were the determination of total proteins, lipids and carbohydrates, to determine the total energy content.

2.2.1 Determination of Humidity

To determine moisture, 5 g of fresh samples were placed in porcelain capsules for 6 hours at 105 °C to constant mass, and then cooled in desiccator to room temperature (IAL, 2008).

$$\text{Humidity (g 100 g}^{-1}\text{)} = ((P' - P'')/(P' - P)) \cdot 100$$

being: P = weight of porcelain capsule (g); P' = weight of the porcelain capsule + fresh sample (g); P'' = weight of the capsule + sample after the oven (g).

2.2.2 Determination of ashes

To determine the ash in the samples, the methodology proposed for the food analysis of IAL (2008) with modifications was used, where 5 g of the lyophilized samples were weighed. These were placed in preheated porcelain crucibles in an oven at 110 °C for one hour, to remove moisture, and cool them in a desiccator to room temperature. The samples were incinerated at 600 °C in a FDG 3P-S EDG muffle for 16 hours, after which the samples were left in the desiccator until reaching room temperature.

$$\% \text{ashes} = ((N \cdot 100)/M)$$

N = mass in grams of ash and M = mass of the sample in grams.

2.2.3 Determination of total proteins

Protein determination is performed from the total nitrogen analysis by Kjeldahl distillation, in which the existing organic matter is transformed into ammonia. The nitrogen content of the different proteins is approximately 16%, which introduces the empirical factor of 5.75 (conversion factor for vegetable protein), this will transform the number of grams of nitrogen, found with the number of grams of protein (IAL, 2008).

$$\% \text{proteins} = \% \text{N} \cdot 5.75$$

2.2.4 Determination of lipids

To determine the total amount of lipids, 20 g of each sample was weighed, and placed in the Soxhlet extractor apparatus with hexane as the solvent for six hours. The solvent was recovered in a rotary evaporator (IAL, 2008).

$$\% \text{lipids} = ((N \cdot 100) / M)$$

Where: N = mass in grams of lipids and M = mass of the sample in grams.

2.2.5 Determination of Carbohydrates

The carbohydrate content is achieved by the difference of the value 100 subtracted from the sum of the already obtained values of moisture, ashes, lipids and proteins.

$$\text{Carbohydrates} = 100 - (\% \text{moisture} + \% \text{ash} + \% \text{lipids} + \% \text{proteins})$$

2.2.6 Energetic value

In order to quantify the energy value, it was necessary to use the protein (P), lipid (L) and carbohydrate (C) contents of each sample. The result should be expressed in kcal 100g⁻¹ (Mendes-Filho, Carvalho, Chiste, & de Souza, 2014).

$$\text{Energy value (kcal 100 g}^{-1}) = (P \cdot 4) + (L \cdot 9) + (C \cdot 4)$$

P = value of protein (%), L = lipid value (%), C = carbohydrate value (%), 4 = conversion factor in kcal determined in calorimetric pump for proteins and

carbohydrates and 9 = conversion factor in kcal determined in a calorimetric pump for lipids.

2.3 MINERALOGICAL ANALYSIS

The extraction of the minerals into the pulps was done according to the methodology described by Embrapa (2009) in which the perchloric nitric digestion (3:1) was used in TECNAL model TE 0079 digester block, washed with distilled water up to 25 mL for subsequent analysis.

Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and aluminum (Al) were determined by Flame Atomic Absorption Spectrophotometry (FAAS) Shimadzu AA-7000, coupled with ASC-7000 auto sample. Calibration was performed with standard solutions prepared from commercial standards P.A. of 1000 mg L⁻¹ Qhemis High Purity PACU 1000-0125, according to the specific conditions of each element (Table 2).

Table 2- Analytical Parameters

Element	Technique	(λ) nm	Calibration Line
Ca	FAAS	422.70	y= 0.0092 x - 0.0005 r ² = 0.999
Mg	FAAS	285.21	y= 0.2353 x - 0.0658 r ² = 0.997
P	UV-Vis spectroscopy	660.00	y= 0.2181 x - 0.0005 r ² = 0.999
K	AES	766.50	y= 0.1231 x - 0.0013 r ² = 0.993
S	UV-Vis spectroscopy	420.00	y= 0.0213 x - 0.0012 r ² = 0.998
Fe	FAAS	248.33	y= 0.0399 x + 0.0067 r ² = 0.996
Zn	FAAS	213.80	y= 0.060 x - 0.0171 r ² = 0.991
Mn	FAAS	279.48	y= 0.0282 x + 0.0041 r ² = 0.999
Cu	FAAS	324.75	y= 0.0512 x - 0.0099 r ² = 0.997
Na	FAAS	589.0	y= 1.00 x + 0.0005 r ² = 0.999
Al	FAAS	309.3	y= 0.0088 x + 0.0005 r ² = 0.998
B	UV-Vis spectroscopy	420.00	y= 0.0537 x + 0.0002 r ² = 0.999
Co	FAAS	240.73	y= 0.0286 x - 0.0066 r ² = 0.997

FAAS = Flame Atomic Absorption Spectroscopy. AES = Flame Atomic emission Spectroscopy.

As the ionization suppressor for the Ca, Mg and K elements, 0.1% of the lantane oxide solution (La₂O) was used. In the case of sodium (Na), it was

determined in the same equipment, but in atomic emission mode. As for potassium (K), it was determined by means of flame photometry on the Digimed Flame Photometer DH-62, calibrated using a Digimed standard solution whose concentration range was 2-100 mg L⁻¹.

For the determination of the phosphorus (P), boron (B) and sulfur (S) elements, the ultraviolet molecular absorption spectrophotometry technique was used using a SHIMADZU UV-1800 model. The determination of P was carried by formation of the blue complex with ammonium molybdate ((NH₄)₂MoO₄) the readings were made at λ = 660 nm. The determination of boron was carried out by the formation of a yellow complex with azomethine-H, the readings being made at 460 nm. The determination of S was performed by forming a precipitate with BaCl₂, the readings being made in Uv-visivel at λ = 420 nm by calibration with potassium sulphate according with Embrapa (2009).

Nitrogen determination was carried out by the distillation method followed by titration (Kjeldahl) according with described methodology with Embrapa (2009).

2.4 STATISTICAL ANALYSIS

Correlations between the amounts of the different minerals in the different parts of the fruit were evaluated using the Pearson statistical test using INFOSTAT (Rienzo, Casanovas, Balzarini, González, Tablado & Robleda, 2016) for significance levels of 5%, 1% and 0.1% respectively, as well as the principal component analyzes (PCA) and Hierarchical Component Groupings (HCA).

3 RESULTS AND DISCUSSION

3.1 NUTRITIONAL ANALYSIS FROM AMAZONIAN FRUITS

Table 3 presents the nutritional analysis values for the pulps of the different Amazonian fruits studied.

The first parameter analyzed is moisture, which according to Welti & Vergara, (1997), the moisture content is used as a factor indicative of propensity for food spoilage, and may think that the greater stability of the food is in the control of the minimum humidity, It is therefore important to dry the food to minimize physical and chemical changes of the product (Herrera, Gabas & Yamshita, 2001). The amount of moisture in the pulps varies from (64.22-95.21%), where the highest moisture values

are for *camu-camu* ($95.21 \pm 0.14\%$) and *acerola* ($94.21 \pm 0.11\%$) and the lowest value for *araçá* with ($64.22 \pm 0.12\%$). Regarding the values of moisture for *abiu*, *acerola* and *graviola*, are slightly lower, but close to those presented Canuto, Xavier, Neves & Benassi (2010) and in the case of *araçá* lower than those presented by the same author.

Table 3- Nutritional composition in Amazonian fruits pulps.

FRUIT	NUTRITIONAL CONTRIBUTION					
	MOISTURE	ASHES	LIPIDS	CARBOHYDRATES	PROTEINS	ENERGETIC VALUE
			%			Kcal 100 g ⁻¹
<i>Abiu</i>	92.43 ± 0.02	0.13 ± 0.07	0.12 ± 0.01	6.48 ± 0.02	0.84 ± 0.01	30.36 ± 0.03
<i>Acerola</i>	94.21 ± 0.01	0.16 ± 0.03	0.06 ± 0.00	4.45 ± 0.02	1.12 ± 0.01	22.82 ± 0.02
<i>Araçá</i>	59.21 ± 0.08	0.21 ± 0.07	0.17 ± 0.09	30.87 ± 0.03	3.93 ± 0.07	18.09 ± 0.02
<i>Bacuparí</i>	86.61 ± 0.11	0.19 ± 0.01	0.07 ± 0.00	12.36 ± 0.01	0.77 ± 0.01	53.15 ± 0.02
<i>Biribá</i>	91.34 ± 0.01	0.31 ± 0.02	0.22 ± 0.02	6.95 ± 0.03	1.18 ± 0.04	34.5 ± 0.01
<i>Camu-camu</i>	95.21 ± 0.14	0.25 ± 0.11	0.05 ± 0.00	3.14 ± 0.02	1.35 ± 0.04	18.41 ± 0.02
<i>Fruta-do-conde</i>	88.27 ± 0.05	0.29 ± 0.07	0.18 ± 0.07	10.08 ± 0.01	1.18 ± 0.04	46.66 ± 0.02
<i>Graviola</i>	80.77 ± 0.07	0.31 ± 0.11	0.23 ± 0.08	17.34 ± 0.01	1.35 ± 0.02	76.83 ± 0.02
<i>Taperebá</i>	88.23 ± 0.10	0.15 ± 0.01	0.05 ± 0.01	10.01 ± 0.03	1.56 ± 0.01	46.73 ± 0.03

Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95%.

The presented values of moisture are within the percentage humidity range given by the IAL (2008) that establishes the values in fruits between 65-90%. In the case of *camu-camu*, Maeda, Pantoja, Yuyama & Chaar (2006), determined the percentage of moisture in the *camu-camu* pulp of 92.65%, close to that found in the present study.

The ash content reflects the amount of minerals present in the food. According to Moreto (2008), the amount of ash in food may vary depending on the food or the conditions in which it is present. On the other hand, by IAL (2008) establishes the values of percentage of fruit ashes between 0.3 - 2.1%, being the values of ashes studied in this work according to this range. For proteins, it is those of animal origin that have higher biological value compared to proteins of plant origin Kinupp & Barros (2008), and the identification of plant species with a certain content of proteins, are important to satisfy the nutritional deficiencies of people with different dietary habits and diets (Aletor & Adeogun, 1995), plus many native Amazonian species have not yet been studied to evaluate their protein potential.

The protein content in the fruit pulps studied in this study ranged from 0.77% for the *bacuparí* pulp to 3.93% for the *araçá* pulp. *Camu-camu* has a protein content of 1.35%. Compared to the table proposed by Aguiar (1996) on the food composition of the Amazon, the *camu-camu* has a value of 0.45 g 100 g⁻¹ of proteins and the *araçá* 0.60 g 100 g⁻¹.

The three groups of primary metabolites in fruits, carbohydrates are the major ones, presenting values between 3.14-30.87% for pulps, being the one with the lowest value is the *camu-camu* and the highest is the *araçá* pulp. Among the factors that characterize the quality of the fruits, we have one of the most important that is the flavor, given by the balance between soluble sugars and organic acids, occurring as the fruit ripens, an increase of soluble sugars, and therefore increases the sweet taste, and at the same time, the amount of organic acids decreases (Medlicott & Thompson, 1985).

The lipid content observed in the fruits cultivated in the Northern Amazon was observed in the pulps of *camu-camu* 0.05%, *taperebá* 0.05%, and *biribá* 0.22%, with lipid values being relatively low in fruit pulps. Among the fruits of the Annonaceae family, the species that had the highest lipid content was *graviola* with 0.23%, followed by the *biribá* with 0.22% and the *fruta-do-conde* with 0.18%. The values obtained for the *biribá* are close to those determined by Berto, da Silva, Visentainer &

De Souza (2015). In addition to the biological benefits that the aforementioned oils present, the main use of oils and fats are, according to Ribeiro, Moura, Grimaldi & Gonçalves (2007) in the human diet as essential nutrients, thus being vital in providing essential fatty acids and energy.

3.2 MINERAL ANALYSIS

In tables 4 and 5, the values of macronutrients and micronutrients are presented for the different pulps studied

Among the macroelements, the high values of potassium in the fruits studied were presented for the *graviola* pulp, with 541.16 ± 0.24 mg 100 g⁻¹ and 468.21 ± 0.13 mg 100 g⁻¹ for the *biribá* pulp. The levels of potassium daily, according to the Food and Nutrition Board of the Institute of Medicine (2013) are 4700 mg day⁻¹, with consumption of potassium-rich foods beneficial for controlling blood pressure, type II diabetes and bone health.

Phosphorus is an essential element that, besides appearing in fruits, its main contribution to the organism is the source of animal origin, mainly in red, white and viscera meats (Cozzolino, 2007). According to Tomassi (2002), in fruits, the phosphorus levels oscillate between 20-100 mg 100 g⁻¹. The fruits in the study presented low values of phosphorus, being the *camu-camu* pulp, which presents a lower value 6.21 ± 0.04 mg 100 g⁻¹ and for *taperebá* pulps 24.12 ± 0.11 mg 100 g⁻¹. According to Tomassi (2002), the recommended dose of phosphorus per day is 800 mg.

Table 4 - Macronutrients analyzed in fruits pulps in the Northern Amazon.

Fruit	Macronutrients					
	Calcium (Ca)	Magnesium (Mg)	Phosphorous (P)	Potassium (K)	Sulfur (S)	Nitrogen (N)
	mg 100 g ⁻¹					
<i>Abiu</i> (<i>Pouteria caimito</i>)	4.51 ± 0.02	1.71 ± 0.07	8.21 ± 0.04	255.21 ± 0.03	11.11 ± 0.04	0.15 ± 0.01
<i>Acerola</i> (<i>Malpighia emarginata</i>)	11.23 ± 0.12	18.41 ± 0.21	11.93 ± 0.04	154.34 ± 0.18	34.13 ± 0.14	0.19 ± 0.01
<i>Araçá</i> (<i>Psidium cattleianum</i>)	24.13 ± 0.03	12.21 ± 0.08	6.32 ± 0.04	137.11 ± 0.08	9.02 ± 0.01	0.68 ± 0.07
<i>Bacuparí</i> (<i>Rheedia gardneriana</i> Planch & Triana)	32.41 ± 0.02	14.21 ± 0.08	12.31 ± 0.14	329.12 ± 0.04	5.21 ± 0.04	0.13 ± 0.01
<i>Biribá</i> (<i>Rollinia mucosa</i>)	32.11 ± 0.08	112.32 ± 0.12	23.41 ± 0.01	468.21 ± 0.13	21.31 ± 0.12	0.21 ± 0.04
<i>Camu-camu</i> (<i>Myrciaria dúbia</i> (Kunth) Mc Vaugh)	9.51 ± 0.02	8.49 ± 0.04	6.21 ± 0.04	124.13 ± 0.12	7.21 ± 0.04	0.23 ± 0.04
<i>Fruta-do-conde</i> (<i>Annona squamosa</i>)	52.21 ± 0.13	32.12 ± 0.09	17.30 ± 0.12	431.21 ± 0.17	27.78 ± 0.13	0.21 ± 0.04
<i>Graviola</i> (<i>Annona muricata</i>)	39.21 ± 0.13	27.11 ± 0.15	19.24 ± 0.16	541.16 ± 0.24	29.31 ± 0.08	0.23 ± 0.02
<i>Taperebá</i> (<i>Spondias mombin</i> L.)	38.12 ± 0.12	16.32 ± 0.09	24.12 ± 0.11	149.13 ± 0.23	4.38 ± 0.08	0.27 ± 0.01

Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95%.

Table 5- Micronutrients analyzed in fruits pulps in the Northern Amazon.

FRUIT	MICRONUTRIENTS							
	Iron (Fe)	Zinc (Zn)	Manganese (Mn)	Copper (Cu)	Sodium (Na)	Aluminum (Al)	Boron (B)	Cobalt (Co)
	mg 100 g ⁻¹							
<i>Abiu</i> (<i>Pouteria caimito</i>)	0.18 ± 0.04	3.71 ± 0.22	6.61 ± 0.11	0.12 ± 0.02	0.22 ± 0.01	0.17 ± 0.02	0.27 ± 0.07	N.D.
<i>Acerola</i> (<i>Malpighia emarginata</i>)	0.80 ± 0.12	0.08 ± 0.01	0.24 ± 0.05	0.17 ± 0.01	35.13 ± 0.12	0.93 ± 0.04	0.11 ± 0.03	N.D.
<i>Araçá</i> (<i>Psidium cattleianum</i>)	3.04 ± 0.02	1.14 ± 0.02	1.25 ± 0.07	1.73 ± 0.02	1.93 ± 0.02	0.14 ± 0.06	0.10 ± 0.02	0.012 ± 0.003
<i>Bacuparí</i> (<i>Rheedia gardneriana</i> Planch & Triana)	0.71 ± 0.02	3.46 ± 0.02	0.24 ± 0.01	0.15 ± 0.03	0.09 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.021 ± 0.000
<i>Biribá</i> (<i>Rollinia mucosa</i>)	1.82 ± 0.11	1.23 ± 0.04	0.33 ± 0.04	1.14 ± 0.13	18.44 ± 0.21	0.06 ± 0.01	0.51 ± 0.05	0.006 ± 0.001
<i>Camu-camu</i> (<i>Myrciaria dúbia</i> (Kunth) Mc Vaugh)	0.29 ± 0.03	0.13 ± 0.04	2.39 ± 0.02	0.17 ± 0.08	1.91 ± 0.04	0.09 ± 0.01	0.11 ± 0.06	0.067 ± 0.001
<i>Fruta-do-conde</i> (<i>Annona squamosa</i>)	0.91 ± 0.09	0.22 ± 0.03	0.12 ± 0.02	0.31 ± 0.08	4.24 ± 0.31	0.04 ± 0.01	0.12 ± 0.03	0.018 ± 0.001
<i>Graviola</i> (<i>Annona muricata</i>)	0.87 ± 0.12	0.39 ± 0.02	0.09 ± 0.00	0.19 ± 0.04	8.76 ± 0.31	0.07 ± 0.01	0.17 ± 0.02	0.012 ± 0.001
<i>Taperebá</i> (<i>Spondias mombin</i> L.)	1.13 ± 0.05	0.19 ± 0.03	0.04 ± 0.00	0.07 ± 0.00	3.24 ± 0.83	0.02 ± 0.00	0.19 ± 0.01	N.D.

N.D. not detected. Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95%

On the other hand, an element that can influence the absorption of phosphorus is calcium, and it is estimated that the absorption of both elements is optimal when the relation between both is equal to 1 (Douglas, 2002). The relation between the two elements is closer to 1 for *acerola* pulp with 1.06 and for *biribá* pulp with 0.72. Of the fruits studied, it is the *abiu* that presents a lower concentration of calcium $4.51 \pm 0.02 \text{ mg } 100 \text{ g}^{-1}$ and the higher concentration of calcium for *fruta-do-conde* pulp with $52.21 \pm 0.13 \text{ mg } 100 \text{ g}^{-1}$. The nutritional contribution of Ca in adults according to Pereira, Genaro, Pinheiro, Szenjfeld & Martini (2009) is the 1000-1200 mg day⁻¹.

Magnesium is other important macroelement in fruits, and it appears within a very variable range in the fruits studied. In the *abiu* pulp, it is in low concentrations $1.71 \pm 0.07 \text{ mg } 100 \text{ g}^{-1}$, presenting the highest concentration of magnesium for the *biribá* pulp with $112.32 \pm 0.12 \text{ mg } 100 \text{ g}^{-1}$. The main function of magnesium in the body is to stabilize the structure of ATP in enzymatic reactions, as cofactor in enzymatic reactions, in neuromuscular transmission (Iseri & French, 1984) and in reactions of the dark phase of photosynthesis are activated by manganese (Malavolta, 2006). The recommendations for magnesium, is 310-320 mg day⁻¹ for women and 410-420 mg day⁻¹ for men (Yuyama, 1992).

As for sulfur, it is required in small concentrations, being an element that forms part of the structure of essential amino acids such as cysteine and methionine and enzymatic activator (Silva, Pereira, Do Carmo, De Albuquerque, Van Raji & Silva, 2004). Of the fruits studied, it is the *acerola* pulp that has the highest concentrations of sulfur, $34.13 \pm 0.14 \text{ mg } 100 \text{ g}^{-1}$, being of *taperebá* pulp, which presents lower concentrations with $4.38 \pm 0.08 \text{ mg } 100 \text{ g}^{-1}$.

Nitrogen in fruits is important in its size, as well as being part of amino acids, proteins, coenzymes, nucleic acids and vitamins, and is part of the processes of photosynthesis, cellular respiration and multiplication (Malavolta, 2006). Nitrogen is not one of the most studied micronutrients in fruits, being more studied associated with the proteins of the fruit. Its fruit pulp quantity is low, presenting the lowest value for the *bacuparí* pulp with $0.13 \pm 0.01 \%$, being the fruit that presents a higher value the *araçá* pulp with $0.68 \pm 0.07\%$.

Leterme, Buldgen, Estrada & Londoño (2006) analyzed the *graviola* pulps and the *fruta-do-conde* pulps. In the case of *graviola*, the values obtained for the case of Ca, K and Mg are close to those obtained by Leterme, Buldgen, Estrada & Londoño,

(2006) in the sodium and potassium are lower than those obtained by the same author and in the case of sulfur the value obtained is bigger.

In the *fruta-do-conde*, the value obtained from Ca, K and S, are similar to those obtained by the same author. For Mg and Na are many lower than those presented by Leterme, Buldgen, Estrada & Londoño (2006).

Feitosa & Maker (2007) determine the concentration of Ca, P and Fe in the *taperebá*, being the values determined by them superior in the case of Ca and P. There are studies for other Amazonian fruits where nitrogen values are evaluated, such as in the case of the *Pitaya vermelha* with 11.30% nitrogen (Cordeiro, da Silva, Mizobutsi, Mizobutsi & da Mota, 2015) and are therefore larger than those presented in this study.

Among the micronutrients, iron is very important in the human diet, once its deficiency can cause anemia, fatigue and impairment in neurological growth and development (Carvalho, 2006). According to the World Health Organization (WHO), the required iron dose per adult person and day is 20-45 mg. The highest values of iron presented in this work are for *araçá* pulp with concentrations of 3.04 ± 0.02 mg.100 g⁻¹, the lowest concentration of iron was for the *abiu* pulp with 0.18 ± 0.04 mg 100g⁻¹. Another of the rich fruits in iron is of *biribá* pulp with 1.82 ± 0.11 mg 100 g⁻¹.

The zinc, it's important in the organism at the physiological level as an antioxidant (Powell, 2000), as well as developing a fundamental role in the polymer organization of macromolecules such as DNA and RNA, as well as their synthesis (Vallee & Falchuk, 1993). According to Food and Nutrition Board (2001) the zinc recommendations for the population are 8 mg day⁻¹ for women and 11 mg day⁻¹ for men. In the fruits studied, the highest pulp concentration was *abiu* pulp with 3.71 ± 0.22 mg 100 g⁻¹ and the lowest concentration of zinc with for *acerola* pulp being 0.08 ± 0.01 mg 100 g⁻¹.

According with Leterme, Buldgen, Estrada & Londoño (2006) studied several Amazonian fruits, among them the *araçá* and *acerola*, presenting values of zinc concentration in the *araçá* of 0.17 mg.100 g⁻¹ e for *acerola* of 0.19 mg 100 g⁻¹ for the edible fraction of fruit, being for *acerola* lesser than that obtained in this work (table 5). For *araçá* pulp is smaller than the value presented in Table 5.

Other important microelement in enzymatic metabolic reactions is manganese which, according to Panziera, Dorneles, Durgante & da Silva, (2011) is part of two metalloenzymes, carboxylase pyruvate and Mn-superoxide dismutase. It also

participates in mitochondrial energy production, activating other enzymes such as superoxide dismutase, and phosphoenolpyruvate carboxylase of great importance in gluconeogenesis (Mahan & Escott-Stump, 2002).

Among the studied fruits, *abiu* pulps presents the high concentrations with $6.61 \pm 0.11 \text{ mg } 100 \text{ g}^{-1}$. Other fruits with considerable concentrations of manganese are the *camu-camu* pulp with $2.39 \pm 0.02 \text{ mg } 100 \text{ g}^{-1}$, with the lowest values manganese in the *taperebá* pulp with $0.04 \pm 0.00 \text{ mg } 100 \text{ g}^{-1}$. Almeida, de Souza, Fonseca, Magalhães, Lópes & de Lemos (2009) studied minerals in tropical fruits and found values of manganese for *graviola* of $0.07 \pm 0.02 \text{ mg } 100 \text{ g}^{-1}$ next to in relation to the value found in the present work for the pulp $0.09 \pm 0.00 \text{ mg } 100 \text{ g}^{-1}$ and for the *fruta-do-conde* $0.16 \pm 0.00 \text{ mg } 100 \text{ g}^{-1}$, a value close to the finding in this work for the *fruta-do-conde* pulp $0.12 \pm 0.02 \text{ mg } 100 \text{ g}^{-1}$. On the other hand Leterme, Buldgen, Estrada & Londoño (2006) evaluated fruits cultivated in Colombia and found manganese values for the *araçá* $0.08 \text{ mg } 100 \text{ g}^{-1}$, the value found in this work for the *araçá* pulp of $1.25 \pm 0.07 \text{ mg } 100 \text{ g}^{-1}$ slightly higher than the value described by the previous author and for *acerola* $0.09 \text{ mg } 100 \text{ g}^{-1}$, being lower than that found in the present study with a value of $0.24 \pm 0.05 \text{ mg } 100 \text{ g}^{-1}$.

Copper is a trace element that may exhibit various oxidation states and within the cell predominates the cuprous ion (Bairele, Valentini, Paniz, Moro, Junior & García, 2010). The same is present in the skeletal muscles, presenting on average 40% of all the content of the body of this mineral, presenting an important role in the production of mitochondrial energy, as well as protective action against oxidizing agents and free radicals, thus promoting the synthesis of melanin and catecholamines (Macdle & Katch, 2003). Copper levels, compared to the other elements, are low, with the exception of *araçá* that presents copper concentrations of $1.73 \pm 0.02 \text{ mg } 100 \text{ g}^{-1}$ for the pulp, and the *taperebá* is the one with the lowest concentration of copper, with only $0.07 \pm 0.00 \text{ mg } 100 \text{ g}^{-1}$.

The need for copper is $1\text{-}2 \text{ mg } \text{dia}^{-1}$, and $10 \text{ mg } \text{dia}^{-1}$ is tolerated according to (DRIs) (Dietary Reference Intakes, 2004) for the maintenance of the human organism, the above fruits being above tolerable levels for the organism. Almeida, de Souza, Fonseca, Magalhães, Lópes & de Lemos (2009) determined copper concentrations in the *graviola* with $0.15 \pm 0.03 \text{ mg } 100 \text{ g}^{-1}$, being close to that found in the present study with $0.19 \pm 0.04 \text{ mg } 100 \text{ g}^{-1}$ and for $0.22 \pm 0.03 \text{ mg } 100 \text{ g}^{-1}$, the

concentration of copper for the *fruta-do-conde* pulps in $0.31 \pm 0.08 \text{ mg } 100 \text{ g}^{-1}$, slightly higher to the value found in the literature.

An important trace element is boron, being related to the cerebral metabolism (Penland, 1994), among other functions. In the case of fruits, boron has an important function of stimulating the germination and generation of pollen and pollen tube growth, being a fundamental factor for the adequate formation of fruits (SangHyun L., WolSoo, K. & TaeHo H., 2009). The highest concentration of boron was found in the *biribá* pulp with $0.51 \pm 0.05 \text{ mg } 100 \text{ g}^{-1}$ and the lowest concentration was the *aracá* pulp with $0.10 \pm 0.02 \text{ mg } 100 \text{ g}^{-1}$.

The aluminum is a toxic metal, whose concentration in food is low, of the order of 5 mg kg^{-1} (Dantas, Saron, Dantas, Yamashita & Kiyataka, 2007). The consumption of foods contaminated by this metal may be related to Alzheimer's disease (Martyn, Coggan, Inskip, Laeey & Young, 1997). Thus, the fruits analyzed had relatively low concentrations, varying between $0.02 - 0.17 \text{ mg } 100 \text{ g}^{-1}$ in pulps, being within the recommended levels.

Among all the evaluated minor elements, cobalt is the lowest concentration in relation to the microconstituents, only present in some of the studied fruits and the highest values show the *camu-camu* pulp with $0.067 \pm 0.001 \text{ mg } 100 \text{ g}^{-1}$. According to Vaitsman, Alonso & Dutra (2001) the estimated cobalt doses are between $0.5\text{-}1.4 \text{ mg dia}^{-1}$, therefore, the levels found in the fruits studied would be below the recommended levels.

Berto, da Silva, Visentainer & de Souza (2015) present a study of determination of seven minerals in different Amazonian fruits in the different parts of the same, among them the *biribá*, being the values obtained for this fruit, very close to those presented by Berto, da Silva, Visentainer & De Souza (2015), with the exception of the sodium concentration obtained for the pulp of the *biribá* that presents a much lower value.

For the *camu-camu*, Yuyama, Rocha & Cozzolina (2013) studied six minerals in different samples and comparing them with the mineral concentrations in the pulps, the values are within the range, except sodium, whose value is lower than the values presented by said author and also for the cobalt, the concentrations obtained by the author are higher than the values presented in this work.

Freitas, Maia, Da Costa, Figueireiro & De Souza (2014) developed a study of mineral determination in *acerola in natura* juice, presenting very close values for Mg,

P, K and Zn, and for the Fe, Na and Cu elements we obtained larger values compared to the author.

The results obtained in this study were similar to those obtained by Caldeira, Hiane, Ramos & Filho (2004), and the values for Ca, Mg and Ca were higher for P, K, Na, Fe, Mn and Cu. Feitosa & Fabricante (2007), determined the concentration of Ca, P and Fe in *taperebá*, the values being determined by them superior in the case of Ca and P.

3.3 STATISTIC ANALYSIS

3.3.1 Pearson correlation coefficient

Table 6 presents the Pearson correlation matrix between the different elements for the pulps of the different fruits.

Table 6- Pearson correlation matrix between the different elements for the pulps of Amazon fruits.

	Ca	Mg	P	K	S	N	Fe	Zn	Mn	Cu	Na	Al	B	Co
Ca	1													
Mg	0.76*	1												
P	0.40ns	0.31ns	1											
K	0.69*	0.43ns	0.36ns	1										
S	0.22ns	0.23ns	-0.29ns	0.42ns	1									
N	0.02ns	-0.08ns	0.60ns	0.12ns	-0.14ns	1								
Fe	0.28ns	0.22ns	0.76*	0.31ns	-0.04ns	0.92**	1							
Zn	-0.25ns	-0.23ns	0.37ns	0.11ns	-0.47ns	0.02ns	0.10ns	1						
Mn	-0.66*	-0.41ns	-0.43ns	-0.28ns	-0.32ns	-0.10ns	-0.26ns	0.56ns	1					
Cu	0.10ns	0.08ns	0.68*	0.27ns	-0.09ns	0.94**	0.97**	0.22ns	-0.06ns	1				
Na	-0.03ns	0.28ns	0.01ns	-0.02ns	0.66*	0.21ns	0.34ns	-0.34ns	-0.34ns	0.27ns	1			
Al	-0.48ns	-0.21ns	-0.25ns	-0.40ns	0.52ns	-0.11ns	-0.10ns	-0.17ns	-0.05ns	-0.10ns	0.80**	1		
B	0.42ns	0.80**	0.17ns	0.40ns	-0.03ns	0.09ns	0.35ns	0.26ns	0.13ns	0.32ns	0.15ns	-0.27ns	1	
Co	0.21ns	0.45ns	-0.14ns	0.08ns	-0.15ns	-0.09ns	-0.04ns	-0.18ns	-0.08ns	0.02ns	-0.11ns	-0.33ns	0.34ns	1

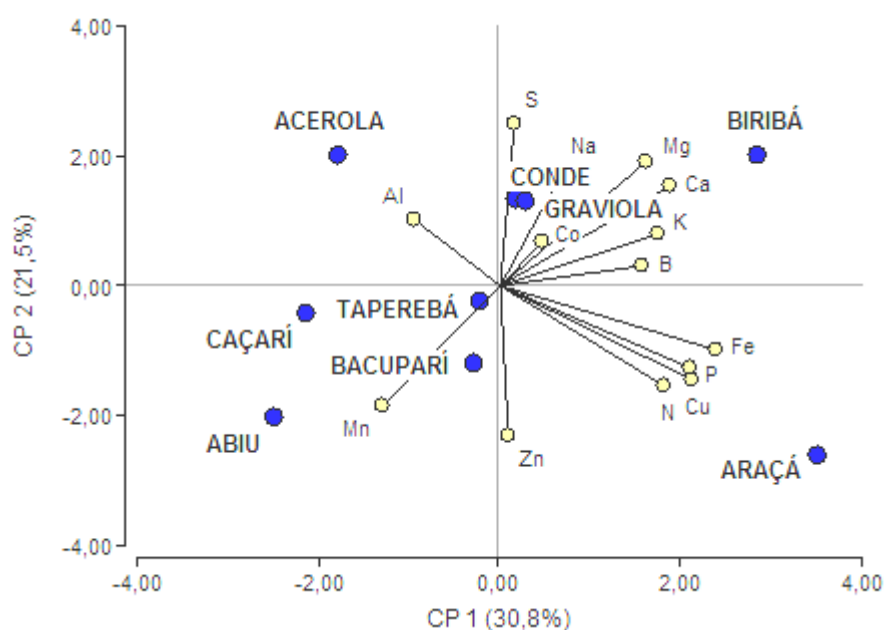
Subtitle: ns (not significant) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$

In Table 6, Pearson correlation coefficient showed highly significant correlation values at 1% significance for the following elements: copper with iron (0.97), iron with nitrogen (0.92), copper with nitrogen (0.94) and aluminum with sodium (0.80). Still, there are significant interactions at the significance level of 5%, calcium with magnesium (0.76), calcium with potassium (0.69), and at the same time, calcium with manganese (0.66). Phosphorus also has a significant interaction with iron and copper. In the case of the interaction of iron with phosphorus presents with Pearson correlation coefficient (0.76) and for the interaction of phosphorus with copper (0.68). Sodium with sulfur also has a significant interaction of (0.66). The other elements do not present significant interactions between them.

3.3.2 Principal component analysis (PCA)

The analyzes of main components were carried out jointly for the evaluated systems (*abiu*, *bacupari*, *acerola*, *graviola*, *camu-camu*, *araçá*, *biribá* and *taperebá*), in for pulps fruits, in order to find a new set of variables (main principals components), uncorrelated, that explain the structure of the variation, being represented the weight of each variable analyzed in each component (axes).

Figure 2. Distribution of the original variables between the different pulps fruits for the first and second component (CP1 and CP2)



In figure 2, the correlation of the two main components PC1 and PC2 is shown, where PC1 shows more information, with a higher variance value (30.8%)

and PC2 carries the maximum part of residual information with a value of 21.5%, being explained 52.3% of the total variance between the different minerals in the different fruit pulps studied.

The *araçá* pulp variables with *abiu* and *camu camu* (*caçari*) are inversely related and are the three variables that are related to the main component primer. Planes PC1 and PC2 reveal that time and quality of life are positive for the first principal component and therefore, variations in the means. Regarding the power of discrimination, we have the *araçá* pulp, together with the *abiu* pulp, we present a greater power of discrimination.

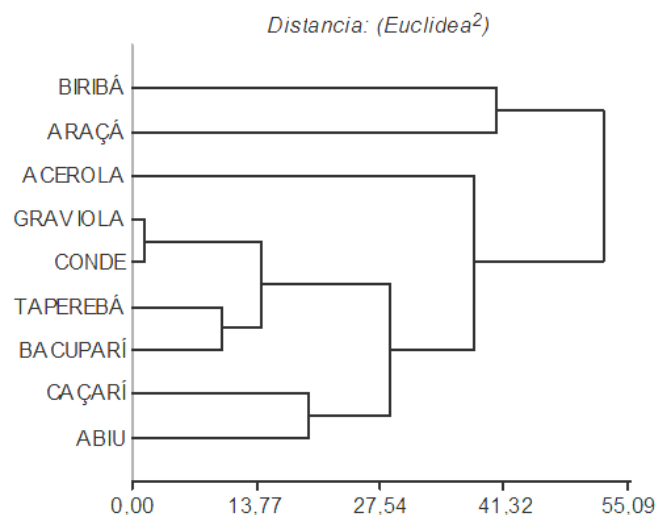
In the second main component (PC2) the *biribá* is related to *fruta-do-conde* and *graviola*, both of the same family for the concentrations of elements (S, Na, Mg, Ca, Co, K and B). The *araçá* pulp, presents the best contribution to the CP2. For the elements Fe, P, N, Cu and Zn as opposed to the others.

In the second quadrant, the *acerola* is located, which disagrees with the aluminum concentration of the other fruits and in the third quadrant the Mn is found, which correlates with the *taperebá*, *camu-camu* (*caçari*), *bacuparí* and *abiu* pulp.

3.3.3 Hierarchical Component Groupings (HCA)

With HCA, we can display the two-dimensional space in order to emphasize the seu groupings and natures, associating the samples so that more semelhantes are related to each other, presenting the samples in the dendrogram, grouping the amostras and variáveis of accord com sua similirade.

Figure 3- Dendrogram by HCA, Euclidean distance and incremental connection technique for the minerals present in the fruit pulps studied.



For the pulps of the studied fruits, the trends observed through the analysis of main components were observed through the HCA, observing that the biribá and araçá are grouped together, and the graviola, with the fruit of the count, taperebá, bacupari, caçarí and abiu are also grouped together. For the distance 27.54, being the value of half of the maximum distance, the biribá, araçá and acerola separate of the rest.

4. CONCLUSIONS

The present work establishes the nutritional importance of Amazonian pulps fruits, which present a great richness in minerals especially in micronutrients, establishing a correlation between the different constituents, as well as establishing methods of multivariate analysis to establish the relationship between the different studied variables.

Due to the nutritional importance of fruits, they could be used to develop bioproducts with interest being at the same time a part with high energetic potential.

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Conflict of interest.

Ismael Montero Fernández, corresponding author of the manuscript “**Bromatological and mineralogical study in fruit pulps cultivated in the northern Amazon**”, declares that she has not received any financial support, or have consulting or

personal relationships with other people or organizations, writing assistance, grant support or numbers, or statements of employment that could influence this work. The authors declare that they have no conflict of interest.

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CAPÍTULO II

USE OF AMAZON FRUIT BARKS AS SOURCE OF NUTRIENTS²

ABSTRACT

The barks of fruits are usually discarded as organic waste. A valuable source of nutrients obtained is used as source of raw material in the preparation of functional foods. In this work, the physicochemical properties (pH, titrable acidity and soluble solids), mineral and bromatological analysis of nine Amazonian fruits were studied: *abiu*, *acerola*, *araçá*, *bacupari*, *biribá*, *camu-camu*, *fruta-do-conde*, *araçá* and *taperebá*. The most acidic values stand out for the different fruits, with the exception of the *abiu* bark (pH = 4.7). As for its nutritional contribution, it was the *araçá* barks that presented the highest energy value of 276.29 Kcal 100 g⁻¹. Among the macrominerals, the potassium concentration stands out, being the highest concentration for the *graviola* bark, 521.04 mg 100 g⁻¹ followed by magnesium, where the concentration in the *biribá* was 64.21 mg 100 g⁻¹. Instead, the barks are rich in micronutrients, highlighting the concentration of zinc in the bark of *araçá*, 12.23 mg 100 g⁻¹ and manganese in the bark of *abiu*, 6.84 mg 100 g⁻¹. The Pearson correlation coefficient presented a highly significant correlation for Fe-Al (0.96), P-Fe (0.94) and Fe-Zn (0.89). O bligpot of principal components (PCA) explains 56% of the cases, being the minerals Mg, Na, Co, K, S and Ca highly associated for the *graviola* and *bacupari*

Keywords: Minerals; Sustainability; Bromatology; Foods.

1 INTRODUCTION

Brazil produces about 140 million tons of food per year, being the largest exporters of agricultural products, but at the same time there are problems with waste (Godim et al., 2005). From the foods wasted, 32 million tons are fruit (Maia et al., 2007). Among those industrial waste we have the part of the fruit barks, rich in nutrients, they are source of compounds with antioxidant activity (Montero et al., 2018). In addition, they have minerals both in high concentrations and in moth concentrations, as well as source of vitamins.

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In Brazil, an important amount of barks is generated in the processing of fruits, whose common destination is being discarded or destined for the production of fertilizers, occupying a total volume of 40% of processed fruits (Silva, 2014; Ajila et al., 2007).

Due to the nutritional importance of barks, in this study, of nine fruits were evaluated in Northern Amazonia: *abiu* (*Pouteria caimito*), *acerola* (*Malpighia emarginata*), *araçá* (*Psidium cattleianum*), *bacuparí* (*Rheedia gardneriana*), *biribá* (*Rollinia mucosa*), *camu-camu* (*Myrciaria dubia*), *fruta-do-conde* (*Annona squamosa*), *graviola* (*Annona muricata*) and *taperebá* (*Spondias mombin* L.), the nutritional value, macro and microminerals, as well as the physico-chemical properties (pH, titratable acidity, total soluble solids) and reducing and non-reducing sugars in order to be considered the use of the barks for the production of bioproducts due to its chemical composition.

2 MATERIALS AND METHODS

2.1 PREPARATION OF SAMPLES

The samples of the different fruits studied were collected in randomized points of the State of Roraima (Brazil) to guarantee the representativeness of the sample. Each of the fruits was collected in the corresponding production period and were collected in the ripening stage suitable for consumption. From all the samples collected at the different sampling points, a single composite sample was prepared for each of the fruits where they were taken to the Environmental Chemistry Laboratory of the Federal University of Roraima, where those that presented an optimum conservation status were selected washed with 1% sodium hypochlorite solution and again with distilled water.

Subsequently, a representative sample of each fruit was selected according to the following criteria: *acerola*, *camu-camu* and *taperebá* was selected 1 kg of fresh fruit: *abiu*, *araçá* and *bacuparí* was selected 2 kg of fruit and for *biribá*, *fruta-do-conde* and *graviola* were selected 10 units according with NTON 17002-02 (2002). The barks of the different fruits was separated of the fruits and were placed in Ultrafreezer at -80°C and then lyophilized in lyophilizer LIOTOP model L 101 for 48 hours until complete drying of the material and subsequently ground in LABOR model SP31

punch mill and stored material in airtight bags in the absence of light until the moment of performing the different analyzes.

Table 1- Names and families of fruits cultivated in the Northern Amazon under study.

Scientific name	Family	Name in Brazil
<i>P. caimito</i>	Sapotaceae	<i>Abiu</i>
<i>M. emarginata</i>	Malpighiaceae	<i>Acerola</i>
<i>P. cattleianum</i>	Myrtaceae	<i>Araçá</i>
<i>R. gardneriana</i>	Clusiaceae	<i>Bacuparí</i>
<i>R. mucosa</i>	Annonaceae	<i>Biribá</i>
<i>M. dubia</i> (Krunth)	Myrtaceae	<i>Camu-camu</i>
<i>A. squamosa</i>	Annonaceae	<i>Fruta-do-conde</i>
<i>A. muricata</i>	Annonaceae	<i>Graviola</i>
<i>S. mombin</i> L.	Anacardiaceae	<i>Taperebá</i>

2.2 PHYSICO-CHEMICAL PARAMETERS: PH, TITRATABLE ACIDITY AND SOLUBLE SOLIDS

The pH was determined by potentiometry using a pH meter previously calibrated. The titratable acidity (AT) was determined by diluting 5 g of lyophilized material, dissolved in 100 mL of distilled water with NaOH titration (0.1 M) until the phenolphthalein was turned (pH 8.1) and the results expressed as g citric acid in 100 g of pulp. Soluble solids (SS) were determined by refractometry with the fresh samples, expressed in °Brix and lastly, the SS/TA ratio were determined by the ratio between soluble solids content and titratable acidity (IAL, 2008).

2.3 NUTRITIONAL ANALYSIS

The physical parameters evaluated to determine the nutritional composition were the percentage of moisture and ash. The other nutritional parameters evaluated were the determination of total proteins, lipids and carbohydrates, to determine the total energy content.

2.3.1 Determination of Humidity

To determine moisture, 5 g of fresh samples were placed in porcelain capsules for 6 hours at 105 °C to constant mass, and then cooled in desiccator to room temperature (IAL, 2008).

$$\text{Humidity (g/100 g)} = ((P' - P'')/(P' - P)) \cdot 100$$

being: being: P = weight of porcelain capsule (g); P' = weight of the porcelain capsule + fresh sample (g); P'' = weight of the capsule + sample after the oven (g)

2.3.2 Determination of ashes

To determine the ash in the samples, the methodology proposed for the food analysis (IAL, 2008) with modifications was used, where 5 g of the lyophilized samples were weighed. These were placed in preheated porcelain crucibles in an oven at 110 °C for one hour, to remove moisture, and cool them in a desiccator to room temperature. The samples were incinerated at 600 °C in a FDG 3P-S EDG muffle for 16 hours, after which the samples were left in the desiccator until reaching room temperature.

$$\% \text{ ashes} = ((N \cdot 100)/M)$$

N = mass in grams of ash and M = mass of the sample in grams.

2.3.3 Determination of total proteins

Protein determination is performed from the total nitrogen analysis by Kjeldahl distillation, in which the existing organic matter is transformed into ammonia. The nitrogen content of the different proteins is approximately 16%, which introduces the empirical factor of 5.75 (conversion factor for vegetable protein), this will transform the number of grams of nitrogen, found with the number of grams of protein (IAL, 2008).

$$\% \text{ proteins} = \% \text{ N} \cdot 5.75$$

2.3.4 Determination of lipids

To determine the total amount of lipids, 20 g of each sample was weighed, and placed in the Soxhlet extractor apparatus with hexane as the solvent for six hours. The solvent was recovered in a rotary evaporator (IAL, 2008).

$$\% \text{ lipids} = ((N \cdot 100)/m)$$

Where: N = mass in grams of lipids and M = mass of the sample in grams.

2.3.5 Determination of Carbohydrates

The carbohydrate content is achieved by the difference of the value 100 subtracted from the sum of the already obtained values of moisture, ashes, lipids and proteins.

$$\text{Carbohydrates} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ lipids} + \% \text{ proteins})$$

2.3.6 Energetic value

In order to quantify the energy value, it was necessary to use the protein (P), lipid (L) and carbohydrate (C) contents of each sample. The result should be expressed in kcal 100 g⁻¹ (Mendes-Filho et al., 2014).

$$\text{Energy value (kcal 100 g}^{-1}\text{)} = (P * 4) + (L * 9) + (C * 4)$$

P = value of protein (%), L = lipid value (%), C = carbohydrate value (%), 4 = conversion factor in kcal determined in calorimetric pump for proteins and carbohydrates and 9 = conversion factor in kcal determined in a calorimetric pump for lipids.

2.4 MINERALOGICAL ANALYSIS

The extraction of the minerals into the epidermis was done according to the methodology described by Embrapa (2009) in which the perchloric nitric digestion (3:1) was used in TECNAL model TE 0079 digester block, washed with distilled water up to 25 mL for subsequent analysis.

Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and aluminum (Al) were determined by Flame Atomic Absorption Spectrophotometry (FAAS) Shimadzu AA-7000, coupled with ASC-7000 auto sample. Calibration was performed with standard solutions prepared from commercial standards of 1000 mg L⁻¹ Qhemis High Purity PACU 1000-0125, according to the specific conditions of each element (Table 2).

Table 2-Analytical Parameters of calibration.

Element	Technique	(λ) nm	Correlation coefficient (r^2)	LOD mg L ⁻¹	LOQ mg L ⁻¹
Ca	FAAS	422.70	0.999	0.481	2.004
Mg	FAAS	285.21	0.997	0.571	1.992
P	UV-Vis spectroscopy	660.00	0.999	0.113	1.773
K	AES	766.50	0.993	0.571	1.754
S	UV-Vis spectroscopy	420.00	0.998	0.074	0.897
Fe	FAAS	248.33	0.996	0.002	0.011
Zn	FAAS	213.80	0.991	0.002	0.071
Mn	FAAS	279.48	0.999	0.001	0.603
Cu	FAAS	324.75	0.997	0.003	0.010
Na	AES	589.0	0.999	0.098	1.103
Al	FAAS	309.3	0.998	0.0008	0.078
B	UV-Vis spectroscopy	420.00	0.999	0.089	0.123
Co	FAAS	240.73	0.997	0.0005	0.0008

FAAS = Flame Atomic Absorption Spectroscopy. AES = Flame Atomic emission Spectroscopy. LOD = detection limit. LOQ = Quantification limit.

As the ionization suppressor for the Ca, Mg and K elements, 0.1% of the lantane oxide solution (La_2O) was used. In the case of sodium (Na), it was determined in the same equipment, but in atomic emission mode. As for potassium (K), it was determined by means of flame photometry on the Digimed Flame Photometer DH-62, calibrated using a Digimed standard solution whose concentration range was 2-100 mg L⁻¹.

For the determination of the phosphorus (P), boron (B) and sulfur (S) elements, the ultraviolet molecular absorption spectrophotometry technique was used using a SHIMADZU UV-1800 model. The determination of P was carried by formation of the blue complex with ammonium molybdate ($(\text{NH}_4)_2\text{MoO}_4$) the readings were made at $\lambda = 660$ nm. The determination of boron was carried out by the formation of a yellow complex with azomethine-H, the readings being made at 460 nm. The determination of S was performed by forming a precipitate with BaCl_2 , the

readings being made in Uv-visivel at $\lambda = 420$ nm by calibration with potassium sulphate according with Embrapa (2009).

Nitrogen determination was carried out by the distillation method followed by titration (Kjeldahl) according with described methodology with Embrapa (2009).

2.5 STATISTICAL ANALYSIS

Correlations between the amounts of the different minerals in the epidermis of the fruit were evaluated using the Pearson statistical test using INFOSTAT (Rienzo et al., 2016) for significance levels of 5%, 1% and 0.1% respectively, as well as the principal component analyzes (PCA) and Hierarchical component analysis (HCA).

3 RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL CHARACTERIZATION

The parameters of physicochemical analysis studied in this work (pH, titratable acidity (TA), total soluble solids (SS) and SS/TA ratio) are parameters that serve to characterize the quality of the fruit (Canuto et al., 2010). In Table 3, the results of the physicochemical parameters for the different fruits are presented, with their standard deviation made for three repetitions using the value of the t-student for the 95% probability.

Table 3-Physicochemical parameters for the barks of different fruits.

Fruit	pH	TA (g citric acid 100 g ⁻¹)	SS (°Brix)	SS/TA
<i>Abiu</i>	4.7 ± 0.1	5.6 ± 0.1	2.7 ± 0.2	0.48 ± 0.1
<i>Acerola</i>	2.1 ± 0.2	1.2 ± 0.1	2.9 ± 0.1	2.41 ± 0.1
<i>Araçá</i>	4.3 ± 0.1	0.4 ± 0.1	4.5 ± 0.2	11.25 ± 0.2
<i>Bacuparí</i>	3.1 ± 0.2	1.9 ± 0.2	7.1 ± 0.1	3.74 ± 0.2
<i>Biribá</i>	3.0 ± 0.2	2.4 ± 0.2	8.1 ± 0.1	3.38 ± 0.1
<i>Camu camu</i>	2.4 ± 0.1	1.7 ± 0.2	4.3 ± 0.2	2.52 ± 0.2
<i>Fruta-do-conde</i>	2.9 ± 0.1	2.7 ± 0.1	8.7 ± 0.1	3.22 ± 0.1
<i>Graviola</i>	3.1 ± 0.1	2.1 ± 0.1	8.4 ± 0.1	4.0 ± 0.1
<i>Taperebá</i>	2.7 ± 0.2	1.7 ± 0.2	5.7 ± 0.1	3.35 ± 0.1

The pH value for the different fruits studied ranges from 2.1 for *acerola* barks, reaching 4.7 for the *abiu* barks. The titratable acidity expressed in mg of citric acid. 100 g⁻¹ presents values of 0.4 ± 0.1 for the *araçá* barks up to 5.6 ± 0.1 for the *abiu*. This parameter is important, since it indicates the maturity of the fruit, measuring the titratable hydrogens contained in the fruits of all the acids that constitute it until they are neutralized at a fixed pH value. It is expressed as the equivalent of citric acid since it is the predominant acid in fruits and according to Fernández et al. (2006), this parameter can not be less than 0.4. The value of the SS expressed in °Brix varies between 2.7 ± 0.2 for the *abiu* barks, reaching the value of 8.7 ± 0.1 for the *fruta-do-conde* barks. Finally, the SS/TA ratio gave values between 0.48 for the *abiu* to values of 11.25 for the *araçá*. This parameter relates the quality of the fruit in terms of maturity and flavor (Chitarra & Chitarra, 2005).

There are few data in the literature about the physicochemical parameters in the barks of these Amazonian fruits, being limited to the study of pulps. In the case of *camu-camu*, studies carried out by Maeda et al. (2006), the physicochemical parameters for the pulp of the *camu-camu*, being the pH of the pulp of 2.64 slightly higher than that of the shell, the solids solids also, with 6.20°Brix and the titratable acidity is also higher for the pulp (3.40 g 100 g⁻¹ of citric acid). In the case of the *fruta-do-conde*, Bonfim et al. (2014) study said fruit in different stages of fruit maturity, finding values in the mature state of soluble solids between 17.25-20.22°Brix values higher than those found for the barks and potential acidity value between (0.18-0.23) % also higher than those found for the bark in this work.

There is a work developed by Carlone et al. (2016), where they prepare a *bacuparí* flour made from the pulp and the seeds finding pH values of 3.18 similar to those determined in this work and titratable acidity of 7.82 g 100 g⁻¹ of citric acid, greater than those presented here, since in this work not only the bark of the fruit is being evaluated, but also the pulp is being evaluated.

For the *graviola*, if studies were found that evaluate the bark of the same, made by Silva (2016), where the pH value determined is approximately one unit lower than the one determined in this work and for the titratable acidity, it finds a value of 3.70 g 100 g⁻¹ of citric acid, somewhat higher than what we determine, since acidity influences the degree of ripeness of the fruit. However, Sacramento et al. (2003), study of the *graviola* pulp, where the determined value of pH is 3.44 being approximate to the one we determined in this work for the barks and acidity titratable

in the case of the pulp it was approximately 1.00 g 100 g⁻¹ of citric acid less than that determined in the barks.

In the case of *taperebá* bark, no results have been found for physicochemical analysis in the bark, only for the pulp being determined by Freitas (2017), pH values for the pulp between 2.60 - 2.95, being within the range of pH determined for the barks in this work. The titratable acidity is slightly lower 0.60 - 1.40 g. 100 g⁻¹ of citric acid to the one determined for the barks and the soluble solids in the pulp are slightly larger (9.96 - 11.30).

The *araçá* presents studies for the pulp, whose pH values vary between 3.0 - 4.0, titratable acidity between 1.80 - 1.87 g. 100 g⁻¹ of citric acid and soluble solids between 4.5 - 11 °Brix (Andrade et al., 1993; Canuto et al., 2010). The pH values determined for the pulps are close but slightly lower than those of the barks of the fruits evaluated in this work, the titratable acidity is much lower for the bark and in the case of the SS, these are close to the determined by Canuto et al. (2010) for the pulp.

In the case of *abiu* barks, the pH of the barks is close to that of the pulp determined by Canuto et al. (2010), who obtains a pH value of 5.0 The titratable acidity is lower for the pulps than for the seeds but with very close value found by the same author 5.9 g. 100 g⁻¹ of citric acid and for soluble acids these are greater for the pulp (3.8) according to the same author as for the barks. For acerola, Godoy et al., (2008) and Canuto et al., (2010), the pH value for pulp (2.8 - 3.4) is slightly higher than that found for the bark in this work, the potential acidity for the bark is within the range determined by these authors (0.92 - 1.90 g. 100 g⁻¹ of citric acid) and the soluble solids for the bark of this work are lower than those found by the previous authors for the pulp of these fruits with values of 3.5-8.24°Brix.

3.2 BROMATOLOGICAL ANALYSIS FROM BARKS OF AMAZON FRUITS

Of all the parameters that make up the bromatological analysis (Table 4), moisture is the majority in the epidermis of the fruits studied compared to the other parameters, ranging from 32.12% for *araçá* barks to 88.99% for *acerola* barks. The content of ashes in the fruit barks does not reach 1%, with the lowest concentration for the *taperebá* barks with 0.24% and 0.89% for the *fruta-do-conde* barks.

The content of lipids in the husk is low, in relation to other parts of the fruit, being in lower concentration for the *taperebá* with a percentage of 0.12% and the

highest concentration for the bacuparí with 1.41%. The carbohydrate content varies according to the fruit in a high percentage range, determining a percentage of 9.62% for the *biribá* to 65.58% for the *araçá* bark.

The proteins are another one of the nutrients that are in low concentration in the barks of the fruits, being only in concentration of 0.04% for the *acerola*, reaching values of 0.41% for the *abiu*. The energy contribution of the barks varies from 48.06 kcal 100 g⁻¹ for *acerola* to 276.29 kcal 100 g⁻¹ for *araçá*.

Table 4 presents the nutritional analysis values for the barks of the different Amazonian fruits studied.

Table 4- Nutritional composition in bark of Amazon fruits.

FRUIT	NUTRITIONAL CONTRIBUTION					
	MOISTURE	ASHES	LIPIDS	CARBOHYDRATES	PROTEINS	ENERGETIC VALUE
	%					Kcal 100 g ⁻¹
<i>Abiu</i>	82.49 ± 0,09	0.41 ± 0.02	1.27 ± 0.02	15.42 ± 0.01	0.41 ± 0.02	74.75 ± 0.01
<i>Acerola</i>	88.89 ± 0.05	0.27 ± 0.03	0.94 ± 0.02	9.86 ± 0.01	0.04 ± 0.00	48.06 ± 0.01
<i>Araçá</i>	32.12 ± 0.12	0.52 ± 0.09	1.37 ± 0.13	65.58 ± 0.01	0.41 ± 0.02	276.29 ± 0.01
<i>Bacuparí</i>	84.37 ± 0.21	0.38 ± 0.11	1.41 ± 0.03	13.52 ± 0.02	0.32 ± 0.02	68.05 ± 0.01
<i>Biribá</i>	88.32 ± 0.07	0.77 ± 0.17	1.12 ± 0.06	9.62 ± 0.02	0.17 ± 0.02	49.24 ± 0.02
<i>Camu-camu</i>	83.12 ± 0.09	0.31 ± 0.09	1.12 ± 0.04	15.37 ± 0.01	0.08 ± 0.00	71.88 ± 0.01
<i>Fruta-do-conde</i>	85.43 ± 0.02	0.89 ± 0.03	1.27 ± 0.01	12.30 ± 0.02	0.11 ± 0.01	61.07 ± 0.03
<i>Graviola</i>	74.16 ± 0.08	0.72 ± 0.04	1.04 ± 0.04	23.91 ± 0.02	0.17 ± 0.03	105.68 ± 0.05
<i>Taperebá</i>	73.21 ± 0.13	0.24 ± 0.12	0.12± 0.04	26.32 ± 0.02	0.11 ± 0.01	106.80 ± 0.02

Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95 %

3.3 MINERAL ANALYSIS

In the Table 5 and the Table 6, the values of macronutrients and micronutrients are presented for the different barks studied. Among the macronutrients detected in the barks of different fruits, potassium stands out as the majority, being in the *graviola* barks where it is in the highest concentration 521 mg 100 g⁻¹, and in lower concentration for the *taperebá* barks with concentration of 111.34 mg 100 g⁻¹. These values of K in the edible fraction are in agreement with those established by Almeida et al. (2009), where they establish that K levels in the edible fraction of fruits ranges between 143.67 -790.11 mg 100 g⁻¹. Elcinto (2000), notes that this element is found in high concentrations in fresh fruits and vegetables, especially in the bark and stem of edible fruits. Its importance in the organism is in the maintenance of the hydroelectric balance with sodium, the concentrations of these elements being regulated inside and outside the cell Cuppari and Bazanelli (2010). The concentrations of sodium in the fruits studied are lower than the potassium concentrations, with the highest concentration for the *camu-camu* barks being 18.26 mg 100 g⁻¹ and the lowest concentration for the *abiu* barks with 0.36 mg 100 g⁻¹. Studies of this mineral in plants of the Annonaceae family, determined Na concentration for the *graviola* of 3.11 mg 100 g⁻¹, slightly higher than that found in this work and for the *fruta-do-conde* of 9.94 mg 100 g⁻¹ also superior to the one found in this work (Bramont et al., 2008).

Unlike what happens with potassium, calcium in the barks of the fruit is of great importance, since it is the element that gives it firmness, being associated with high levels of calcium to a good quality of the fruit (Johnston et al. al., 2002; Poovaiah et al., 1988). In this study, Ca is the second element in abundance after K, with the highest concentration in the *camu-camu* barks with 52.21 mg 100 g⁻¹ and the lowest concentration for *abiu* barks with 22.11 mg 100 g⁻¹. The next element in abundance in the fruits studied is the Mg, being the *biribá* bark with 64.21 mg 100 g⁻¹ who presents a higher concentration of Mg, even superior to that of Ca. Berto et al. (2015) determined Mg concentrations of 69.07 mg 100 g⁻¹ for *biribá*, a value close to that determined in this work.

The importance of phosphorus in the organism lies in its involvement in metabolic functions such as the synthesis of ATP, synthesis of carbohydrates, nucleic acids and coenzymes (Epstein and Bloom, 2006), being found in the body

between 0.8 - 1.1% (Monteiro and Vannucchi , 2010). The highest concentration of phosphorus was in *araçá* bark 43.47 mg 100 g⁻¹ and the lowest concentration of *abiu* bark with 4.3 mg 100 g⁻¹. The concentration of minerals in *biribá* fruits was studied by Berto et al. (2015) who determined P concentrations in this fruit of 25.32 mg 100 g⁻¹, next to the value found in this work.

Sulfur is within the macrominerals, found in a wide range of values for the fruits studied, from 3.14 mg 100 g⁻¹ for the *bacuparí* bark, to 37.22 mg 100 g⁻¹ for acerola bark. This element is necessary for the human body since it is part of amino acids such as cysteine and methionine present in hair and nails, being found in the body in concentrations of 140 g of this element (Lisboa, 2015).

The last element to consider in this work is nitrogen, being a constituent in several components of plants and the sea in the form of amino acids, nucleic acids and chlorophyll, as well as part of numerous microbiological reactions (Novais et al. 2007), In the fruits studied they are stored in the *biribá* barks whose concentration is 8.56 mg 100 g⁻¹.

Table 5- Macronutrients analyzed in barks fruit in the Northern Amazon.

Fruit	Macronutrients						
	Calcium (Ca)	Magnesium (Mg)	Phosphorous (P)	Potassium (K)	Sodium (Na)	Sulfur (S)	Nitrogen (N)
	mg 100 g ⁻¹						%
<i>Abiu</i> (<i>Pouteria caimito</i>)	22.11± 0.14	13.21± 0.15	4.31± 0.11	242.11± 0.14	0.36 ± 0.02	15.44 ± 0.12	0.07 ± 0.02
<i>Acerola</i> (<i>Malpighia emarginata</i>)	27.12 ± 0.13	37.21 ± 0.05	7.21 ± 0.11	121.33 ± 0.22	17.81 ± 0.04	37.22± 0.14	6.96.10 ⁻³ ± 0.00
<i>Araçá</i> (<i>Psidium cattleianum</i>)	27.23 ± 0.01	15.27 ± 0.11	43.47 ± 0.04	123.11 ± 0.07	6.83 ± 0.11	7.31 ± 0.02	0.07 ± 0.02
<i>Bacuparí</i> (<i>Rheedia gardneriana</i> Planch & Triana)	44.12 ± 0.10	35.55± 0.12	6.21 ± 0.09	411.08 ± 0.07	7.13 ± 0.01	3.14± 0.08	0.06 ± 0.01
<i>Biribá</i> (<i>Rollinia mucosa</i>)	47.91 ± 0.12	64.21 ± 0.11	20.22 ± 0.01	441.12 ± 0.12	17.13 ± 0,31	19.14 ± 0.14	8.56 ± 0.02
<i>Camu-camu</i> (<i>Myrciaria dúbia</i> (Kunth) Mc Vaugh)	52.21 ± 0.13	32.12 ± 0.09	17.30 ± 0.12	431.21± 0.17	18.26 ± 0.11	27.78 ± 0.13	0.21 ± 0.04
<i>Fruta-do-conde</i> (<i>Annona squamosa</i>)	50.11 ± 0.04	28.07 ± 0.12	15.11 ± 0.01	417.09 ± 0.11	3.48 ± 0.07	23.12 ± 0.09	0.02 ± 0.00
<i>Graviola</i> (<i>Annona muricata</i>)	33.12 ± 0.04	21.08 ± 0.09	16.11 ± 0.21	521.04 ± 0.15	2.16 ± 0.08	13.11± 0.05	0.03 ± 0.00
<i>Taperebá</i> (<i>Spondias mombin</i> L.)	45.21 ± 0.02	28.11± 0.04	16.22± 0.08	111.34 ± 0.04	6.56 ± 0.07	3.21 ± 0.03	0.02 ± 0.00

Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95 %.

Table 6- Micronutrients analyzed in barks fruits in the Northern Amazon.

Fruit	Iron (Fe)	Zinc (Zn)	Manganese (Mn)	Copper (Cu) mg 100 g ⁻¹	Aluminum (Al)	Boron (B)	Cobalt (Co)
<i>Abiu</i> (<i>Pouteria caimito</i>)	0.07 ± 0.00	3.04 ± 0.01	6.84 ± 0.11	1.24 ± 0.05	0.08 ± 0.01	0.74 ± 0.03	N.D.
<i>Acerola</i> (<i>Malpighia emarginata</i>)	0.44 ± 0.02	0.04 ± 0.01	0.78 ± 0.07	0.09 ± 0.02	0.16 ± 0.04	0.22 ± 0.04	N.D.
<i>Araçá</i> (<i>Psidium cattleianum</i>)	4.41 ± 0.03	12.23 ± 0.02	0.31 ± 0.03	3.38 ± 0.02	0.03 ± 0.00	0.19 ± 0.01	N.D.
<i>Bacuparí</i> (<i>Rheedia gardneriana</i> Planch & Triana)	0.32 ± 0.04	2.94 ± 0.09	0.50 ± 0.07	0.82 ± 0.06	0.22 ± 0.04	0.12 ± 0.03	0.031 ± 0,006
<i>Biribá</i> (<i>Rollinia mucosa</i>)	1.32 ± 0,12	0.94 ± 0.09	0.57 ± 0.07	0.87 ± 0.06	0.27 ± 0.04	0.22 ± 0.04	0.011 ± 0,006
<i>Camu-camu</i> (<i>Myrciaria dúbia</i> (Kunth) Mc Vaugh)	0.21 ± 0.08	0.71 ± 0.03	1.07 ± 0.07	0.72 ± 0.02	0.04 ± 0.01	0.23 ± 0.02	0.061 ± 0.002
<i>Fruta-do-conde</i> (<i>Annona squamosa</i>)	0.23 ± 0.07	0.19 ± 0.01	2.55 ± 0.01	2.48 ± 0.04	0.18 ± 0.03	0.29 ± 0.17	N.D.
<i>Graviola</i> (<i>Annona muricata</i>)	0.81 ± 0.04	0.32 ± 0.01	0,64 ± 0.05	0.39 ± 0.02	0.38 ± 0.01	0.37 ± 0.03	0.010 ± 0.001
<i>Taperebá</i> (<i>Spondias mombin</i> L.)	0.45 ± 0.08	0.07 ± 0.01	0.87 ± 0,05	1.03 ± 0.07	0.14 ± 0.02	0.79 ± 0.06	N.D.

N.D. not detected. Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95 %

Analyzes of microminerals in Amazonian fruit barks are scarce or nonexistent. In Table 6, the values of micronutrient concentrations for the nine fruits studied are presented.

Zinc is one of the important micronutrients since among other functions it is present in the liver mobilization of vitamin A, sexual maturation, fertility and reproduction as well as participating in more than 300 metalloenzymes (Manganaro, 2008; Cominetti, 2009) being found in low concentrations in the fruits studied with the exception of *araçá* where the concentration of this element in the *araçá* bark is 12.23 mg 100 g⁻¹ followed by *abiu* bark with 3.04 mg 100 g⁻¹ and the lowest concentrations of This element was obtained in the *acerola* barks with concentrations of 0.04 mg 100 g⁻¹. Studies conducted by Berto et al. (2015) on barks of different Amazonian fruits obtain values of concentration of Zn for the *biribá* barks with 1.04 mg 100 g⁻¹ being a value practically similar to that obtained in this work. The recommendations of this element according to the DRI (2001) for adulthood are of 9.4 mg dia⁻¹ for men and 11 mg dia⁻¹ for women.

Another of the micronutrients found in the highest concentration in some fruits is the Mn, being the *abiu* barks, which presents a higher concentration with 6.84 mg 100 g⁻¹, followed by *fruta-do-conde* barks with concentrations of 2.55 mg 100 g⁻¹. This mineral presents lower concentrations for the *araçá* barks with only 0.31 mg 100 g⁻¹. Berto et al. (2015) studied micronutrients in the different Amazonian fruits barks, obtaining for the *biribá* barks concentrations of 0.47 mg 100 g⁻¹, close to the concentration determined in this work (Table 6). For the *taperebá*, Sena et al. (2014), study the composition of micronutrients in flour obtained from residues of fruit processing, with concentrations of Mn of 0.04 mg 100 g⁻¹, concentration lower than that found in its barks pure. This element is interesting for its implication with diverse metabolic reactions in the organisms of immune response, synthesis of ATP and as cofactor in metalloenzymes (Burton and Guillarte, 2009) being the recommendations of this element for adults of 2.3 mg dia⁻¹ for men and 1.8 mg dia⁻¹ for women (DRI, 2001).

Iron is another essential micronutrient whose recommendations according to DRI (2001) for adults are 8 mg dia⁻¹ and from the age of fifty, these amounts are reduced to 8 mg dia⁻¹ being found in the human body at concentrations of 3-5 g (Fantisi et al. 2008).

Copper is another microelement of interest in the bark of the fruits studied, being found in a higher concentration in the *araçá* bark with a value of 3.38 mg 100 g⁻¹

¹ and in a lower concentration in the *acerola* bark with a concentration of 0.09 mg 100 g⁻¹. The deficiency of this element, has important series implications for the organism as is the case of the diseases of Wilson and Menkes (Amancio, 2017) being the recommendations of Cu in adulthood according to the DRI (2011) of 700 µg day⁻¹.

Boron is another essential element for man, related to maintaining the integrity of the plasma membrane and involved with bone metabolism (Brown et al. 2002). According to the DRI (2001), the recommendations of B for adults are of 20 mg dia⁻¹. The highest concentrations of B found in the fruit bark studied in this study are in the *taperebá* barks whose concentration is 0.79 mg 100 g⁻¹ and for *abiu* barks with concentrations of 0.74 mg 100 g⁻¹. The lowest concentrations of this element are in the *bacuparí* barks whose concentration is of 0.12 mg 100 g⁻¹. Ribeiro et al. 2016, studied the concentrations of B in dry *camu-camu* fruits and obtained values of 1.7-1.8 mg 100 g⁻¹, values higher than only for the isolated barks (Table 6).

The cobalt is the element found in ultra-trace concentrations in the barks of the studied fruits, being only detected in *bacuparí*, *biribá*, *camu-camu* and *graviola* whose values oscillate between 10 µg 100 g⁻¹ for the *graviola* barks being the highest concentration of this element in *camu-camu* barks with concentration of 61 µg 100 g⁻¹. These values are lower than those recommended by FAO/WHO, which should be ingested 0.58 mg kg⁻¹ as a function of the individual's body size (FAO, 2013). This element in higher concentrations can cause toxicity as is the case of cardiomyopathy, as well as linked to other nervous and blood clotting problems (Seghizzi et al. 1994).

Finally, the aluminum was also identified in the fruit skin studied, being one of the metals that must be found in low concentrations in foods since this metal being a neurotoxic substance, is involved with Alzheimer's disease (Armstrong, 2002). The concentrations of Al in this work are low, being the highest value for the *graviola* bark with Al concentration of 0.38 mg 100 g⁻¹ and the lowest concentration of Al for *camu-camu* bark with concentration of 0.03 mg 100 g⁻¹.

3.4 STATISTIC ANALYSIS

Pearson correlation coefficient

Table 7 presents the Pearson correlation matrix between the different elements for the barks of the different fruits.

Table 7-Pearson correlation matrix between the different elements for the bark of Amazonian fruits.

	Ca	Mg	P	K	S	N	Fe	Zn	Mn	Cu	Na	Al	B	Co
Ca	1													
Mg	0.66*	1												
P	0.00ns	-0.10ns	1											
K	0.55ns	0.41ns	-0.19ns	1										
S	0.05ns	0.38ns	-0.25ns	0.09ns	1									
N	0.39ns	0.82**	0.14ns	0.36ns	0.17ns	1								
Fe	-0.10ns	-0.08ns	0.94**	-0.21ns	-0.16ns	0.11ns	1							
Zn	-0.26ns	-0.32ns	0.76*	-0.28ns	-0.30ns	-0.12ns	0.89**	1						
Mn	-0.28ns	-0.38ns	-0.38ns	-0.05ns	0.13ns	-0.19ns	-0.30ns	0.00ns	1					
Cu	0.12ns	-0.29ns	0.73*	-0.13ns	-0.19ns	-0.12ns	0.69*	0.75*	0.13ns	1				
Na	-0.19ns	0.44ns	0.01ns	-0.34ns	0.23ns	0.44ns	-0.01ns	-0.20ns	-0.51ns	-0.38ns	1			
Al	0.00ns	-0.09ns	0.88**	0.00ns	-0.14ns	0.02ns	0.96**	0.85**	-0.33ns	0.65*	-0.19ns	1		
B	-0.03ns	-0.30ns	-0.25ns	-0.24ns	-0.16ns	-0.20ns	-0.31ns	-0.22ns	0.58ns	-0.08ns	-0.49ns	-0.34ns	1	
Co	-0.36ns	-0.17ns	-0.18ns	-0.06ns	-0.49ns	-0.03ns	-0.26ns	-0.17ns	-0.26ns	-0.32ns	0.46ns	-0.32ns	-0.39ns	1

Subtitle: ns (not significant) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$

Table 7 presents the Pearson interaction values for the different fruit constituents in the barks of the fruit, where highly significant interactions are found at a significance level of 1% for nitrogen systems with magnesium (0.82), aluminum with phosphorus (0.88), iron with phosphorus (0.94), aluminum with iron (0.96), zinc with iron (0.89) and aluminum with zinc (0.85). On the other hand, there are significant interactions at the significance level of 5% for the magnesium systems with calcium (0.66), zinc with phosphorus (0.76), copper with phosphorus (0.73), copper with iron (0.69), copper with zinc (0.75) and aluminum with copper (0.65). For the remaining elements there is no significant interaction.

3.4.1 Principal component analysis (PCA)

The analyzes of main components were carried out jointly for the evaluated systems (*abiu*, *bacupari*, *acerola*, *graviola*, *camu-camu*, *araçá*, *biribá* and *taperebá*), independently for barks of the fruit, in order to find a new set of variables (main components), uncorrelated, that explain the structure of the variation, being represented the weight of each variable analyzed in each component (axes).

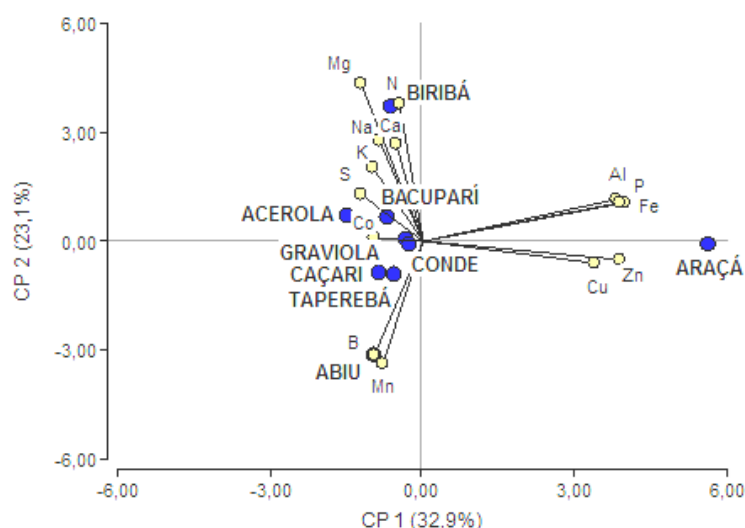
Figure 1 shows the correlation of the main components PC1 and PC2 of PC1 plus information, with higher value of variance (32.9%) and PC2 is the most important part of residual information with a value of 23.1%, being explained to 56.0% of the total of the variant between the different minerals in the different fruit barks studied.

The araçá barks variables with abiu barks are inversely related and are the three variables that contribute to the first principal component. The PC1 and PC2 planes reveal that araçá barks presents a positive score for the first principal component and, therefore, present higher than average variations. Regarding the power of discrimination, we have that araçá barks, together with the abiu barks, have a greater power of discrimination.

In the second main component, the biribá barks with bacupari barks and acerola barks are related in the second quadrant for the concentrations of elements (N, Mg, Na, Ca, K, S and Co) with negative contribution to CP1, being the biribá barks who else contributes to the second main component. On the other hand, the elements such as Al, P and Fe, do not present any correlation in the different parts of the fruit barks studied.

The B and Mn are strongly correlated for the abiu, graviola, camu camu (çaçari) and taperebá barks and negatively correlated with the biribá barks.

Figure 1. Distribution of the original variables among the different fruits for the barks on the first and second main component (CP1 and CP2).

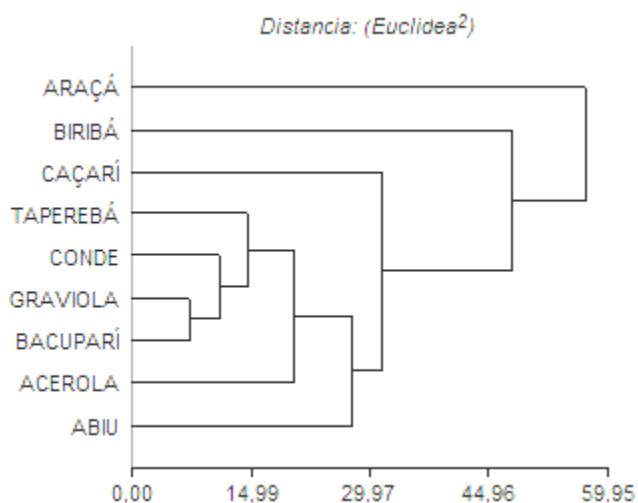


3.4.2 Hierarchical Component Analysis (HCA)

Through the HCA, data can be displayed in a two-dimensional space in order to emphasize their natural groupings and patterns, relating the samples so that the most similar are related to each other, presenting the samples in dendrogram, grouping the samples and variables according to with its similarity.

In Fig.2 the dendrogram for the HCA analyzes of the different fruit barks studied is presented.

Figure 2-Dendrogram by HCA, Euclidean distance and incremental connection technique for the minerals present in the fruit barks studied.



For the production of tested fruits, the trends observed through or analysis of principal components, observed through HCA, observing that either *taperebá*, *fruta-do-conde*, *graviola*, *bacupari* and *abiu* are not grouped between them, and for distance. 29.98, sendo o valor de metade gives maximum distance, or *araçá* e *biribá* separated rest.

4. CONCLUSIONS

Given the values obtained from nutritional intake and minerals for the barks of the fruits studied, which in some cases are superior to those of the edible parts, the barks could be used as an alternative source of nutrients, taking advantage of the source of nutrients and at the same time, other products can be prepared from these samples such as jellies, sweets and flours.

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CAPÍTULO III

CHEMICAL CHARACTERIZATION OF AMAZON FRUITS SEEDS AND ITS NUTRITIONAL CONTRIBUTION AS FOODS FUNCTIONAL AND BIOTECHNOLOGICAL INTEREST³

ABSTRACT

The fruit seeds of the Northern Amazon region: *abiu*, *acerola*, *araçá*, *bacupari*, *biribá*, *camu-camu*, *fruta-do-conde*, *graviola* and *taperebá*, were evaluated bromatologically, nutritionally both at the level of macroelements, elements in trace concentrations and the fatty acids present in them. Bromatologically, of *camu-camu* seeds with 369.08 Kcal 100 g⁻¹ and *biribá* seeds with 364.78 cal 100 g⁻¹ have the highest energy value. In relation to the macroelements is the majority potassium of all with concentration of 554.23 mg 100 g⁻¹ for the *graviola*, followed by magnesium, with concentration of 123.11 mg 100 g⁻¹ for the *biribá*. In terms of micronutrients, the highest concentrations are zinc 4.14 mg 100 g⁻¹ and manganese 4.12 mg 100 g⁻¹ for *abiu* seeds. All metal contents do not pose a risk to health. On the other hand, the profile of fatty acids, presents in most of the seeds studied a higher percentage of unsaturated fatty acids than saturated ones. Among the most saturated is palmitic acid with a concentration of 40.4% for *taperebá* seeds and among the unsaturated is oleic acid with a concentration of 47.4% for *bacupari* seeds. The Pearson correlation coefficient was evaluated, as well as the statistical treatment by multivariate analysis PCA and HCA.

Keywords: Oleic acid; Bromatological, Minerals, PCA, HCA.

1 INTRODUCTION

Brazil is one of the countries with the largest fruit production in the world, generating about 43.5 million tons of fruit for the year 2017. This is due to present on the one hand a large territorial extension, on the other hand conditions adequate climate and soil and a strong investment by the public and private sector in both infrastructure and logistics innovation, with the export of fresh fruits and by-products for the year 2017 of 878,400 thousand tons with US \$ 946,792 million (Brazilian fruit yearbook, 2018).

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According to the Brazilian Institute of Fruit Growing (IBRAF, 2013), of the total fruit produced in Brazil, 53% is destined to commercialization as fresh fruits and the remaining 47% go to the agroindustrial sector.

Among the main Brazilian fruit-producing states are São Paulo (39%), Bahia (12%), Rio Grande do Sul (6%), Minas Gerais (6%) and Pará (3.7%) according to the data provided by (IBRAF, 2013).

In the production of food of plant origin we have the problem of residues and wastes in the production chain. At harvesting, it is estimated a loss of 10%, in the transport and industrialization stages 50% of the losses, and in the preparation of the food, 10% of the plant purchased is not used (Roriz, 2012). The large residues are generated in the industries producing pulps and commercial juices, mainly the husks and seeds.

According with Gondim, Moura, Dantas, Medeiros & Santos (2005), food waste along with hunger is one of the biggest problems facing Brazil, being such waste an important source of nutrients such as minerals, fatty acids, compounds with antioxidant activity and source of vitamins (Scherer, Rybka & Godoy, 2008), which can be used in the food industry, and can be used in the elaboration of functional foods with great biotechnological potential, since these nutrients are usually found in higher concentrations in seeds and in the husks than in the pulps. food products for the development of new products, thus contributing to valorize the products obtained with positive impact on both the economic and social side (Ayala-Zavala, Vega-Vega, Rosas-Domínguez, Palafox-Carlos, Villa-Rodríguez, Wasim, Dávila-Aviña & González-Aguilar et al., 2011).

The objective of this work was to evaluate the bromatological, mineralogical composition and characterization of fatty acids from seeds extracted from fruits occurring in the northern Amazon: *abiu* (*Pouteria caimito*), *acerola* (*Malpighia emarginata*), *araçá* (*Psidium cattleianum*), *bacuparí* (*Rheedia gardneriana*), *biribá* (*Rollinia mucosa*), *camu-camu* (*Myrciaria dubia*), *fruta do conde* (*Annona squamosa*), *graviola* (*Annona muricata*) and *taperebá* (*Spondias mombin* L.), to be used as substrates for the recovery of compounds or for the development of new products with interest as functional food and to study the correlation between existing using the Pearson test as well as to use multivariate analysis methods such as Principal Component Analysis (PCA) and Hierarchical Component Groupings (HCA) for concentration of mineral seeds fruits.

2 MATERIALS AND METHODS

2.1 PREPARATION OF SAMPLES

The samples of the different fruits studied were collected in randomized points of the State of Roraima (Brazil) to guarantee the representativeness of the sample. Each of the fruits was collected in the corresponding production period and were collected in the ripening stage suitable for consumption. From all the samples collected at the different sampling points, a single composite sample was prepared for each of the fruits where they were taken to the Environmental Chemistry Laboratory of the Federal University of Roraima, where those that presented an optimum conservation status were selected washed with 1% sodium hypochlorite solution and again with distilled water.

Subsequently, a representative sample of each fruit was selected according to the following criteria: *acerola*, *camu-camu* and *taperebá* was selected 1 kg of fresh fruit; *abiu*, *araçá* and *bacuparí* was selected 2 kg of fruit and for *biribá*, *fruta-do-conde* and *graviola* were selected 10 units according with NTON 17002-02 (2002). The seeds of the different fruits were separated from the different parts of the fruits and were placed in Ultrafreezer at -80°C and then lyophilized in lyophilizer LIOTOP model L 101 for 48 hours until complete drying of the material and subsequently ground in LABOR model SP31 punch mill and stored material in airtight bags in the absence of light until the moment of performing the different analyzes.

Table 1- Names and families of fruits cultivated in the Northern Amazon in study.

Scientific name	Family	Name in Brazil
<i>Pouteria caimito</i>	Sapotaceae	<i>Abiu</i>
<i>Malpighia emarginata</i>	Malpighiaceae	<i>Acerola</i>
<i>Psidium cattleianum</i>	Myrtaceae	<i>Araçá</i>
<i>Rheedia gardneriana</i>	Clusiaceae	<i>Bacuparí</i>
<i>Rollinia mucosa</i>	Annonaceae	<i>Biribá</i>
<i>Myrciaria dúbia</i> (Krunth)	Myrtaceae	<i>Camu-camu</i>
<i>Annona squamosa</i>	Annonaceae	<i>Fruta-do-conde</i>
<i>Annona muricata</i>	Annonaceae	<i>Graviola</i>
<i>Spondias mombin</i> L.	Anacardiaceae	<i>Taperebá</i>

2.2 NUTRITIONAL ANALYSIS

The physical parameters evaluated to determine the nutritional composition were the percentage of moisture and ash. The other nutritional parameters evaluated were the determination of total proteins, lipids and carbohydrates, to determine the total energy content.

2.2.1 Determination of Humidity

To determine moisture, 5 g of fresh samples were placed in porcelain capsules for 6 hours at 105°C to constant mass, and then cooled in desiccator to room temperature (IAL, 2008).

$$\text{Humidity (g/100 g)} = ((P' - P'')/(P' - P)) \cdot 100$$

being:

P = weight of porcelain capsule (g)

P' = weight of the porcelain capsule + fresh sample (g)

P'' = weight of the capsule + sample after the oven (g)

2.2.2 Determination of ashes

To determine the ash in the samples, the methodology proposed for the food analysis of (IAL, 2008) with modifications was used, where 5 grams of the lyophilized samples were weighed. These were placed in preheated porcelain crucibles in an oven at 110 °C for one hour, to remove moisture, and cool them in a desiccator to room temperature. The samples were incinerated at 600 °C in a FDG 3P-S EDG muffle for 16 hours, after which the samples were left in the desiccator until reaching room temperature.

$$\% \text{ ashes} = ((N \cdot 100)/M)$$

N = mass in grams of ash and M = mass of the sample in grams.

2.2.3 Determination of total proteins

Protein determination is performed from the total nitrogen analysis by Kjeldahl distillation, in which the existing organic matter is transformed into ammonia. The nitrogen content of the different proteins is approximately 16%, which introduces the empirical factor of 5.75 (conversion factor for vegetable protein), this will transform

the number of grams of nitrogen, found with the number of grams of protein (IAL, 2008).

$$\% \text{ proteins} = \% \text{ N} \cdot 5.75$$

2.2.4 Determination of lipids

To determine the total amount of lipids, 20 g of each sample was weighed, and placed in the Soxhlet extractor apparatus with hexane as the solvent for six hours. The solvent was recovered in a rotary evaporator (IAL, 2008).

$$\% \text{ lipids} = ((N \cdot 100) / m)$$

Where: N = mass in grams of lipids and M = mass of the sample in grams.

2.2.5 Determination of Carbohydrates.

The carbohydrate content is achieved by the difference of the value 100 subtracted from the sum of the already obtained values of moisture, ashes, lipids and proteins.

$$\text{Carbohydrates} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ lipids} + \% \text{ proteins})$$

2.2.6 Energetic value.

In order to quantify the energy value, it was necessary to use the protein (P), lipid (L) and carbohydrate (C) contents of each sample. The result should be expressed in Kcal 100 g⁻¹ (Mendes-Filho, Carvalho, Chiste & de Souza, 2014).

$$\text{Energy value (Kcal 100 g}^{-1}\text{)} = (P \cdot 4) + (L \cdot 9) + (C \cdot 4)$$

P = value of protein (%), L = lipid value (%), C = carbohydrate value (%), 4 = conversion factor in Kcal determined in calorimetric pump for proteins and carbohydrates and 9 = conversion factor in Kcal determined in a calorimetric pump for lipids.

2.3 MINERALOGICAL ANALYSIS

The extraction of the minerals into the seeds was done according to the methodology described by Embrapa (2009) in which the perchloric nitric digestion (3:1) was used in TECNAL model TE 0079 digester block, washed with distilled water up to 25 mL for subsequent analysis.

Calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and aluminum (Al) were determined by Flame Atomic Absorption Spectrophotometry (FAAS) Shimadzu AA-7000, coupled with ASC-7000 auto sample. Calibration was performed with standard solutions prepared from commercial standards of 1000 mg L⁻¹ Qhemis High Purity PACU 1000-0125, according to the specific conditions of each element (Table 2).

Table 2- Analytical Parameters of calibration.

Element	Technique	(λ) nm	Correlation coefficient (r ²)	LOD mg L ⁻¹	LOQ mg L ⁻¹
Ca	FAAS	422.70	0.999	0.481	2.004
Mg	FAAS	285.21	0.997	0.571	1.992
P	UV-Vis spectroscopy	660.00	0.999	0.113	1.773
K	AES	766.50	0.993	0.571	1.754
S	UV-Vis spectroscopy	420.00	0.998	0.074	0.897
Fe	FAAS	248.33	0.996	0.002	0,011
Zn	FAAS	213.80	0.991	0.002	0.071
Mn	FAAS	279.48	0.999	0.001	0.603
Cu	FAAS	324.75	0.997	0.003	0.010
Na	AES	589.0	0.999	0.098	1.103
Al	FAAS	309.3	0.998	0.0008	0.078
B	UV-Vis spectroscopy	420.00	0.999	0.089	0.123
Co	FAAS	240.73	0.997	0.0005	0.0008

FAAS = Flame Atomic Absorption Spectroscopy. AES = Flame Atomic emission Spectroscopy. LOD = detection limit. LOQ = Quantification limit.

As the ionization suppressor for the Ca, Mg and K elements, 0.1% of the lantane oxide solution (La₂O) was used. In the case of sodium (Na), it was determined in the same equipment, but in atomic emission mode. As for potassium (K), it was determined by means of flame photometry on the Digimed Flame Photometer DH-62, calibrated using a Digimed standard solution whose concentration range was 2-100 mg L⁻¹.

For the determination of the phosphorus (P), boron (B) and sulfur (S) elements, the ultraviolet molecular absorption spectrophotometry technique was

used using a SHIMADZU UV-1800 model. The determination of P was carried by formation of the blue complex with ammonium molybdate ((NH₄)₂MoO₄) the readings were made at λ = 660 nm. The determination of boron was carried out by the formation of a yellow complex with azomethine-H, the readings being made at 460 nm. The determination of S was performed by forming a precipitate with BaCl₂, the readings being made in Uv-visivel at λ = 420 nm by calibration with potassium sulphate according with Embrapa (2009).

Nitrogen determination was carried out by the distillation method followed by titration (Kjeldahl) according with described methodology with Embrapa (2009).

2.4 COMPOSITION OF FATTY ACIDS BY GC-FID

An aliquot (10 mg) of samples was transferred to a 2 mL cryotube, which contained 100 μ L of a mixture made of ethanol (95%) and KOH 1 mol L⁻¹ (5%). After vortexing for 10 s, esters in the oil were hydrolyzed in a microwave oven (Panasonic Piccolo) at 80 W (power 2) for 5 min. After cooling and neutralization with 400 μ L of hydrochloric acid 20%, 20 mg NaCl and 600 μ L of ethyl acetate were added. Afterwards, free fatty acids were obtained by using an adapted protocol of the one reported by Christie (1989) with adaptation. After vortexing for 10 s and rest for 5 min, aliquots (300 μ L) of the ethyl acetate layer were taken, placed in microcentrifuge tubes and dried by evaporation. Free fatty acids were methylated using 100 μ L of BF₃/methanol (14%) and the reaction mixture was heated for 10 min in a water-bath at 60 °C. After dilution with 400 μ L methanol, fatty acid methyl esters were analyzed by Gas Chromatography.

Free fatty acids profile was resolved by Gas Chromatography using HP7820A (Agilent) system equipped with flame ionization detector. An Innowax column (HP) 15 m $\times$ 0.25 mm $\times$ 0.20 μ m was used and the following temperature gradient: 100 °C min and 0.7 °C min⁻¹ up to 240 °C; injector (1/30 split) to 250 and 260 °C detector. Hydrogen was used as carrier gas (3 mL min⁻¹) and injection volume was 1 μ L. The data acquisition program used was EZChrom Elite Compact (Agilent). The peaks were identified using FAME Mix C₁₄-C₂₂, CRM18917 Supelco fatty acid methyl esters standard.

2.5 STATISTICAL ANALYSIS

Correlations between the amounts of the different minerals in the seeds of the fruit were evaluated using the Pearson statistical test using INFOSTAT (Rienzo, Casanovas, Balzarini, González, Tablado & Robleda, 2016) for significance levels of 5%, 1% and 0.1% respectively, as well as the principal component analyzes (PCA) and Hierarchical component analysis (HCA).

3 RESULTS AND DISCUSSION

3.1 NUTRITIONAL ANALYSIS FROM SEEDS OF AMAZON FRUITS

Table 3 presents the nutritional analysis values for the seeds of the different Amazonian fruits studied.

The first parameter analyzed is moisture, which according to Welti & Vergara, (1997), the moisture content is used as a factor indicative of propensity for food spoilage, and may think that the greater stability of the food is in the control of the minimum humidity. The determination of humidity is important, since the amount of water exerts a pronounced influence on the physical and chemical properties of the seeds, being important its determination in all stages of the process of seeds technology, from its handling to its processing and storage (Carvalho, 2005). In this work, the seeds have low moisture values, all of them less than 50%, with the lowest value of the *camu-camu* seed with 8.21% and the one with the highest value is the *bacuparí* with 41.77%. The variation in the concentrations of the different elements may vary according to fruit maturity, geographical origin, seasonal variation and processing conditions.

The seeds have high energy content, the values are between 252.68 Kcal 100 g⁻¹ for *bacuparí* seeds and 369.08 Kcal 100 g⁻¹ for *camu-camu* seeds. For the case of *acerola*, the energy value determined in this work is close to that found by Aguiar, Rodrigues, Santos & Sabaa-Srur (2010) with 332.0 Kcal 100 g⁻¹.

The lipids in the seeds vary from 0.02% for the *taperebá* seeds to 21.03% obtained for the *fruta-do-conde* seeds. Compared with other works, such as *abiu* seeds, for this fruit the value obtained is lower than that determined by de Melo Filho, da Costa, Montero, dos Santos, Chagas, Chagas, Takahashi & Ferraz (2018) which obtains a yield of extraction of 14.01%, as it happens for the *camu camu* seeds,

being the yield of 0.84% compared to the value obtained of 2.98% (de Melo Filho, dos Santos, Chagas, E., Chagas, P., Montero & Sousa, 2018).

Proteins are macromolecules of great importance in living cells and constituted from amino acids. They present diverse biological functions and at the same time they are the molecular instruments by means of which the genetic information is expressed (Nelson & Cox, 2002). The protein values in this work are relatively low with 0.04% for the *taperebá* seeds, with the highest value being the *fruta-do-conde* seeds with 7.32%. In other Amazonian fruits, the values of proteins determined by Souza, Pereira Filho & Oishi, (2009) were of 6.1% in the *araçá* slightly higher than those found in this work, for the *acerola* residue determined 5%, very close to the value of 5.41% found in this work, for the *graviola* residue determined 6.3%, slightly lower than value determined in this work of 7.29% and for *camu-camu* seeds determined 6.3%, slightly higher than found in this work of 7.29%.

Of the nutritional parameters, it is the carbohydrates that contribute a greater energetic percentage to the fruit seeds analyzed. Through the reaction of photosynthesis, there is the conversion of solar energy into chemical energy where it is assimilated from carbon in organic compounds, mainly carbohydrates, which are used in turn in the synthesis of other organic compounds such as amino acids and lipids (Geigenberger, Kolbe & Tiessen, 2005). The determined values of carbohydrates in this work, are between 0.81% for the *araçá* seeds reaching up to 84.41% for the *camu-camu* seeds. For *acerola* seeds, the value determined in this work is 78.13% higher than that determined by Aguilar, Rodrigues, Santos & Sabaa-Srur (2010).

Finally, the last parameter analyzed as a bromatological component, are the ashes, where after subjecting the seeds to a calcination process, in the ashes remains the inorganic fraction in which the minerals are found being ash values that vary between 0.21% for the *bacuparí* seeds up to 2.31% for the *araçá* seeds. In studies undertaken by Geigenberger, Kolbe & Tiessen (2005), the amount of ash for the *acerola* residue is 1.9% slightly higher than this work and for the *araçá* seeds 1.6% slightly lower than the one found in this work. The same authors determined the amount of ash in *graviola* residues with 1.6% ash, slightly higher than the one determined in this work with 0.95% swimming in this work with 0.95%.

Table 3-Nutritional composition in Amazon fruit seeds.

FRUIT	NUTRITIONAL CONTRIBUTION					
	MISTURE	ASH	LIPIDS	CARBOHYDRATES	PROTEINS	ENERGETIC VALUE
			%			Kcal 100 g ⁻¹
Abiu	38.53 ± 0,04	0.27 ± 0.04	4.89 ± 0.11	53.20 ± 0.01	3.11 ± 0.01	269.25 ± 0.02
Acerola	15.34 ± 0.07	0.41 ± 0.12	0.75 ± 0.12	78.13 ± 0.03	5.41 ± 0.03	340.55 ± 0.02
Araçá	9.31 ± 0.01	2.31 ± 0.04	0.74 ± 0.12	0.81 ± 0.04	1.24 ± 0.02	20.86 ± 0.02
Bacuparí	41.77 ± 0.09	0.21 ± 0.06	4.12 ± 0.12	51.52 ± 0.01	2.38 ± 0.01	252.68 ± 0.02
Biribá	31.11 ± 0.04	1.52 ± 0.07	19.06 ± 0.12	41.07 ± 0.04	7.24 ± 0.02	364.78 ± 0.05
Camu-camu	8.21 ± 0.03	0.57 ± 0.07	0.84 ± 0.02	84.41 ± 0.03	5.97 ± 0.08	369.08 ± 0.02
Fruta-do-conde	38.11 ± 0.09	0.92 ± 0.04	21.05 ± 0.03	32.60 ± 0.01	7.32 ± 0.01	349.13 ± 0.04
Graviola	38.29 ± 0.14	0.95 ± 0.13	20.62 ± 0.17	32.85 ± 0.01	7.29 ± 0.02	346.14 ± 0.02
Taperebá	32.12 ± 0.23	1.34 ± 0.05	0.02 ± 0.00	66.48 ± 0.01	0.04 ± 0.00	266.26 ± 0.08

Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95%.

3.2 MINERAL ANALYSIS

In the Table 2 and the Table 3, the values of macronutrients and micronutrients are presented for the different seeds studied.

The levels of calcium in the seeds fruits studied vary between 12.42 mg 100 g⁻¹ for *abiu* seeds, arriving to find high concentrations in fruits of the family of the Anonaceae as it is the case of the *fruta-do-conde* seeds with value of 74.41 mg 100g⁻¹ and 63.13 mg 100 g⁻¹ for the *biribá* seeds. The calcium nutritional intake for adults according to Pereira, Genero, Pinheiro, Szenjfeld & Martini (2009) is between 1000-1200 mg dia⁻¹ being therefore the calcium levels found in these significant seeds to incorporate it in the development of new products since calcium is an element of utmost importance for participating in various physiological processes in the body as is its role in the chemical synapse, muscle contraction processes, blood coagulation and nerve impulse transmission (Guéguen & Pointillart, 2000; Henry, 2006).

Related to the absorption of calcium is or phosphorus, since according to Douglas, (2002) and Hossain & Yoshimatsu (2014). The absorption of both elements is optimal when the relationship between both elements is equal to unity. In the fruits studied in this work the values of this relationship are the following: *abiu*: 0.39; *acerola*: 1.00; *araçá*: 0.35; *bacupari*: 0.41; *biribá*: 0.99; *camu-camu*: 0.39; *fruta-de-conde*: 3.23; *graviola*: 2.25 and *taperebá*: 1.95; it can be observed that of all of them who presents exactly the value of the unit is the *acerola* and next to the unit is the *biribá* seeds, being therefore the two seeds that facilitate the greater absorption of these elements. According with Tomassi (2002), phosphorus levels range between 20-100 mg 100 g⁻¹, with the highest phosphorus values found for *biribá* with 64.02 mg 100 g⁻¹, with the lowest value for *graviola* seeds being 21.03 mg 100 g⁻¹, being these values within the normal values and at the same time, the recommended phosphorus levels per day are 800 mg dia⁻¹ (Tomassi, 2002).

Another element related to physiological activities in the human body is magnesium, whose main function is to stabilize the structure of ATP and as a cofactor in enzymatic reactions acting on neuromuscular transmission (Iseri & French, 1984) and as an activator in the reactions of the dark phase of photosynthesis (Malavolta, 2006). In this work, the concentrations of magnesium in the *biribá* seeds with 123.11 mg 100 g⁻¹ are surprisingly high, being the lowest value for the *abiu* seeds with 7.21 mg 100 g⁻¹ being the recommendations of this element of

310-320 mg dia⁻¹ for women and 410-420 mg dia⁻¹ for men (Yuyama, 1992). For the *acerola* seeds in this work were certain concentrations in the seeds of 22.04 mg 100 g⁻¹, being a value similar to that determined by Aguiar, Rodrigues, Santos & Salaa-Shur (2010) who find a magnesium value in the *acerola* seed of 22.24 mg 100 g⁻¹.

Potassium is another of the most abundant elements in the body, being an electrolyte that contributes in the body with cellular contraction, as well as together with sodium in the sodium/potassium pump in the nervous impulse (Berne, 2000) and on the other hand, it participates in the metabolism of carbohydrates, proteins (Czajka-Narins, 1998). In the fruits studied, high potassium values were found, with the lowest values found for *taperebá* with 11.34 mg 100 g⁻¹ and the highest for *graviola* with 554.23 mg 100 g⁻¹. Other studies only that for the case of the *graviola* seeds find high levels of potassium with 523 mg 100 g⁻¹ (Leterme, Buldgen, Estrada & Londoño, 2006).

Sulfur is classified as macronutrient, but it is required in low concentrations, being an element whose main function is as part of the amino acids cysteine and methionine, as well as enzymatic activator (Silva, Pereira, do Carmo, de Albuquerque, Van Raji & Silva, 2004). Of the fruits studied, the highest sulfur levels are found for *acerola* seed with 41.22 mg 100 g⁻¹ and the lowest value for *taperebá* seeds with 1.12 mg 100 g⁻¹.

Finally, among the macro-constituents, there is nitrogen, an element of great biological importance because it is part of proteins, with coenzyme function, nucleic acids and vitamins, as well as being part of processes of photosynthesis and cellular respiration (Malavolta, 2006). Generally, it is not studied in an isolated way in fruits, but it is studied associated to the protein form. In this work, the highest protein values were detected in the *biribá* seeds with 6.44% and in *taperebá* seeds, they were determined in trace concentrations.

Table 4-Macronutrients analyzed in seeds fruit in the northern Amazon.

Fruit	Macronutrients					
	Calcium (Ca)	Magnesium (Mg)	Phosphorous (P)	Potassium (K)	Sulfur (S)	Nitrogen (N)
	mg 100 g ⁻¹					
Abiu (<i>Pouteria caimito</i>)	12.42± 0.14	7.21± 0.11	31.45± 0.14	324.56± 0.14	23.14 ± 0.14	0.54 ± 0.01
Acerola (<i>Malpighia emarginata</i>)	31.48 ± 0.11	22.04 ± 0.12	31.34 ± 0.04	164.11 ± 0.04	41.22 ± 0.18	0.94 ± 0.01
Araçá (<i>Psidium cattleianum</i>)	17.21 ± 0.04	17.11 ± 0.03	49.32 ± 0.04	361.24 ± 0.02	12.03 ± 0.02	0.04 ± 0.01
Bacuparí (<i>Rheedia gardneriana</i> Planch & Triana)	17.24 ± 0.04	21.11± 0.01	42.11± 0.12	378.02 ± 0.11	1.17± 0.01	0.41 ± 0.01
Biribá (<i>Rollinia mucosa</i>)	63.13 ± 0.09	123.11± 0.21	64.02 ± 0.02	513.33 ± 0.09	32.11 ± 0.08	6.44 ± 0.01
Camu-camu (<i>Myrciaria dúbia</i> (Kunth))	18.8 ± 0.07	9.21± 0.09	47.86± 0.12	338.45 ± 0.11	15.11 ± 0.02	1.26 ± 0.02
Fruta-do-conde (<i>Annona squamosa</i>)	72.41 ±0.04	37.21± 0.02	22.41 ± 0.16	421.23 ± 0.13	34.11 ± 0.08	1.27 ± 0.01
Graviola (<i>Annona muricata</i>)	47.32 ± 0.12	32.41 ± 0.17	21.03 ± 0.19	554.23 ± 0.14	38.11± 0.08	1.27 ± 0.02
Taperebá (<i>Spondias mombin</i> L.)	57.24 ± 0.01	33.11 ± 0.02	29.22± 0.03	11.34 ± 0.05	1.12 ± 0.03	6.9.10-3 ± 0.00

Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95 %.

Table 5- Micronutrients analyzed in seeds fruits in the Northern Amazon.

FRUIT	MICRONUTRIENTS							
	Iron (Fe)	Zinc (Zn)	Manganese (Mn)	Copper (Cu)	Sodium (Na)	Aluminum (Al)	Boron (B)	Cobalt (Co)
	mg 100 g ⁻¹							
Abiu (<i>Pouteria caimito</i>)	0.14 ± 0.08	4.14 ± 0.08	4.12 ± 0.05	2,07 ± 0,05	0.41 ± 0.03	0.04 ± 0.00	0.17 ± 0.05	N.D.
Acerola (<i>Malpighia emarginata</i>)	0.63 ± 0.04	0.14 ± 0.06	0.65 ± 0.09	1.58 ± 0.02	22.03 ± 0.11	0.05 ± 0.01	0.74 ± 0.03	N.D.
Araçá (<i>Psidium cattleianum</i>)	3.78 ± 0.01	0.74 ± 0.01	0.94 ± 0.02	1.04 ± 0.01	16.98 ± 0.01	0.12± 0.04	0.11 ± 0.03	N.D.
Bacuparí (<i>Rheedia gardneriana</i> Planch & Triana)	0.84 ± 0.02	2.62 ± 0.07	0.42 ± 0.03	0.07 ± 0.02	5.98 ± 0.14	0.13 ± 0.02	0.57 ± 0.09	0.024 ± 0.004
Biribá (<i>Rollinia mucosa</i>)	2.92 ± 0.11	0.87 ± 0.08	0.74 ± 0.04	1.71 ± 0.06	12.24 ± 0.06	0.12 ± 0,06	0.18 ± 0.04	0.009 ± 0.001
Camu-camu (<i>Myrciaria dúbia</i> (Kunth))	0.79 ± 0,07	0.22 ± 0.03	0.17 ± 0.03	1.12 ± 0.04	17.04 ± 0.18	0.39 ± 0.07	0.12 ± 0.08	0.077 ± 0.001
Fruta-do-conde (<i>Annona squamosa</i>)	1.76 ± 0.06	0,85 ± 0.08	0.88 ± 0.07	0.16 ± 0.02	7.54 ± 0.12	0.03 ± 0.00	0.06 ± 0.02	N.D.
Graviola (<i>Annona muricata</i>)	1.30 ± 0.06	2.37 ± 0.08	0.91 ± 0.07	0.09 ± 0.01	5.84 ± 0.24	0.05 ± 0.01	0.08 ± 0.01	0.026 ± 0,004
Taperebá (<i>Spondias mombin</i> L.)	1.33 ± 0.02	1.37 ± 0.04	0.73 ± 0,03	1.52 ± 0.02	6.11 ± 0.06	0.31 ± 0.08	N.D.	N.D.

N.D. not detected. Analyzes performed in triplicate and using as a standard deviation the value of the t-student for 95 %

Among the micronutrients, iron is very important in the human diet, because its deficiency can cause anemia, fatigue and impairment in neurological growth and development (Carvalho, 2006). According to the World Health Organization (WHO), the required iron dose per adult person and day is 20-45 mg. The highest values of iron presented in this work are for *araçá* with concentrations of 3.78 mg.100 g⁻¹, finding the lowest concentration of iron for *abiu* seeds with only 0.14 mg.100 g⁻¹.

Given that in Brazil, anemia mainly in children, is a worrisome problem that affects their development, these fruits would be an important source of iron for the development of food supplements to correct those deficiencies in children. As for zinc, it is important in the organism at the physiological level as an antioxidant (Powell, 2000), as well as developing a fundamental role in the polymer organization of macromolecules such as DNA and RNA, as well as their synthesis (Vallee & Falchuk, 1993). According to Food and Nutrition Board (2001), the zinc recommendations for the population are 8 mg day⁻¹ for women and 11 mg day⁻¹ for men. In this work, the highest zinc concentration is in the *abiu* seeds with 4.14 mg.100 g⁻¹ and the lowest value for the *acerola* seeds with 0.14 mg.100 g⁻¹. In a work developed by Aguiar, Rodrigues, Santos & Salaa-Shur (2010) on *acerola* seeds, determine zinc values of 0.09 mg 100 g⁻¹.

Other important microelement in enzymatic metabolic reactions is manganese which, according to Panziera, Dorneles, Durgante & da Silva (2011), is part of two metalloenzymes, carboxylase pyruvate and Mn-superoxide dismutase. In this work, the concentrations of manganese found in the seeds vary between 0.17 mg 100 g⁻¹ for the *camu-camu* seeds, up to 4.12 mg.100 g⁻¹ for the *abiu* seeds, being in the *abiu* the values of Mn very close with the values of Zn. Copper is a trace element that may exhibit various oxidation states and within the cell predominates the cuprous ion (Bairele, Valentini, Paniz, Moro, Junior & Garcia, 2010). For the seeds studied in this paper, the copper values are very low, being the lowest value for the *bacuparí* seeds with 0.07 mg 100 g⁻¹ up to 2.07 mg.100 g⁻¹ for the *abiu* seeds. The need for copper is 1-2 mg dia⁻¹, and 10 mg dia⁻¹ is tolerated according to (DRIs) (Dietary Reference Intakes, 2004) for the maintenance of the human organism, the above fruits being above tolerable levels for the organism (Almeida, de Souza, Fonseca, Magalhães, Lópes & de Lemos 2009).

Sodium is an important element in the organism since it works as a regulator of osmotic pressure and prevents dehydration since it acts in the maintenance of

cellular permeability. According with Food and Nutrition Board, Institute of Medicine (2005) sodium recommendations in the human diet are 1.2 - 1.5 g day⁻¹. The sodium levels in the fruits studied, vary in a wide range, from 0.41 mg 100 g⁻¹ for the *abiu* seeds to 22.03 mg 100 g⁻¹ for the *acerola* seeds. An important trace element is boron, being related to the cerebral metabolism (Penland, 1994), among other functions. In the case of fruits, boron has an important function of stimulating the germination and generation of pollen and pollen tube growth, being a fundamental factor for the adequate formation of fruits (SangHyun L., WolSoo, K. & TaeHo H., 2009). The highest concentration of boron in the seeds studied is in *acerola* seeds with concentration of 0.74 mg 100 g⁻¹, being in excessively low concentrations in the *frutadoconde* 0.06 mg 100 g⁻¹ not being detected in the *taperebá* seeds.

The aluminum is a toxic metal, whose concentration in food is low, of the order of 5 mg kg⁻¹ (Dantas, Saron, Dantas, Yamashita & Kiyataka, 2007). The consumption of foods contaminated by this metal may be related to Alzheimer's disease (Martyn, Coggan, Inskip, Laeey & Young, 1997). Thus, the seeds of fruits analyzed had relatively low concentrations, varying between 0.04 - 0.36 mg 100 g⁻¹ being within the recommended levels.

Among all the evaluated minor elements, cobalt is the lowest concentration in relation to the microconstituents, The aluminum is a toxic metal, whose concentration in food is low, of the order of 5 mg kg⁻¹. According to Vaitsman, Alonso & Dutra (2001), the estimated cobalt doses are between 0.5-1.4 mg dia⁻¹, therefore, the levels found in the fruits studied would be below the recommended levels.

3.3 FATTY ACIDS IN OILS AND FATS FROM AMAZONIAN FRUIT SEEDS

The information provided by the chromatograms (Appendix B), of crude oils and fats of the Amazon fruit seeds were placed in Table 6, in this way it is possible to verify the saturated fatty acids (SFA) and unsaturated fatty acids (UFA) present in the samples.

Of the fatty acids analyzed, the concentration of (SFA) is lower than the concentration of (UFA) with the exception of the seeds of *taperebá* that contains a higher percentage of (SFA) (74.60%) than (UFA), therefore, of the seeds studied in this work is the only solid state at room temperature.

Among the saturated fatty acids identified in the oils and fats presented in Table 6, the majority is palmitic acid, being in the range of 8.1% for *araçá* seeds up to

40.4% in the *taperebá* seed. After palmitic acid the following in abundance is stearic acid, being in concentrations of 3.2% for the *araçá* reaching up to 30.7% for the *taperebá*. The SFA's identified in this work are considered long chain fatty acids, and for them to be metabolized, undergo a process of esterification, forming the triglycerides, where they are taken to the heart and transported by the bloodstream, by the chylomicrons, being stored as fat in the organism (Santos, Marmesat, Brito, Alves & Dobarganes, 2013). Within the SFA (Lottenberg, 2009) points out that the ingestion of palmitic and myristic acid, causes increase of cholesterol levels in the blood, but the other major SFA, stearic acid does not promote hypercholesterolemin due to it being converted in the liver to oleic acid.

The minority SFA's detected are the myristic whose concentrations vary between 0.1% for *araçá*, *camu-camu* and *fruta-do-conde* up to 0.6% for the seeds of the *taperebá* and araquidic acid presents concentrations of 0.3% for the *araçá* seeds arriving up to 2.9% for the *taperebá* seeds. This fatty acid in animal tissues is found in low concentrations, less than 1% but its concentration increases in milk being between 7-12% and in palm oil it is between 15-23%, being an acid that increases in the plasma concentrations of low-density LDL proteins (Kromhout, Bioemberg, Feskens, Menotti & Nissinen, 2000).

Table 6-Composition of fatty acids in oil seeds fruits in the Northern Amazon.

Fatty acids (%)	TR (min)	Abiu	Acerola	Araçá	Bacuparí	Biribá	Camu-camu	Fruta-do-conde	Graviola	Taperebá
C14:0 (myristic)	4.10	0.2	0.4	0.1	0.3	0.2	0.1	0.1	0.4	0.6
C16:0 (palmitic)	5.65	33.8	21.4	8.1	35.3	24.9	12.3	15.8	19.0	40.4
C16:1 (palmitoleic)	5.87	0.4	1.8	0.9	1.7	0.7	0.1	0.5	1.5	1.5
C18:0 (stearic)	7.26	5.4	11.1	3.2	7.5	6.0	7.9	11.7	4.3	30.7
C18:1 (oleic (ω -9))	7.46	45.0	33.7	14.3	47.4	43.1	11.6	46.9	41.9	19.2
C18:2 (linoleic (ω -6))	7.84	10.9	27.1	69.0	4.4	21.8	63.7	22.2	28.2	2.2
C18:3 (linolenic (ω -3))	8.19	0.7	2.2	2.6	0.8	0.6	0.7	0.8	1.5	0.1
C20:0 (arquidic)	8.82	0.8	1.1	0.3	0.4	0.4	0.9	0.8	0.4	2.9
Others		2.8	1.2	1.5	2.2	2.3	2.7	1.2	2.8	2.4
Σ Saturated fatty acids (SFA)		40.2	34.00	11.70	43.50	31.50	21.2	28.40	24.10	74.60
Σ Unsaturated Fatty Acids (UFA)		57.0	64.80	86.80	54.30	66.20	76.10	70.40	73.10	23.00
Σ Monounsaturated Fatty Acids (MFA)		45.4	35.50	15.20	49.10	43.80	11.70	47.40	43.40	20.70
Σ Polyunsaturated Fatty Acids (PUFA's)		11.60	29.30	71.60	5.20	22.40	64.40	23.00	29.70	2.30
Ratio ω -6/ ω -3		15.57	12.32	26.54	5.50	36.33	91.00	27.75	18.80	22.00

The percentage of saturated oils and fats found in this work are compared in Table 7, with the oils of the same species and with other vegetable oils. Only the *camu-camu*, *bacupari*, *araçá*, and *taperebá* species were not compared, since no such information was found for the seeds.

Table 7- Comparison of the concentration of SFA in oils and fats.

Sample	Fatty acids (%)			
	C14:0	C16:0	C18:0	C20:0
Acerola ^{a,b}	-	21.80-23.70	2.27-13.90	-
Biribá ^c	35.24	15.83	23.75	6.16
Abiu ^d	0.80	27.30	6.20	-
Graviola ^{e,f}	-	19.31-25.5	4.56-6.00	0.50
Fruta-do-conde. ^{g,h}	0.70	12.10-15.20	9.30-13.60	0.90-1.50
Olive oil ⁱ	-	11.20-20.70	1.68-4.30	0.00-0.83
Soy oil ^j	0.065	9.70	2.02	-

a) (da Cunha, Freitas, Godoy, Cabral & Tonon, 2017). b) (Aguiar, Rodrigues, Santos & Sabaa-Srur, 2010). c) (Berto, da Silva, Visentainer & Sousa, 2015). d) (de Melo Filho, da Costa, Fernández, dos Santos, Chagas, Chagas, Takahashi & Ferraz, 2018). e) (Pinto, de Souza, de Souza, da Silva, da Silva, Cerqueira-Lima, da Silva, Medeiros, Bittencourt, Bradão, Druzian, Conceição, Lopes & Figueireiro, 2018). f) (Solis-Fuentes, Amador-Hernández, Hernández-Medel & Durán-de-Bazua, M.C, 2010). g) (Rana, 2014). h) (Mariod, Elkheir, Ahmed & Martha, 2010). i) (Zarrouk, Baccouri, Taamalli, Trigui, Daouda & Zarrouka, 2009). j) (Sultan, Dikshit & Vaidya, 2015).

The concentration of myristic acid present in the *abiu* and the *fruta-do-conde* count are lower than those determined in the literature but higher than the amount present in soy oil. On the other hand, for the palmitic and myristic acids, for the seeds of *acerola*, *abiu*, *graviola* and *fruta-do-conde*, the determined values are close to the compounds with the same species (Table 7), the values for the acid being palmitic within the range in which said acid is found in olive oil, but for stearic acid are higher than those found for olive oil. On the other hand, for the palmitic and myristic acid, for the seeds of *acerola*, *abiu*, *graviola* and *fruta-do-conde*, the determined values are

close to the compounds with the same species (Table 7), the values for the acid being palmitic within the range in which said acid is found in olive oil, but for steric acid are higher than those found for olive oil.

The unsaturated fatty acids determined in this work were palmitoleic acid, oleic acid (ω -9), linoleic acid (ω -6) and linolenic acid (ω -3), (Table 6). The concentration of unsaturated fatty acids in the studied seeds varies between 23.0% for *taperebá* and 86.80% for *araçá*. With the exception of tapereba seed, the concentrations of unsaturated fatty acids are much higher than the concentrations of saturated fatty acids. The benefits of unsaturated fatty acids are known as protectors and as a risk reducer of different diseases. Linolenic acid, reduces the risk of cancer and heart disease, in addition to being anti-inflammatory, antithrombotic, anti-arrhythmic and with vasodilating properties, on the other hand linoleic acid when deficient in a diet can cause skin diseases such as squamous dermatitis, or bad healing in a wound, so a balanced relationship of both acids are important to prevent chronic diseases, the levels of these two acids in the body being around 1-4:1 (Fagundes, 2002; Göğüs & Chris, 2010; Gómez-Candela, López & Kohen, 2011) but according to criteria of other organizations such as the World Health Organization or the Food and Agriculture Organization, they establish values of relation between acids ω 6/ ω 3 of 5:1-10:1 (WHO, 1995).

Of the fatty acids analyzed in this work, the lowest concentration found is palmitoleic acid whose concentrations vary from 0.1% for the oil of the *camu-camu* seeds to 1.7% for the *bacuparí* seeds. This acid can be found in its two isomeric forms, the *cis* form being associated with insulin sensitivity and the *trans* form is found in dairy products and hydrogenated oils associated with lower incidence of diabetes (Cao, Mayers, Wiest, Watkins & Hotamisligil, 2008; Ouchi, Parker, Lugus & Walsh, 2011). This acid is produced in liponeogenesis in humans synthesized mainly in the liver and later incorporated into adipose tissue to become part of the phospholipids, triacylglycerols, waxes and cholesterol esters (Frigolet & Gutierrez-Aguilar, 2017). This acid is found in concentrations higher than those determined in this work, in fish oils such as salmon (6%), cod liver (7%) and macadamia oil (17%) and in vegetables in sea buckthorn oil, plant that develops in Asia and Europe, reaching concentrations of up to 32-42% (Maguire, O'Sullivan, Galvin, O'Connor & O'Brien, 2004; Fatima, Snyder, Schroeder, Cram, Datlha, Wishart, Weselake & Krishna, 2012).

Oleic acid is the major unsaturated fatty acid with the exception of the *camu-camu* and *araçá* seeds, where the majority is linoleic. The concentrations of this acid vary between 11.6% for the *camu-camu* seed, being the highest concentrations in the *bacupari* seeds with 47.4%, but in spite of being the most abundant their levels in these seeds are below the concentrations of oleic acids in olive oil whose values are between 70-80% (Owen, Mier, Giacosa, Spiegelhalder & Bartsch, 2000). The daily consumption of this acid is important since it contributes to reduce the risk of suffering from cardiovascular diseases such as the reduction of blood pressure, decrease in cholesterol levels and arteriosclerosis (Ferrara, Raimondi, d'Episcopo, Guida, Dello-Russo & Marotta, 2000; Owen, Mier, Giacosa, Spiegelhalder, Bartsch & 2000; Panagiotakos, Dimakopoulou, Katsouyanni, Bellander, Grau, Koenig, Lanki, Pistelli, Schneider & Peters, 2009) and on the other hand, it is shown to have a protective effect against breast cancer and strengthens the immune system (Assmann, de Backer, Bagnara, Betteridge, Crepaldi, Fernandez-Cruz, Godtfredsen, Jacotot, Paoletti, Renaud, Ricci, Rocha, Trautwein, Urbinati, Varela & Williams, 1997; Simonsen, Fernandez-Crehuet, Martin-Moreno, Strain, Huttunen, Martin, Thamm, Kardinaal, Van't Veer, Kok & Kohlmeier, 1998). Therefore, due to these properties, it is important to consume it in the diet.

Or unsaturated acid ω -6, and two other important unsaturated fatty acids in the diet. Seeds studied to fruit that apresentou or lower percentage was to *taperebá* seeds with barely 2.2%, presenting the highest values the *camu-camu* seeds with 63.7% and *araçá* seeds com 69%, both seeds of the same family *Myrtaceae*. This fatty acids together with the ω -3, is considered essential, since the organism does not synthesize them and they have to be incorporated into the diet. Linoleic acid, like oleic acid, has positive effects on cardiovascular risk factors, reducing the levels of triacylglycerol and plasma cholesterol, improving insulin sensitivity (Lee, Kritchevsky & Pariza, 1994). Finally, linolenic acid has low concentrations, whose values vary between 0.1% for *taperebá* seeds and 2.6% for *araçá*. The linolenic acid, in addition to serving to prevent cardiovascular diseases, is involved with the best development of visual quality (Wu, Zhou, Ma, Yuan & Peng, 2015).

Oil studied can be compared (Table 8) with the same of their species, in local oils and commercial oils, as well as unsaturated fatty acids (palimoleic and omega 3, 6 and 9). As soon as you know *camu-camu*, *bacupari*, *araçá* and *taperebá* were not compared, for they were not found for these seeds.

Table 8-Comparison of the concentration of UFA in oils and fats.

Sample	Fatty acids (%)			
	C16:1	C18:1	C18:2	C18:3
Acerola ^{a,b}	1.29	31.9-47.64	24.15-29.2	0.90-1.30
Biribá ^c	0.0057	58.6	4.4	0.19
Abiu ^d	-	43.1	8.6	0.4
Graviola ^{e,f}	1.50 – 1.57	39.21-39.50	27.10-32.99	1.24
Fruta-do-conde. ^{g,h}	Trace	47.4-49.2	22.3-22.9	-
Olive oil ⁱ	1.29	31.9-47.64	24.15-29.2	0.90-1.30
Soy oil ^j	0.0057	58.6	4.4	0.19

a) (da Cunha, Freitas, Godoy, Cabral & Tonon, 2017). b) (Aguiar, Rodrigues, Santos & Sabaa-Srur, 2010). c) (Berto, da Silva, Visentainer & Sousa, 2015). d) (de Melo Filho, da Costa, Fernández, dos Santos, Chagas, Chagas, Takahashi & Ferraz, 2018). e) (Pinto, de Souza, de Souza, da Silva, da Silva, Cerqueira-Lima, da Silva, Medeiros, Bittencourt, Bradão, Druzian, Conceição, Lopes & Figueireiro, 2018). f) (Solis-Fuentes, Amador-Hernández, Hernández-Medel & Durán-de-Bazua, M.C, 2010). g) (Rana, 2014). h) (Mariod, Elkheir, Ahmed & Martha, 2010). i) (Zarrouk, Baccouri, Taamalli, Trigui, Daouda & Zarrouka, 2009). j) (Sultan, Dikshit & Vaidya, 2015).

The concentration of palmitoleic acid present in *acerola* and *graviola* present great proximity compared to the same species in Table 8. *Fruta-do-conde* and *abiu* detected only concentrations of trace of palmitoleic. For *acerola* and *graviola* the levels of palmitoleic acid found are within the range where this acid is found in olive oil.

The concentration of omega-9 present in the seeds of *acerola*, *biribá*, *abiu*, *graviola* and *fruta-do-conde* compared to the same species (Table 8), present a great proximity, being the omega 9 acid concentration higher than in the soy oil, and for the *fruta-do-conde* and *abiu*, are close to the concentration determined in olive oil.

On the other hand, the omega 6 fatty acid, the values of this acid in the seeds of *fruta-do-conde*, *acerola*, *abiu* and *graviola*, are close to the seeds of the same

species (Table 8) and inferior to the concentration of said acid in soy seeds, but are in the same range as that found in olive oil. Finally, for the omega 3 fatty acid present in *acerola*, *abiu* and *graviola* are close to those that present the same species (Table 8) being much lower than the value of said acid in soy oil and close to those found in the olive oil.

3.4 STATISTIC ANALYSIS

Pearson correlation coefficient.

Table 9 presents the Pearson correlation matrix between the different elements for the seeds of the different fruits.

Table 9-Pearson correlation matrix between the different elements for the seeds of Amazon fruits.

	Ca	Mg	P	K	S	N	Fe	Zn	Mn	Cu	Na	Al	B	Co
Ca	1													
Mg	0.62ns	1												
P	0.04ns	0.34ns	1											
K	0.05ns	0.36ns	-0.24ns	1										
S	0.27ns	0.27ns	-0.30ns	0.50ns	1									
N	0.40ns	0.93**	0.33ns	0.53ns	0.40ns	1								
Fe	0.61ns	0.56ns	0.79*	-0.12ns	-0.05ns	0.40ns	1							
Zn	-0.38ns	-0.28ns	-0.02ns	0.08ns	-0.26ns	-0.30ns	-0.12ns	1						
Mn	-0.32ns	-0.23ns	-0.10ns	-0.02ns	0.08ns	-0.16ns	-0.23ns	0.76*	1					
Cu	-0.01ns	0.07ns	0.76*	-0.61ns	-0.18ns	0.03ns	0.57ns	0.14ns	0.33ns	1				
Na	0.02ns	0.09ns	0.43ns	-0.29ns	0.24ns	0.18ns	0.28ns	-0.75*	-0.55ns	0.34ns	1			
Al	-0.15ns	-0.13ns	0.22ns	-0.37ns	-0.63ns	-0.11ns	-0.02ns	-0.37ns	-0.42ns	0.10ns	0.23ns	1		
B	-0.46ns	-0.16ns	0.08ns	-0.15ns	0.10ns	-0.10ns	-0.21ns	-0.11ns	-0.16ns	0.01ns	0.47ns	-0.30ns	1	
Co	0.00ns	0.57ns	0.40ns	0.50ns	0.04ns	0.75*	0.22ns	-0.36ns	-0.38ns	-0.06ns	0.28ns	0.42ns	-0.16ns	1

Subtitle: ns (not significant) $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$.

In Table 9 the Pearson correlation coefficient for the seeds of the different fruits studied was presented, being only the interaction between nitrogen and phosphorus, highly significant at a significance level of 1% with a value of (0.93). The interactions of iron with phosphorus (0.79), copper with phosphorus (0.76), cobalt with nitrogen (0.75) and sodium with zinc (0, 75). The remaining elements do not show significant interaction.

3.4.1 Principal component analysis (PCA)

The analyzes of main components were carried out jointly for the evaluated systems (*abiu*, *bacupari*, *acerola*, *graviola*, *camu-camu*, *fruta-do-conde*, *araçá*, *biribá* and *taperebá*), independently for seeds of the fruit, in order to (minerals present in different parts of the fruit), in order to find a new set of variables (main components), uncorrelated, that explain the structure of the variation, being represented the weight of each variable analyzed in each component (axes).

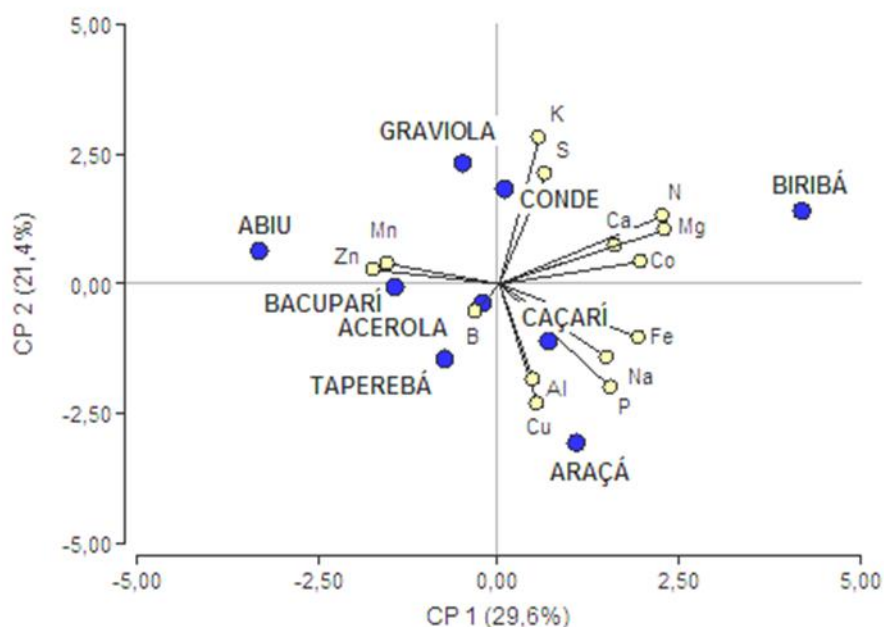
In Figure 1, the correlation of the two main components PC1 and PC2 is shown where PC1 shows more information, with a greater variance value (29.6%) and PC2 carries the maximum part of residual information with a value of 21.4%, being explained 51.0% of the total variance between the different minerals in the different fruit seeds studied.

The seed variables of *araçá* and *camu-camu* are inversely related to *taperebá* seeds are the three variables that contribute to the first main component together with the *biribá* seeds. The PC1 and PC2 planes reveal that the *biribá* presents a positive score for the first main component and, therefore, present variations above the average.

In the second main component, the *graviola* seed contributes the most to that main component. On the other hand, the elements (K, S, N, Ca, Mg and Co) present a strong positive correlation for the *biribá* and the *fruta-do-conde*, for both main components as opposed to *graviola* and *abiu* seeds that present a correlation positive for the second main component for Mn and Zn.

In the fourth quadrant the fruit seeds of *camu-camu* (*caçari*) and *araçá*, present a strong positive correlation for the first main component of the elements (Fe, Na, P, Al and Cu).

Figure 1- Distribution of the original variables among the different fruits for the seeds on the first and second main components (CP1 and CP2).

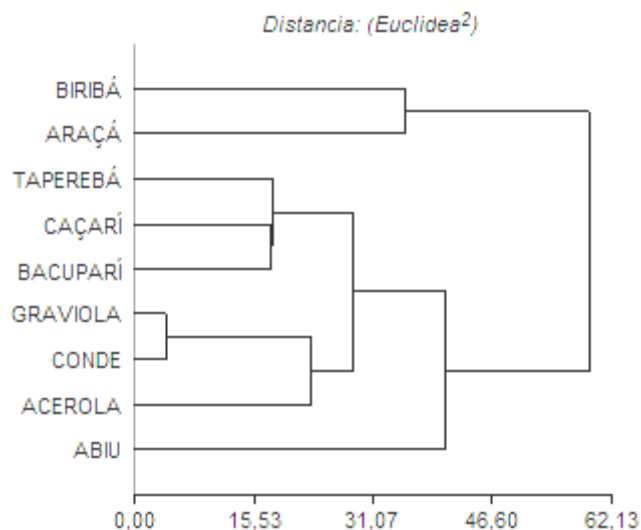


3.4.2 Hierarchical Component Analysis (HCA)

Through the HCA, data can be displayed in a two-dimensional space in order to emphasize their natural groupings and patterns, relating the samples so that the most similar are related to each other, presenting the samples in dendrogram, grouping the samples and variables according to with its similarity.

In Figure 2 the dendrogram for the HCA analyzes of the different fruit seeds studied is presented.

Figure 2- Dendrogram by HCA, Euclidean distance and incremental connection technique for the minerals present in the fruits seeds studied



For the seeds of the studied fruits, the trends observed through the analysis of main components were observed through the HCA, observing that the *biribá* and *araçá* form a group, the *taperebá*, *camu-camu* and *bacuparí* form another group, the *graviola* together with the *fruta-do-conde* are grouped and *acerola* and *abiu* are not grouped with the other fruits. For the distance 31.07 which is the value of half of the maximum distance, the *biribá* and *araçá* are separated from the rest.

4 CONCLUSIONS

The seeds of fruits studied in this work, on the one hand, are an important source of contribution of mineralogical nutrients, both macronutrients and elements in concentrations of traces, being able to be used for enrichment of functional foods or for use them in diets that need a contribution of a certain nutrient with a specific physiological activity.

On the other hand, most of the seeds of the fruits studied in this work, have a high ratio of unsaturated fatty acids in relation to saturated, especially in omega 3 fatty acid, being responsible for reducing cholesterol and triglycerides in the blood, they reduce the formation of thrombi and clots and decrease of cardiovascular diseases, given the profile of fatty acids that they present, being healthy for human

health, those seeds could be incorporated in the elaboration of foods with functional potential.

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CAPÍTULO IV

EVALUATION OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN AMAZON FRUITS⁴

ABSTRACT

In this work, nine fruits cultivated in the northern Amazon were studied: *abiu* (*Pouteria caimito*), *acerola* (*Malpighia emarginata*), *araçá* (*Psidium cattleianum*), *bacuparí* (*Rheedia gardneriana*), *biribá* (*Rollinia mucosa*), *camu-camu* (*Myrciaria dubia*), *fruta-do-conde* (*Annona squamosa*), *graviola* (*Annona muricata*) and *taperebá* (*Spondias mombin* L.). The total phenolic compounds were evaluated in the pulp, seed and bark by means of the colorimetric reaction of Folin Ciocateau, as well as the antioxidant capacity in the different extracts. DPPH method and on the other hand by the iron reduction method. As the fruits that presented a greater quantity of phenolic compounds are in mg gallic acid. 100 g⁻¹ sample, we have: *camu camu* barks (1241.1 ± 12.04), followed by the *abiu* barks with (1132.43 ± 8.10), *araçá* pulp (1080.21 ± 1.1) and *acerola* pulp (1071.4 ± 22.2). Evaluating the antioxidant capacity, the evaluated fruits that present a higher value of antioxidant capacity are the *araçá* seed with EC₅₀ value of (471.23 ± 21.23 g g⁻¹ DPPH) and for iron reduction of (57.21 ± 4.11 μmol Fe₂SO₄ g⁻¹), followed by the EC₅₀ of *abiu* bark (521.71 ± 1.34 g g⁻¹ DPPH) and iron reduction of (411.43 ± 27.12 μmol Fe₂SO₄ g⁻¹), and for the *camu-camu* pulp (549.24 ± 21.13 g g⁻¹ DPPH) and for reduction of iron (235.47 ± 11.44 μmol Fe₂SO₄ g⁻¹). Multivariate analysis methods were applied through Principal Component Analysis (PCA) with pulp having the highest correlation between data variability with 93.6% according to PCA.

1 INTRODUCTION

Phenolic compounds are part of secondary metabolites of plants, mainly showing the function of protecting the plant against organisms and pests, consequently, influencing the nutritional value of food, and sensorial quality, besides conferring physical-chemical attributes such as color, texture, bitterness and astringency (Evertte et al., 2010). From the point of view of health, the phenolic compounds present bioactive potential as anti-inflammatory, antioxidant and antitumor (BOGANI et al., 2007; WENG AND YEN, 2012).

According to Abe et al. (2007), the phenolic compounds can be classified between flavonoids and non-flavonoids, and the flavonoids are catechins, epicatechins, epigallocatechins, caempferol, quercetin, myricetin, anthocyanins,

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rutin and naringenin, and within non-flavonoids, phenolic acids, hydroxybenzoic acid, Hydroxycinnamic acid and resveratrol. The problem with the above phenolic compounds according with Fang and Bhandari (2010), is instability is that insulation techniques increase the physical stability of these compounds, thus increasing the stability, thus protecting the phenolic composition of the interaction of others Compounds in the food, in addition to having their release controlled, thus increasing their bioactivity.

A parameter used to measure the amount of total phenolic compounds in biological samples is the total antioxidant capacity, which can be used for pure compounds and for matrices of food plants (Choi et al., 2002).

In this way, the total phenolic compounds and the antioxidant potential will be evaluated and for nine fruits grown in the northern Amazon (*abiu*, *acerola*, *camu-camu*, *bacupari*, *fruta-do-conde*, *graviola*, *araçá*, *biribá* and *taperebá*). Iron reduction method, and DPPH, as well as total phenolic compounds by the Folin-Ciocateau method, and statistically correlated using multivariate analysis analysis techniques PCA with infostar program version 2016.

2 MATERIALS AND METHODS

2.1 SAMPLE PREPARATION

The samples of the different fruits studied were collected in randomized points of the State of Roraima (Brazil) to guarantee the representativeness of the sample. Each of the fruits was collected in the corresponding production period and were collected in the ripening stage suitable for consumption. From all the samples collected at the different sampling points, a single composite sample was prepared for each of the fruits where they were taken to the Environmental Chemistry Laboratory of the Federal University of Roraima, where those that presented an optimum conservation status were selected washed with 1% sodium hypochlorite solution and again with distilled water.

Subsequently, a representative sample of each fruit was selected according to the following criteria: *acerola*, *camu-camu* and *taperebá* was selected 1 kg of fresh fruit: *abiu*, *araçá* and *bacupari* was selected 2 kg of fruit and for *biribá*, *fruta-do-conde* and *graviola* were selected 10 units according with NTON 17002-02 (2002).

All of them were separated into pulp, barks and seed and were placed in Ultrafreezer at -80°C and then lyophilized in lyophilizer LIOTOP model L 101 for 48 hours until complete drying of the material and subsequently ground in LABOR model SP31 punch mill and stored material in airtight bags in the absence of light until the moment of performing the different analyzes.

Table 1- Names and families of cultivated fruits cultivated in study.

Scientific name	Family	Common Name in Brazil
<i>Pouteria caimito</i>	Sapotaceas	<i>Abiu</i>
<i>Malpighia emarginata</i>	Malpighiaceae	<i>Acerola</i>
<i>Psidium cattleianum</i>	Myrtaceae	<i>Araçá</i>
<i>Rheedia gardneriana</i> Planch & Triana	Clusiaceae	<i>Bacuparí</i>
<i>Rollinia mucosa</i>	Annonaceae	<i>Biribá o fruta da condessa</i>
<i>Myrciaria dubia</i> (Krunth)	Myrtaceae	<i>Camu-camu</i>
<i>Annona squamosa</i>	Annonaceae	<i>Fruta-do-conde</i>
<i>Annona muricata</i>	Annonaceae	<i>Graviola</i>
<i>Spondias mombin</i> L.	Anacardiaceae	<i>Taperebá</i>

2.2 Determination of total phenolic compounds

The determination of the total phenolic compounds (CFT) was done according to the methodology proposed by Wolfre et al. (2013) where methanolic extracts were prepared from the extraction of 4.0 grams of lyophilized material with 35 mL of 80% (v/v) methanol acidified with 0.5% (v/v) hydrochloric acid, in falcon tubes and were subsequently placed in a bath with water at 90 °C for 30 minutes, the supernatant being separated and remaining material was added again 35 mL and treated under the same conditions as above.

The fractions were then pooled and centrifuged at 6000 rpm for 30 minutes. The samples were placed in amber glasses and stored in the refrigerator at 2 °C until the analysis.

2.2 DETERMINATION OF ANTIOXIDANT ACTIVITY

The determination of the antioxidant activity in the different extracts was by different methods: the method of extinguishing the absorption of the radical 1,1-diphenyl-2-picrylhydrazyl (DDPH); and the iron reduction method. The DDPH method was developed using visible ultraviolet molecular absorption spectrophotometry, measured at 515 nm (MIRANDA; FRAGA, 2006) in Shimadzu UV-1800.

The methodology of iron reduction was described by Barros et al. (2010), using different concentrations of the methanolic extracts. 0.5 ml aliquots of each concentration were mixed with 0.5 ml sodium phosphate buffer (200 mmol L⁻¹, pH

6.6) and 0.5 ml potassium ferricyanide (1% w/v, in water). The mixture was incubated for 20 minutes at 50 °C using 0.5 mL of trichloroacetic acid (10% w/v).

3. RESULTS AND DISCUSSION

In the Table 2 shows the values of total phenolic compounds for the different fruit samples studied using the Folin Ciocateau test, using gallic acid as standard, with the calibration curve of $y = 0,0173 x + 0,0431$ $r^2 = 0.994$

Table 2-Total phenolic compounds in Amazonian fruits.

Fruit		mg gallic acid 100 ⁻¹ g sample
<i>Abiu</i>	pulp	900.2 ± 7.3
	bark	1132.43 ± 8.1
	seeds	611.34 ± 6.2
<i>Acerola</i>	pulp	1071.4 ± 22.2
	bark	1042.1 ± 17.4
	seeds	312.3 ± 11.1
<i>Araçá</i>	pulp	1080.21 ± 1.1
	bark	110.14 ± 12.4
	seeds	941.3 ± 11.2
<i>Bacuparí</i>	pulp	31.2 ± 1.1
	bark	78.21 ± 2.1
	seeds	54.11 ± 1.3
<i>Biribá</i>	pulp	101.3 ± 2.2
	bark	85.4 ± 1.1
	seeds	209.4 ± 12.1
<i>Camu camu</i>	pulp	1741.2 ± 34.3
	bark	1241.1 ± 12.0
	seeds	241.2 ± 7.4
<i>Fruta-do-conde</i>	pulp	11.3 ± 0.7
	bark	92.2 ± 1.2
	seeds	58.3 ± 0.4
<i>Graviola</i>	pulp	89.2 ± 2.2
	bark	427.3 ± 11.2
	seeds	632.3 ± 2.4
<i>Taperebá</i>	pulp	524.3 ± 11.4
	bark	558.3 ± 10.12
	seeds	7.13 ± 0.21

Vasco et al. (2008) classify the polyphenolic compounds in different categories according to the content of polyphenols in the samples being from below to 100 mg 100 g⁻¹ EGA, (average of Gallic acid in 100 grams of sample), average for concentrations between 100- 500 mg 100 g⁻¹ EGA and high for values having greater than 500 mg EGA.100 g⁻¹.

In the case of the samples studied in this work, they present low values according to the previous classification of the tapereba seed with values of 7.31 ± 0.21 mg 100 g⁻¹ EGA, the bacupari with low values of phenolic compounds in all parts of the fruit, presenting 31.2 ± 1.1 mg 100 g⁻¹ EGA for the pulp, 85.4 ± 1.1 mg 100 g⁻¹ EGA for the seed and 78.21 ± 2.1 mg 100 g⁻¹ EGA for the waterfall Another fruit that presents a low value of phenolic compounds is the *fruta-do-conde* pulps with $11,3 \pm 0,7$ mg 100 g⁻¹ EGA. Among the fruits that present average values are the *acerola* seed with 312.3 ± 11.1 mg 100 g⁻¹ EGA, the *araçá* bark with 110.4 ± 12.4 mg 100g⁻¹ EGA, the *biribá* pulp with 101.3 ± 2.2 mg 100 g⁻¹ EGA as the seed with 209.43 ± 12.1 mg 100 g⁻¹ EGA, the *camu-camu* seeds with 241.1 ± 7.4 mg 100 g⁻¹ EGA, the bark and seed of *graviola* with 427.3 ± 11.2 mg 100 g⁻¹ EGA for the waterfall and 632.3 ± 2.4 mg 100 g⁻¹ EGA for the seed and finally the *taperebá* pulp with 524.3 ± 11.4 mg 100 g⁻¹ and the *taperebá* bark with 558.3 ± 10.1 mg 100 g⁻¹.

The remaining samples showed high values of total phenolic compounds, with *camu-camu* with a concentration of 1241.1 ± 12.4 mg 100g⁻¹ and pulp with 1741.2 ± 34.3 mg 100 g⁻¹, being the values within the ranges given by Maeda et al. (2007), where they find values between 1100-1800 mg AGE. 100 g⁻¹. Other fruits that present high values of phenolic compounds are the *acerola* pulp with 1071.4 ± 22.2 mg 100 g⁻¹, the *acerola* pulp of *araçá* with 1080.21 ± 1.1 mg 100 g⁻¹ and the bark of *abiu* with 1132.43 ± 8.1 mg 100 g⁻¹.

The high concentration of phenolic compounds in *camu-camu* bark and pulp is related to the high concentration of vitamin C present in the fruit, because according to Yuyama et al. (2002), it possesses up to 6000 mg.100 g⁻¹ of ascorbic acid. In addition to vitamin C, *camu-camu* contains other compounds with antioxidant activity such as anthocyanins, flavonoids such as rutin (1.3 mg 100g⁻¹ fresh weight) and quercetin (2.4 mg 100g⁻¹ fresh weight, and in the presence of a high solubility in the diet.

Table 3 shows the percentage of antioxidant activity done with the DPPH method, iron reduction and EC₅₀ for the different fruits studied, with the calibration curve of DPPH $y = 0.0099x + 0.0077$ with $r^2 = 0.997$ and for the iron reduction method $y = 0.00036x + 0.08172$ with $r^2 = 0.998$.

Table 3- Antioxidant activity EC₅₀ by the DPPH method and reduction of iron.

Fruit		Antioxidant capacity	
		DPPH	Reduction of iron
		EC ₅₀ (g g ⁻¹ DPPH·)	μmol Fe ₂ SO ₄ g ⁻¹
<i>Abiu</i>	pulp	912.04 ± 3.17	170.04 ± 32.04
	bark	521.71 ± 1.34	411.43 ± 27.12
	seeds	781.14 ± 3.4	217.11 ± 7.22
<i>Acerola</i>	pulp	647.11 ± 12.34	124.01 ± 17.04
	bark	712.23 ± 21.12	71.17 ± 0.69
	seeds	1517.18 ± 19.23	42.31 ± 4.38
<i>Araçá</i>	pulp	531.22 ± 12.04	31.54 ± 1.10
	bark	1321.18 ± 23.83	17.31 ± 2.34
	seeds	471.23 ± 21.23	57.21 ± 4.11
<i>Bacuparí</i>	pulp	1231.48 ± 12.45	11.17 ± 1.11
	bark	2121.72 ± 22.23	7.23 ± 1.1
	seeds	1611.32 ± 28.11	9.11 ± 1.1
<i>Biribá</i>	pulp	1411.31 ± 11.04	109.23 ± 17.22
	bark	1591.31 ± 14.28	92.41 ± 14.31
	seeds	1212.15 ± 21.17	217.31 ± 28.11
<i>Camu camu</i>	pulp	549.24 ± 21.13	235.47 ± 11.44
	seeds	612.34 ± 17.05	147.23 ± 8.32
	bark	1231.45 ± 43.76	98.05 ± 15.11
<i>Fruta-do-conde</i>	pulp	1711.04 ± 11.04	165.11 ± 21.12
	seeds	1511.08 ± 7.31	191.24 ± 17.11
	bark	1634.22 ± 21.22	180.12 ± 4.18
<i>Graviola</i>	pulp	1517.31 ± 12.34	104.11 ± 17.21
	bark	1211.21 ± 4.23	215.12 ± 11.21
	seeds	1187.04 ± 12.04	161.11 ± 31.04
<i>Taperebá</i>	pulp	1931.24 ± 23.12	18.04 ± 7.11
	bark	1811.31 ± 17.05	19.11 ± 3.23
	seeds	n.d.	n.d.

According to the above, observing the results presented in table 3 and compared with the results discussed in table 2, there is a correlation between the values of antioxidant activity with the phenolic compounds, due to that when the concentration of phenolic compounds decreases in the samples, the amount of substances that are antioxidant (reducing) substances that reduce iron also decreases, increasing the value of the IC_{50} , being smaller the antioxidant activity.

Comparing the results obtained with other works for Amazon fruits developed by Rufino et al. (2010), where they evaluated different fruit pulps, they determined by the FRAP technique the antioxidant capacity of acerola pulp with a value of $148 \pm 16 \mu\text{mol Fe}_2\text{SO}_4 \text{ g}^{-1}$, a result close to the value found in this work, for the *camu-camu* pulp $279 \pm 1.5 \mu\text{mol Fe}_2\text{SO}_4 \text{ g}^{-1}$, being a value close to that found in this work and for the *tapereba* pulp $11,08 \pm 0,2 \mu\text{mol Fe}_2\text{SO}_4 \text{ g}^{-1}$, a slightly lower value than that found in the present study $18.04 \pm 7.11 \mu\text{mol Fe}_2\text{SO}_4 \text{ g}^{-1}$, but presenting low antioxidant activity.

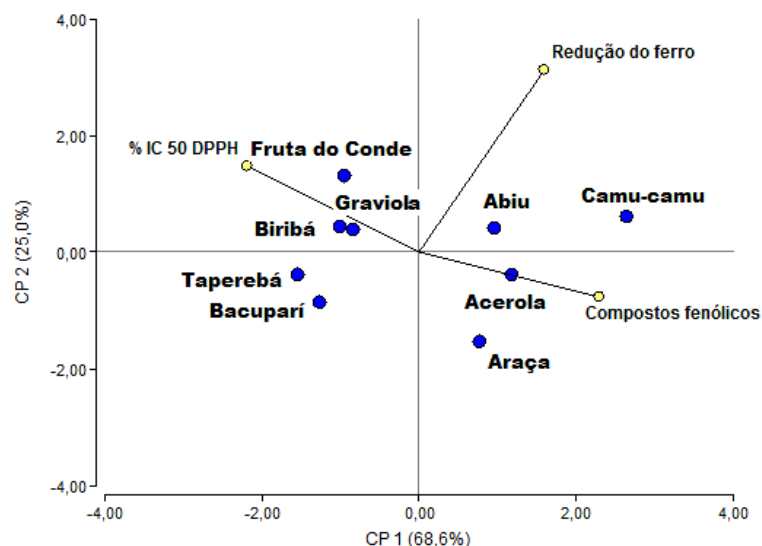
On the other hand, Canuto et al. (2010), evaluated the antioxidant activity of fruit pulps, using trolox, and found antioxidant activity for the *abiu* pulp $0.8 \pm 0.1 \mu\text{mol.L}^{-1}$ trolox, for the *araçá* pulp $0 \pm 0.1 \mu\text{mol.L}^{-1}$ trolox, and for *graviola* pulp $2.2 \pm 0.1 \mu\text{mol l}^{-1}$ trolox, the lowest antioxidant activity values being those determined by the reduction of iron.

3.1. STATISTICAL ANALYSIS

The analyzes of main components were carried out jointly for the evaluated systems (*abiu*, *bacupari*, *acerola*, *graviola*, *camu-camu*, *fruta-do-conde*, *araça*, *biribá* and *taperebá*), independently for each part of the fruit, in order to (% IC_{50} DPPH, reduction of iron and total phenolic compounds in the different parts of the fruit), in order to find a new set of variables (main components), uncorrelated, that explain the structure of the variation, being represented the weight of each variable analyzed in each component (axes).

In the *blipot* (Figure 1), the results of the analysis of the main components (PCA) for the different fruits were explained, explaining the 93.6% of the original variability of the data retained in these components for the pulps, 90.3% for the skin and 81% in the casso of the seeds (Figures 1-3).

Figure 1- Distribution of original variables between the different fruits pulps for the first and second principal component (CP1 and CP2).

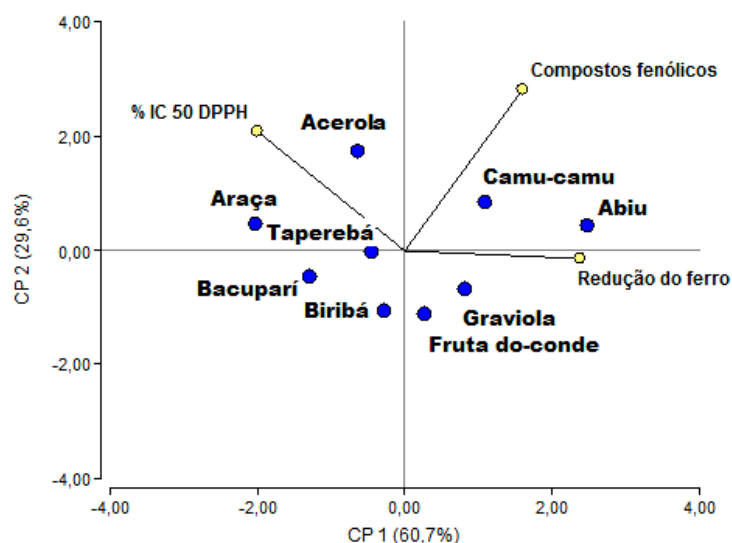


In figure 1, the correlation of the two main components PC1 and PC2 for the antioxidant activity and phenolic compounds for the different fruit pulps is shown, where PC1 shows more information, with higher variance value (68.6%) and PC2 it carries the maximum part of residual information with a value of 25.0%, with 93.6% of the total variance between the different minerals in the different fruit pulps studied being explained.

The variable phenolic compounds and iron reduction have a positive correlation for the first main component, presenting a positive score for the first main component and therefore presenting variations above the average.

In the second main component, iron reduction and IC50 present a positive correlation but inversely related through the first main component. Fruit pulps such as *abiu*, *camu-camu*, *acerola* and *araça* present a positive contribution, being associated with each other and therefore with a greater antioxidant activity. Finally, in the fourth quadrant are the *taperebá* and *bacuparí* pulps that do not correlate with any of the others.

Figure 2- Distribution of original variables between the different fruits for the barks on the first and second principal component (CP1 and CP2).



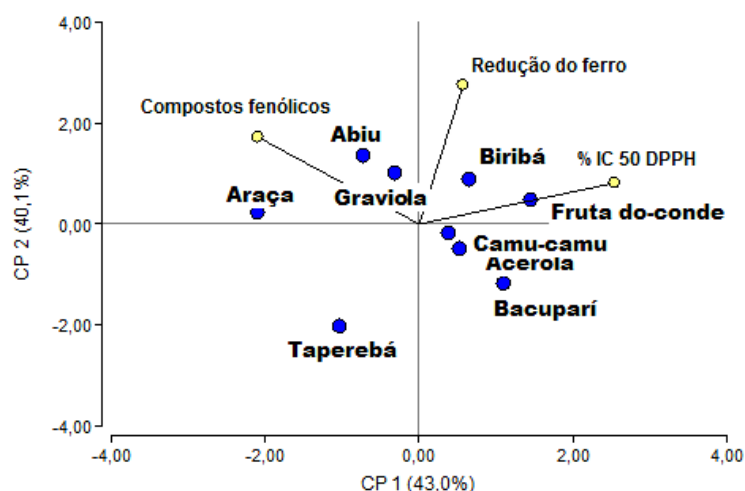
In figure 2, the correlation of the two main components PC1 and PC2 is shown, where PC1 shows more information, with a greater variance value (60.7%) and PC2 carries the maximum part of residual information with a value of 29.6%, being explained 90.3% of the total variance between the total phenolic compounds and antioxidant activity for the different fruit barks studied.

The variable phenolic compounds and iron reduction have a positive correlation for the first main component, presenting a positive score for the first main component and therefore presenting variations above the average.

In the second main component, iron reduction presents a value close to zero for both main components and IC50 presents a positive correlation but inversely related through the first main component.

Fruit barks such as: *abiu*, *camu-camu*, *graviola* and *fruta-do-conde* present a positive contribution, being associated with each other and therefore with greater antioxidant activity. Finally, in the fourth quadrant are the *biribá* barks and *bacupari* barks that do not correlate with any of the others.

Figure 3- Distribution of original variables between the different fruits for the seeds on the first and second principal component (CP1 and CP2).



In figure 3, the correlation of the two main components PC1 and PC2 is shown, where PC1 shows more information, with a greater variance value (43.0%) and PC2 carries the maximum part of residual information with a value of 40.1%, being explained 83.1% of the total variance between the total phenolic compounds and antioxidant activity for the different fruit seeds studied.

The variable phenolic compounds and IC50 present a positive correlation for the first main component, presenting a positive score for the first main component and therefore present variations above the average, being strongly related the *biribá* seeds and the *fruta-do-conde* fruits.

On the other hand, the three variables contribute positively to the second main component, with the phenolic compounds being opposed with the other two variables, there being a strong correlation for phenolic compounds between *abiu*, *graviola* and *araçá*.

4. CONCLUSIONS

It is observed that several fruits such as *camu-camu*, *acerola* and bark of *abiu*, which present a important contribution of phenolic compounds as well as high free antiradical activity, indicating the presence of bioactive compounds, being a great incentive to revalue these Amazonian fruits and elaboration of herbal products with phytotherapeutic interest or as functional foods.

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CAPÍTULO V

CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN NORTHERN AMAZON FRUITS⁵

ABSTRACT

Fruits and vegetables are highly appreciated they are constituted by active phytochemicals with functional properties for the organism acting with modulating pharmacological effect. Due to the pharmacological properties of this type of food, in this work were studied the concentrations of vitamin C, total carotenoids and reducing and non-reducing sugars of nine fruits developed in the northern Amazon region: *abiu*, *acerola*, *araçá*, *bacuparí*, *biribá*, *camu-camu*, *fruta-do-conde*, *graviola* and *taperebá*. The concentration of vitamin C, the highest concentration in the barks of *camu-camu* 2521.51 mg 100 g⁻¹ and for *acerola* with 1731.4 mg 100 g⁻¹ stand out. The highest concentrations of total carotenoids were also found for the *camu-camu*, with concentrations of 0.67 mg 100 g⁻¹ the of *camu-camu* bark and 0.57 mg 100 g⁻¹ for the pulp. The concentrations of sugars are higher for the pulps, with the highest concentrations for the pulp of the *fruta-do-conde* with 16.31 g 100 g⁻¹ followed by the pulp of the *graviola*, both of the Annonaceae family with a concentration of 15.61 g 100 g⁻¹. The different bioactive molecules were correlated for the different parts of the fruit, by means of multivariate analysis techniques (PCA and HCA), where 90.1% of the cases were explained for the pulps, 65.4% for the barks of the fruits and finally the 88.5% of the cases for the seeds. Due to the results obtained in this work, these fruits can be used for the preparation of foods with functional interest.

Keywords: Ascorbic acid, Carotenoids, Total sugars, Multivariate analysis

1 INTRODUCTION

The antioxidant capacity of fruits varies as to its composition in phenolic compounds such as carotenoids and flavonoids as well as the concentrations in vitamins E and ascorbic acid (Saura-Calixto & Goñi, 2006). The phenolic compounds that exist in plant sources are divided into flavonoids and non-flavonoids, while the anthocyanins and carotenoids are within the flavonoids and the color of antioxidants is related to the molecular structure (Rodríguez-Amaya, 2004). Foods rich in these active molecules, have aroused increasing interest among consumers, especially in the European and North American continent, where there is 92% of consumption of this type of products (Sahota, 2015). The bioactive compounds are functional ingredients for foods that provide health benefits, as well as influence physiological

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processes that reduce the risk of chronic diseases, including antioxidants, carotenoids and vitamins (Jiménez-Colmenero, 2013; Cantillano et al., 2013).

Ascorbic acid, also known as vitamin C, is a type of organic acid present in fruits and vegetables, providing the fruit with certain organoleptic characteristics and stability properties, being one of the main characteristics of ascorbic acid, its property as an antioxidant (Sherer et al., 2012). According to Davey et al. (2000), due to the antioxidant capacity of this acid, it is one of the main vitamins for human nutrition, due to its main active molecule, L-ascorbic acid.

Another group of biomolecules of interest in the quality of the fruit are the sugars, since they are related to the flavor of the same, since as the fruit matures, the amount of soluble organic acids decreases and the quantity of soluble sugars increases, acquiring the same sweet taste. Of the simple sugars present in fruits, fructose and glucose stand out, with lower concentrations of sucrose and sorbitol (Barreiros, Bossolan, & Trinidad, 2005).

Objective of this work was to quantify the content of vitamin C, total carotenoids and reducing and non-reducing sugars in pulps, barks and seeds of fruits developed in the northern Amazon and correlate the different biomolecules by chemometric techniques of multivariate analysis.

2 MATERIAL AND METHOD

2.1 COLLECTION AND PREPARATION OF SAMPLES

The samples of the different fruits studied were collected in randomized points of the State of Roraima (Brazil) to guarantee the representativeness of the sample. Each of the fruits was collected in the corresponding production period and were collected in the ripening stage suitable for consumption. From all the samples collected at the different sampling points, a single composite sample was prepared for each of the fruits where they were taken to the Environmental Chemistry Laboratory of the Federal University of Roraima, where those that presented an optimum conservation status were selected washed with 1% sodium hypochlorite solution and again with distilled water.

Subsequently, a representative sample of each fruit was selected according to the following criteria: acerola, camu-camu and taperebá was selected 1 kg of fresh

fruit, abiu, araçá and bacuparí was selected 2 kg of fruit and for biribá, fruta-do-conde and graviola were selected 10 units according with NTON 17002-02 (2002). All of them were separated into pulp, barks and seed and were placed in Ultrafreezer at -80°C and then lyophilized in lyophilizer LIOTOP model L 101 for 48 hours until complete drying of the material and subsequently ground in LABOR model SP31 punch mill and stored material in airtight bags in the absence of light until the moment of performing the different analyzes.

Table 1- Names and families of the cultivated fruits in the Northern Amazon in study.

Scientific name	Family	Common name in Brazil
<i>Pouteria caimito</i>	Sapotaceae	<i>Abiu</i>
<i>Malpighia emarginata</i>	Malpighiaceae	<i>Acerola</i>
<i>Psidium cattleianum</i>	Myrtaceae	<i>Araçá</i>
<i>Rheedia gardneriana</i> Planch & Triana	Clusiaceae	<i>Bacuparí</i>
<i>Rollinia mucosa</i>	Annonaceae	<i>Biribá</i>
<i>Myrciaria dúbia</i> (Krunth) Mc Vaugh	Myrtaceae	<i>Camu-camu</i>
<i>Annona squamosa</i>	Annonaceae	<i>Fruta-do-conde</i>
<i>Annona muricata</i>	Annonaceae	<i>Graviola</i>
<i>Spondias mombin</i> L.	Anacardiaceae	<i>Taperebá</i>

2.2 QUANTIFICATION OF VITAMIN C

Weighed 2.0 g of lyophilized material and transferred to an Erlenmeyer flask with 10 mL of 5% metaphosphoric acid solution and 10 mL of 0.05 M sulfuric acid. Posteriorly, the samples were agitated for 30 minutes and after agitation, 10 g were transferred to a 50 mL volumetric flask and were completed with one volume of water. From there, an aliquot of 10 mL was taken and 5 mL of chloroform was added to the flask and stirred for 1 minute. Then they were left at rest to separate the layers and 5 mL of the aqueous (limpid) layer was pipetted and taken to a separating flask, where 1 mL of the buffer solution and 5 mL of the complexing solution were added. Stir later for 90 seconds and let the layers separate. Remove 3 mL of the top part (isoamyl alcohol) and transfer to a 25 mL in Erlenmeyer. Add 0.5 mL of isoamyl alcohol and shake gently. Prepare the blank, pipetting 5 mL of solution A (pipette 2

mL of 5% metaphosphoric acid solution, transferred to a 100 mL volumetric flask with distilled water) directly into the reaction tube and then with the sample, making the readings at 545 nm. The calibration curve was prepared by diluting aliquots of the standard solution of 0.5: 1.0; 1.5; 2.0 and 2.5 mL of the standard solution $20 \mu\text{g mL}^{-1}$ (This solution is prepared from the dilution of a concentration of ascorbic acid PA of concentration 1 mg mL^{-1} for a 100 mL flask and add 2 mL of the 5% metaphosphoric acid solution completing the volume with distilled water) corresponding to 10, 20, 30, 40 e 50 μg of ascorbic acid and completing up to 5 mL of solution A. Subsequently, 1 mL of the buffer solution and 5 mL of the complexing solution. Shake the solution vigorously and let the layers separate. From the upper layer, 3 mL of the upper part is removed and transferred to a 25 mL Erlenmeyer flask and 0.5 mL of isoamyl alcohol is added, shake and read at 544 nm in UV-visivel molecular absorption spectrophotometer model SHIMADZU UV -1800 (Badolato et al.,1996; Contreras-Guzmán, 1984).

2.3 QUANTIFICATION OF SUGARS

Using the methodology described in IAL (2008), certain carbohydrates are not present in different fruit. This section is determined by the reducing agents and not reducers. The first ones are determined by a Fehling reaction, using the Lane-Aynon method, at where or aldehyde or ketonic free group in position C1, or sugar considered a redutor, thus reducing indicators, as the complexes of (Cu^{2+}) to cuprous form (Cu^+). Or agent in these reactions is an open chain form of aldose or ketoses. However, non-reducing sugars are not determined, but based on reductive sugar (copper sugar sulphate) in the alkaline (Fehling's solution), forming a precipitate of cupric oxide. After hydrolysis acid two non-reducing disaccharides. This method will determine the theory of non-reducing sugars (Macedo, 2005).

2.4 DETERMINATION OF TOTAL CAROTENOIDS

The determination of total carotenoids was also performed by UV-visible molecular spectrophotometry, in a spectrophotometer model SHIMADZU UV-1800 by the technique described by Lichtenthaler and Buschmann (2001) modified, where 1 g of lyophilized material was weighed to which was added 18 mL of acetone, the carotenoids being extracted by shaking for 20 minutes in the absence of light. The

samples are filtered and the absorbance readings are carried out at concentrations of 661 nm, 644 nm and 470 nm respectively, to calculate the carotenoid concentration by means of equations 1-3.

$$C \text{ carotenoids } (\mu\text{g mL}^{-1}) = (1000 A_{470} - 1.90 C_a - 63.14 C_b)/214 \quad (1)$$

$$C_a (\mu\text{g mL}^{-1}) = 11.24 A_{661} - 2.04 A_{644} \quad (2)$$

$$C_b (\mu\text{g mL}^{-1}) = 20.13 A_{644} - 4.19 A_{661} \quad (3)$$

2.5 STATISTIC ANALYSIS

Correlations between the amounts of the different fruit were evaluated using the statistical program sta INFOSTAT, Rienzo et al. (2016) for significance levels of 5%, 1% and 0.1% respectively, as well as the principal component analyzes (PCA) and Hierarchical Component Analysis (HCA).

3 RESULT

Tables 2-4 show the results of ascorbic acid, total carotenoids and sugars (reducing and non-reducing) for the pulp, skin and seeds respectively.

Table 2- Quantification of bioactive molecules in fruits pulps

Fruit	Vitamin C (mg 100 g ⁻¹)	Total carotenoids (mg 100 g ⁻¹)	Sugars (g 100 g ⁻¹)		
			Reducers	No reducers	Totals
<i>Abiu</i>	43.1±0.2	0.018±0.001	7.38±0.03	7.30±0.13	14.68±0.15
<i>Acerola</i>	1104.67±0.31	0.37±0.08	4.3±0.02	0.20±0.04	4.5±0.06
<i>Araçá</i>	117.14±0.18	0.11±0.03	0.57±0.04	0.77±0.01	1.34±0.05
<i>Bacuparí</i>	41.34±0.2	0.021±0.01	8.12±0.07	5.09±0.02	13.21±0.10
<i>Biribá</i>	11.45±0.13	0.19±0.03	10.40±0.12	4.95±0.08	15.35±0.2
<i>Camu-camu</i>	1471.48±0.11	0.57±0.02	1.95±0.11	2.46±0.12	4.41±0.23
<i>Fruta-do-conde</i>	36.1±0.07	0.21±0.07	12.41±0.03	3.90±0.01	16.31±0.04
<i>Graviola</i>	26.15±0.12	0.26±0.01	11.67±0.15	3.94±0.07	15.61±0.22
<i>Taperebá</i>	27.1±0.12	0.022±0.02	4.40±0.2	0.15±0.13	4.55±0.23

Table 3- Quantification of bioactives molecules in fruit bark

Fruit	Vitamin C (mg 100 g ⁻¹)	Total carotenoids (mg 100 g ⁻¹)	Sugars (g 100 g ⁻¹)		
			Reducers	No reducers	Totals
<i>Abiu</i>	45.8±0.18	0.056±0.012	7.54±0.12	2.83±0.07	10.37±0.19
<i>Acerola</i>	1731.4±0.24	0.47±0.02	1.87±0.07	0.59±0.2	2.46±0.27
<i>Araçá</i>	74.17±0.13	0.074±0.008	0.78±0.09	0.29±0.2	1.07±0.29
<i>Bacuparí</i>	45.68±0.21	0.091±0.007	6.41±0.05	1.91±0.2	8.32± 0.25
<i>Biribá</i>	98.71±0.16	0.21±0.03	6.31±0.07	2.40±0.2	8.71±0.27
<i>Camu-camu</i>	2521.51±0.13	0.67±0.02	2.14±0.04	0.99±0.2	3.13±0.24
<i>Fruta-do-conde</i>	88.17±0.12	0.29±0.04	7.12±0.02	3.05±0.2	10.17±0.22
<i>Graviola</i>	119.21±0.17	0.31±0.01	9.36±0.04	2.04±0.01	11.40±0.05
<i>Taperebá</i>	34.17±0.21	0.041±0.07	2.61±0.03	1.17±0.02	3.78± 0.05

Table 4- Quantification of bioactives molecules in fruits seeds

Fruit	Vitamin C (mg 100 g ⁻¹)	Total carotenoids (mg 100 g ⁻¹)	Sugars (g 100 g ⁻¹)		
			Reducers	No reducers	Totals
<i>Abiu</i>	12.31±0.11	0.012±0.002	10.12±0.09	1.09±0.12	11.21±0.20
<i>Acerola</i>	62.12±0.08	0.087±0.013	6.29±0.12	0.86±0.04	7.15±0.16
<i>Araçá</i>	84.22±0.14	0.057±0.011	9.39±0.07	4.78±0.12	14.17±0.19
<i>Bacuparí</i>	16.21±0.07	0.023±0.002	8.21±0.2	2.57±0.2	10.78±0.4
<i>Biribá</i>	7.13±0.02	0.028±0.004	7.89±0.07	1.62±0.12	9.51±0.19
<i>Camu-camu</i>	6231.13±0.05	0.094±0.004	16.42±0.12	4.83±0.08	18.25±0.20
<i>Fruta-do-conde</i>	10.21±0.12	0.021±0.001	8.11±0.12	1.30±0.07	9.41±0.19
<i>Graviola</i>	7.84±0.04	0.029±0.003	7.21±0.11	2.06±0.04	9.27±0.15
<i>Taperebá</i>	n.d.	0.035±0.002	10.74±0.14	1.43±0.04	12.17±0.18

n.d. = no detected

The analyzes of the main components were carried out jointly for the evaluated systems (*abiu*, *acerola*, *araçá*, *bacuparí*, *biribá*, *camu-camu*, *fruta-do-conde*, *graviola* and *taperebá*), independently for each part of the fruit, in order to (vitamin C, carotenoids, reducing and non-reducing sugars in different parts of the fruit), in order to find a new set of uncorrelated variables (main components) that

explain the structure of the variation, and the weight of each variable analyzed in each component (axes) is represented. The major component blip (PCA) for the different parts of the evaluated fruit is shown in Figures 1-3.

Figure 1- Distribution of the original variables between the different fruits for the pulps on the first and second main component (CP1 and CP2)

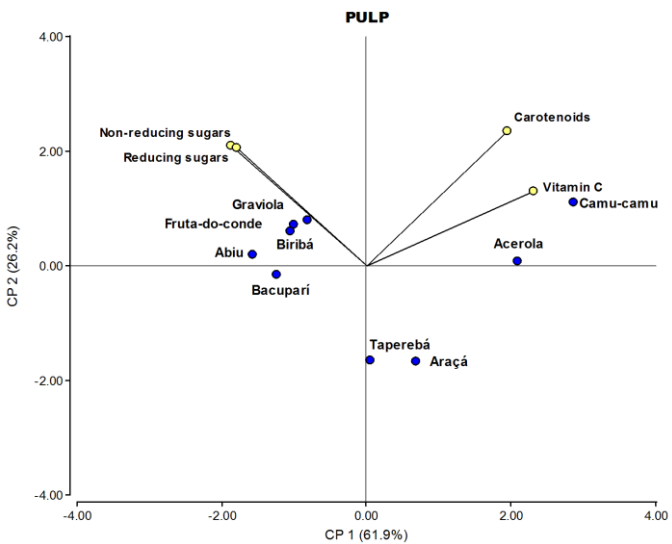


Figure 2- Distribution of the original variables between the different fruits for the barks on the first and second main component (CP1 and CP2)

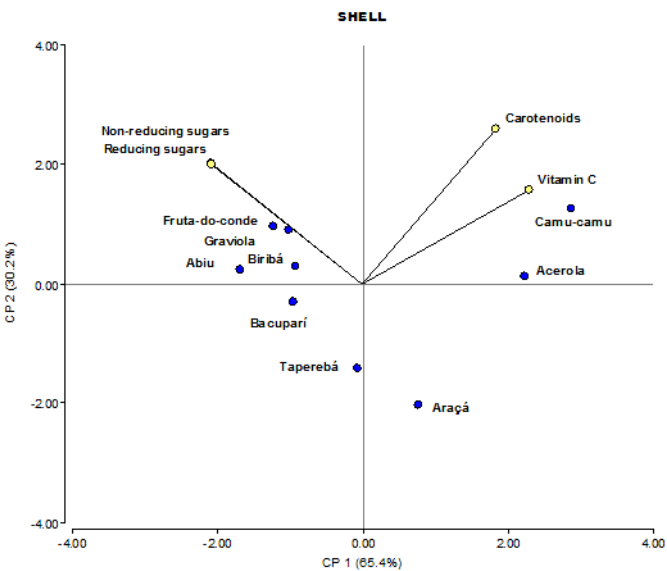


Figure 3- Distribution of the original variables between the different fruits for the seeds on the first and second main component (CP1 and CP2)

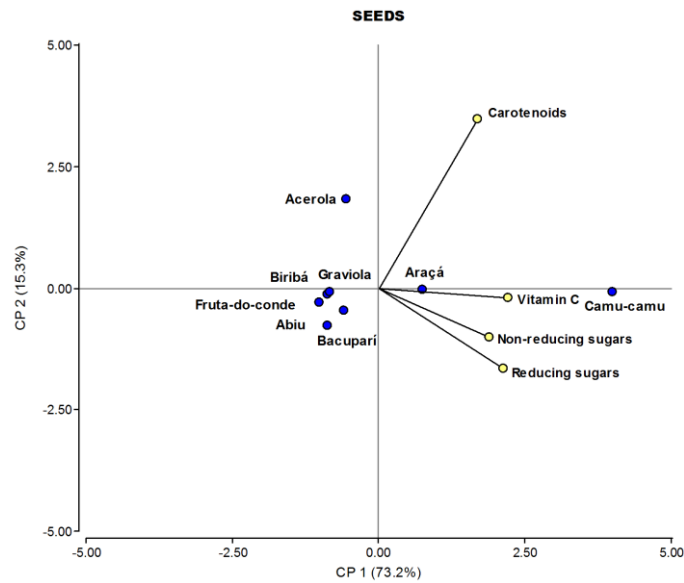


Figure 4- Dendrogram by HCA, Euclidean distance and incremental connection technique for the bioactive molecules in the pulps fruits studied

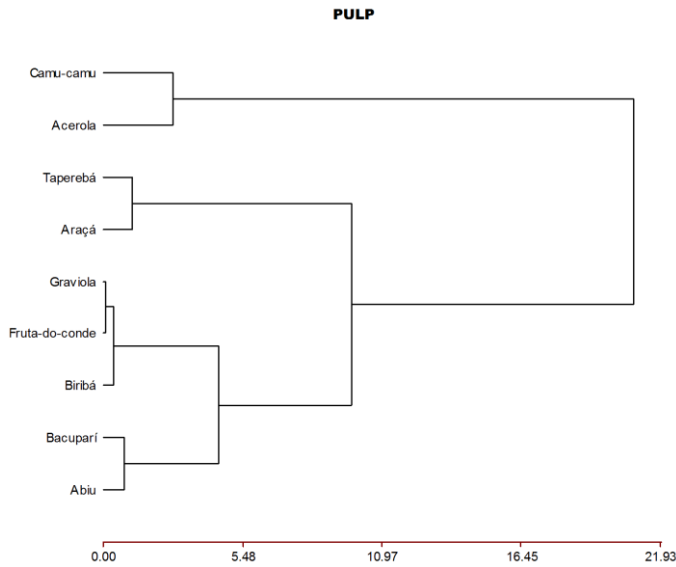


Figure 5- Dendrogram by HCA, Euclidean distance and incremental connection technique for the bioactive molecules in the barks fruits studied

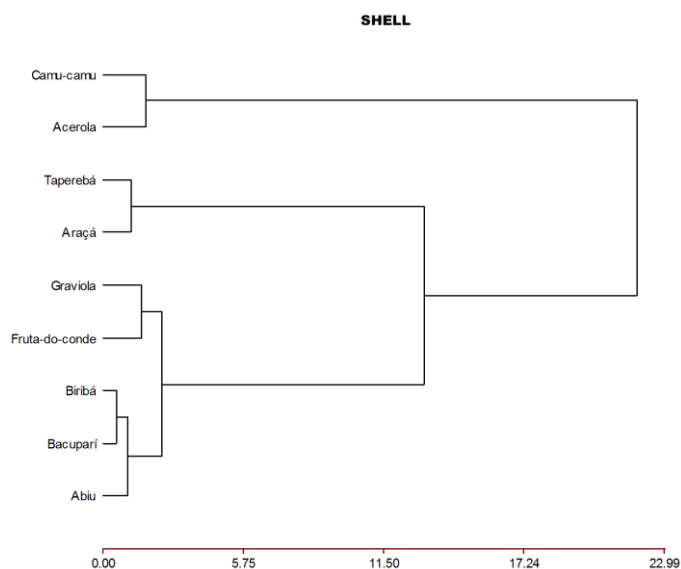
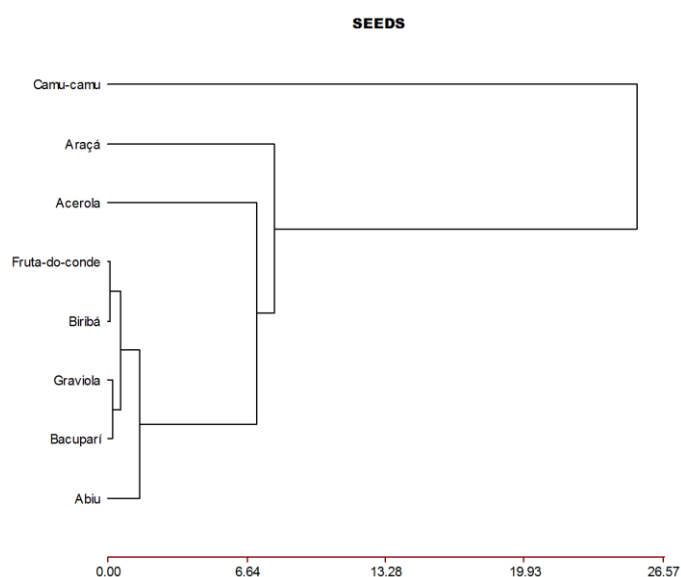


Figure 6- Dendrogram by HCA, Euclidean distance and incremental connection technique for the bioactive molecules in the fruit seeds studied



4 DISCUSSION

4.1 DETERMINATION OF BIOACTIVE MOLECULES IN AMAZONIAN FRUITS

In Table 2, the results of vitamin C, carotenoids and sugars are presented in the different fruits studied. The composition of vitamin C, it is the *camu-camu* pulp that presents the highest concentration of all the pulps studied, with a value close to

that determined by Justi et al. (2000) who obtained an ascorbic acid concentration of 1410.0 mg 100 g⁻¹. The next pulp that presents a high value of vitamin C is *acerola* with a concentration of 1104.67 mg 100 g⁻¹, whose value is within the limits established by Nunes et al. (2002) and Santos et al. (2002). Franco (1999) classifies the concentration of ascorbic acid according to different levels: high sources for those fruits in which the ascorbic acid is in concentrations between 100-300 mg 100g⁻¹, medium sources for concentrations of 25-50 mg 100 g⁻¹ and finally very low sources for those fruits whose concentrations are less than 25 mg 100 g⁻¹ in pulps. In this classification, the *camu-camu* and the *acerola* are above the classification made by Franco (1999), the *araçá* is in high concentration. Instead, the *abiu*, *bacuparí*, *frutadão-conde* and *graviola* in low concentrations and finally, the *biribá* in very low concentrations according to that classification. The other fruit with contains high concentration of vitamin C among those studied is the *araçá* pulp whose concentration was 117.14 mg 100 g⁻¹, being within the values determined by Sánchez et al. (2017) where the concentration of ascorbic acid for the pulp of this fruit varies between 103.6-141.8 mg 100 g⁻¹. The *taperebá*, presents low values of vitamin C, nevertheless these values are lower to those found by Botánico (2016) who finds concentrations of ascorbic acid in the *taperebá* pulp of 31 mg 100 g⁻¹.

The next group of bioactive substances studied in the pulps were the total carotenoids (Table 2) whose values vary in a wide range, with the lowest values for the *abiu* and *taperebá* pulp. The *abiu* had a value of total carotenoids in the pulp extremely low, 0.018 mg 100 g⁻¹, value close to that found by Virgolin et al. (2017) that obtained a total carotenoid value of 25.55 µg 100 g⁻¹ for the *abiu* pulp and the highest values found again for the *camu-camu* and *acerola* pulp. Neves et al. (2015) studied the total carotenoids in Amazonian fruits, including *camu-camu*, where they determined concentrations of 0.6 mg 100 g⁻¹ for the pulp. Rufino et al. (2010) determined carotenoid concentrations of 0.4 mg 100 g⁻¹ for the *camu-camu* pulp, this value being below the concentrations determined in this work. The value of carotenoids for the *acerola* obtained is close to the value determined by Aquino et al. (2011) whose carotenoid concentration was 0.35 mg 100 g⁻¹. *Tapereba* pulp the carotenoid concentration is also relatively low, values close to those found by Mattietto et al. (2010) who obtain values of 0.028 mg 100 g⁻¹. For the *araçá* pulp, the

concentration of carotenoids is 0.11 mg 100 g⁻¹, close to the value determined by Sanches et al. (2017) that obtains 0.129 mg 100 g⁻¹ of carotenoids in the *araçá* pulp.

Finally, the total sugars vary from 4.5 g 100 g⁻¹ for *acerola* to 16.31 mg 100 g⁻¹ for the *fruta-do-conde*. For the *abiu* pulp, the value of total sugars determined in this work is close to that determined by Virgolin et al. (2017) with a value of 14.70 mg 100 g⁻¹. Another of the fruits studied by these authors is *araçá*, whose total sugar value is 1.18 mg 100 g⁻¹, slightly lower than the value found in this study. *Taperebá* pulp, the concentration of total sugars is low compared to other fruits, but with values close to those determined by Mattietto et al. (2010), who found sugar concentrations of 4.54 g and 100 g⁻¹.

In Table 3, the results of the concentrations of the bioactive molecules for the barks of the fruits studied are presented. In comparison, the values of vitamin C between the pulp and the barks of the fruits, it is found that these are superior to those of the pulps, being generally a discarded part of the fruit. For the barks, the lowest vitamin C values were found in the *taperebá* barks with only 34.17 mg 100 g⁻¹ and the highest values again for the *acerola* and *camu-camu* bark. Correa et al. (2011), study the pulp and bark of *camu-camu* in different stages of maturity, where they also determine concentrations of vitamin C in the bark above the pulp, concentrating vitamin C in the bark as it matures. The fruit, being the determined values of vitamin C of 2792.82-2496.23 mg 100 g⁻¹ of *camu-camu* bark, these values being within those found in this work. Carotenoids already have higher concentrations than pulp, with the lowest value for *taperebá* bark 0.041 mg 100 g⁻¹ and the highest values for *camu-camu* bark 0.67 mg 100 g⁻¹ and *acerola* 0.47 mg 100 g⁻¹. The concentration of these compounds is greater in the bark of fruits since these compounds have as a mission to protect plant cells from oxidation and free radicals, in addition to promoting the formation of antibodies that act specifically against substances or elements strangers that can affect the organism (Palencia, 2010).

The concentration of sugars in the bark of the different fruits is lower than that found in the pulps, with the lowest concentrations in the *araçá* bark with 1.07 g 100 g⁻¹ and 10.37 g 100 g⁻¹ the highest concentration, for the *abiu* bark. The sugars together with the acidity influence the sensory quality of the fruit, being found in ripe fruits, simple sugars such as sucrose, glucose and fructose acting as primary source of energy (Miller et al.1986).

In Table 4, the results for the seeds are presented, where the concentration of vitamin C in the seeds of the fruits studied was not detected in the Taperebá seed and in others the values were extremely low as in the case of the *biribá* with 7.13 mg 100 g⁻¹ and the *graviola* with 7.84 mg 100 g⁻¹ being again, the highest values of vitamin C for the *camu-camu* and *acerola* seeds. The vitamin C content in the *camu-camu* seed was studied by Neves et al. (2015), in a flour obtained with the shell residue together with the seed where the concentration of ascorbic acid was 9004 mg 100 g⁻¹, values higher than those of the husk and isolated pulp, as it happens in this work, where the concentration is higher than for the other parts studied. The content of vitamin C decreases in fruits as they mature as well as with storage, since the acid ascorbic acid oxidase (ascorbinase) acts directly or by the action of oxidizing enzymes such as peroxidase (Poliniati, 2010). For *acerola* seeds, the value determined in this work is close to that determined by Aguiar et al. (2010), which obtain a concentration value of ascorbic acid of 66 mg 100 g⁻¹.

The concentration of carotenoids found in the seeds of the fruits studied, these are found in low concentrations compared to other parts of the fruit studied, with the lowest values for the seed of the *abiu* with 0.012 mg 100 g⁻¹ and the highest value for the *camu-camu* seeds with 0.094 mg 100 g⁻¹. Given their characteristic of being soluble in vegetable oils, they give pigmentation to the different vegetable oils concentrated mainly in the seeds. The main carotenoids found in vegetable oils are β -carotene, α -carotene and phytoene (Ferrari, 2001). According to Basu et al. (2001), carotenoids play an important role in the cellular protection against lipid peroxidation, preventing degenerative diseases such as cancer, heart disease or reduction in the creation of cataracts and strengthening of the immune system.

Finally, the concentration of total sugars in the seeds varies between 7.15 g 100 g⁻¹ for the *araçá* seed, 18.25 g 100 g⁻¹ for the *camu-camu* seed. These fruit seeds are richer in reducing sugars than in non-reducing sugars (Table 4). Within these reducing sugars are glucose, fructose, maltose and aldose that can be reduced in the presence of transition elements such as copper or iron (Demiate et al., 2002).

4.2 ANALYSIS OF MAIN COMPONENTS (PCA) IN DIFFERENT PARTS OF THE FRUITS

In the biplot (Figure 1), the results of the principal component analysis (PCA) for the concentration of the bioactive molecules in the different pulps studied are presented, explaining 90.1% of the original variability of the data retained in these components.

The arrangement of the sequence in Figure 1, shows that the systems can be grouped into two sets, the first major component (CP1), contributed with 61.9% of the total variance explained, however most of the variables that were strongly affected contributed positively to CP1 carotenoids and vitamin C, and inverse with reducing and non-reducing sugars. These results indicate that CP1 allowed to distinguish the fruits that are associated with the pulp of camu-camu and acerola which are strongly associated. The second main component (CP2) explained 26.2% of the total data, appearing in the values of reducing and non-reducing sugars. The analysis of this component also showed that this attribute projects negatively on vitamin C and carotenoids. *Graviola*, *fruta-do-conde*, *biribá*, *abiu* and *bacupari* pulps were associated.

In the biplot (Figure 2), the results of the analysis of the main components (PCA) for the bioactive molecules of the bark of the fruits studied are explained, explaining the 95.6% of the original variability of the data retained in these components.

The arrangement of the sequence in Figure 2 shows that the systems can be grouped into two sets, the first major component (CP1), contributed 65.4% of the total variance explained, however most of the variables that were strongly affected contributed positively to CP1 the vitamin C and carotenoids and inverse with the reducing and non-reducing sugars as happens with the pulps studied. These results indicate that CP1 allowed to distinguish that *camu-camu* and *acerola* are strongly associated. The second main component (CP2) explained 30.2% of the total data, appearing in this case the reducing and non-reducing sugars. The analysis of this component also showed that this attribute projects negatively on vitamin C and carotenoids, and the bioactive molecules in the barks of *biribá*, *fruta-do-conde*, *graviola*, *abiu* and *bacupari* showed to be associated.

In the biplot (Figure 3), the results of the main component analyzes (PCA) for the bioactive molecules of the seeds of the fruits studied are represented, accounting for 88.5% of the original variability of the data retained in these components. The arrangement of the sequence in Figure 3 shows that the systems can be grouped into two sets, the first major component (CP1), contributed with 73.2% of the total variance explained, however most of the variables that were strongly affected contributed positively to CP1 vitamin C and sugars and to a lesser extent carotenoids. These results indicate that CP1 allowed to distinguish that *araçá*, *camu-camu* and *acerola* are strongly associated. The second main component (CP2) explained 15.3% of the total data, appearing in this case associated with the remaining fruit seeds.

4.3 HIERARCHICAL ANALYSIS OF COMPONENTS (HCA) IN DIFERENT PARTS OF THE FRUITS

In Figure 4, the hierarchical component analysis (PCA) for fruit pulps is presented. The bioactive molecules in the pulps of the fruits studied, the trends observed through the analysis of PCA main components, were observed through the HCA, mainly observing four large groupings: one of them formed by the association of *graviola* with the *fruta-do-conde* and *biribá* with smaller Euclidean distance, belonging them the same family. Another strong association is the *bacuparí* pulp with *abiu*. *Taperebá* with *araçá* and *acerola* with *camu-camu*. All fruit pulps not counting the *abiu* and *acerola* are associated with the euclidean distance of 10.97, grouping with the other two fruit pulps with euclidean distance of 21.93

In Figure 5 the HCA analysis for the skin of the different fruits is barks. As it ocorre in the pulp of the fruits studied, in the shell there are four groups. On the one hand the *camu-camu* with the *acerola* that are strongly associated and only joins with the rest of fruits at an elevated Euclidean distance of 22.99. On the other hand we have an association between of the *biribá* bark with the *bacuparí* that are associated in turn to a Euclidean distance with the *abiu*, *graviola* with the *fruta-do-conde* are associated and the other grouping is between the *taperebá* and *araçá*.

The dendrogram presented for the seeds (Figure 6) presents atypical behavior if we compare it with the other parts of the fruit. Fruits that appear strongly associated

(less Euclidean distance) are the *graviola* with *bacuparí* and practically at the same distance the *fruta-do-conde* with the *biribá* that in turn the bioactive molecules in this group of fruits are going to be associated *abiu* at a slightly higher Euclidean distance.

5 CONCLUSIONS

The results obtained show that the fruits studied have a good source of bioactive compounds, especially *acerola* and *camu-camu*, where the concentration of ascorbic acid is especially high in the pulp and in the bark. These fruits are at the same time the source of carotenoids and sugars, with the highest concentrations for *abiu*, *fruta-do-conde* and *graviola*. These fruits, due to the concentrations of these compounds, present socioeconomic potential for the region where they are found, and can be consumed in natura or industrialized in the form of foods with high functional potential.

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CAPÍTULO VI

ANTIMICROBIAL ACTIVITY AND ACETILCHOLINESTERASE INHIBITION OF OILS AND AMAZON FRUITS EXTRACTS⁶

ABSTRACT

The present work consists on the evaluation of antimicrobial activity and inhibition of the enzyme acetylcholinesterase (AChE) of fixed oils and hexane extracts of nine fruits with the following native names: *abiu* (*Pouteria caimito*), *acerola* (*Malpighia emarginata*), *araçá* (*Psidium cattleianum*), *bacuparí* (*Rheedia gardneriana*), *biribá* (*Rollinia mucosa*), *camu-camu* (*Myrciaria dubia*), *fruta-do-conde* (*Annona squamosa*), *graviola* (*Annona muricata*) and *taperebá* (*Spondias mombin* L.). Different evaluations were carried out with different parts of the fruits, pulp, seed and barks. The antimicrobial assay was carried out with the following microorganisms: *Candida albicans* ATCC 18804, *Staphylococcus aureus* ATCC 29212, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028. Of these microorganisms, the best inhibition results were obtained for yeast *Candida albicans* with percent inhibition of 94.46% by *taperebá* barks extracts, *acerola* barks (87.12%), *araçá* seed (85.23%) and *taperebá* pulp (85.22%). Against the bacteria tested, percent inhibition was low, showing that the extracts have good antifungal selectivity. Some extracts were able to inhibit the enzyme AChE and high percentage of inhibition was observed for the oils, especially from *biribá* barks, with 86.39% inhibition, *taperebá* seeds with 62.17% and *acerola* pulp with 52.18%. Methods of Multivariate Analysis were applied through Principal Component Analysis (PCA) and Hierarchical Component Analysis (HCA), to establish correlations and groupings between the data obtained, justifying 82.3% of cases for pulps, 73.2% for the barks and 65.7% for the seeds according to the PCA.

Keywords: Bacteria; Yeasts; Alzheimer; PCA; HCA.

1 INTRODUCTION

In Brazil there are ten thousand plants considered medicinal, aromatic and useful, but around 99.6% of these plants are little known about the chemical composition (Silva et al., 2002). Many of these plant species have in their chemical composition secondary metabolites with a defensive function when they are attacked by certain microorganisms such as bacteria, fungi, parasites or virus among others. The compounds with antibacterial action usually are terpenoids, phenolic compounds, alkaloids, polypeptics, coumarins and camphors, being extremely

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numerous and, at the same time, their chemical structures present high selectivity and specificity (Simões, 2003; Reschke et al., 2007; Chen et al., 2015).

Fungi and bacteria are present in plants environment make the latter to act in the fight against these phytopathogens, as well as against insect pests and herbivores (Peixoto et al., 2005). Since natural products have high biological activity, it is increasingly common to use extracts as an alternative, for example against certain diseases such as candidiasis treatment (Reis et al., 2011). Infectious diseases represent an important cause of morbidity and mortality in humans, especially in developing countries, and pharmaceutical industries have been motivated in recent years for the development of new antimicrobial drugs, especially due to the occurrence of microbial resistance to such diseases as the bacteria possess genetic ability and acquire resistance to drugs used as therapeutic agents (Nascimento et al., 2000).

However, there are compounds of natural origin that have the property of inhibiting acetylcholinesterase (AChE), a key enzyme in Alzheimer's disease. These compounds can be either isolated from plants or from microorganisms (dos Santos et al., 2017). Inhibition of AChE *in vitro* is attributed to several reasons, including the structure of phenolic compounds, considering the metabolism suffered by phenolic compounds after their ingestion at gastrointestinal tract and liver level (Roseiro et al., 2012).

In this context, the objective of this work was to perform a bioassay to evaluate inhibition of Gram-positive and Gram-negative bacteria, yeasts and the inhibition of the acetylcholinesterase enzyme by different oils and fruit extracts (*Abiu*, *Acerola*, *Camu-camu*, *Bacupari*, *Graviola*, *Araçá*, *Biribá* and *Taperebá*) in the pulps, barks and seeds fruits of Norther Amazon and to correlate the different results through multivariate analysis techniques (PCA and HCA), aining to collaborate to future pharmaceutical applications.

2 MATERIAL AND METHOD

2.1 SAMPLE PREPARATION

The samples of the different fruits studied were collected in randomized points of the State of Roraima (Brazil) to guarantee the representativeness of the sample.

Each of the fruits was collected in the corresponding production period and were collected in the ripening stage suitable for consumption. From all the samples collected at the different sampling points, a single composite sample was prepared for each of the fruits where they were taken to the Environmental Chemistry Laboratory of the Federal University of Roraima, where those that presented an optimum conservation status were selected washed with 1% sodium hypochlorite solution and again with distilled water.

Subsequently, a representative sample of each fruit was selected according to the following criteria: acerola, camu-camu and taperebá was selected 1 kg of fresh fruit, abiu, araçá and bacuparí was selected 2 kg of fruit and for biribá, fruta-do-conde and graviola were selected 10 units according with NTON 17002-02 (2002). All of them were separated into pulp, barks and seed and were placed in Ultrafreezer at -80°C and then lyophilized in lyophilizer LIOTOP model L 101 for 48 hours until complete drying of the material and subsequently ground in LABOR model SP31 punch mill and stored material in airtight bags in the absence of light until the moment of performing the different analyzes.

Table 1- Names and families of the cultivated fruits cultivated in the Northern Amazon.

Scientific name	Family	Common name in Brazil
<i>Pouteria caimito</i>	Sapotaceae	<i>Abiu</i>
<i>Malpighia emarginata</i>	Malpighiaceae	<i>Acerola</i>
<i>Psidium cattleianum</i>	Myrtaceae	<i>Araçá</i>
<i>Rheedia gardneriana</i> Planch & Triana	Clusiaceae	<i>Bacuparí</i>
<i>Rollinia mucosa</i>	Annonaceae	<i>Biribá</i>
<i>Myrciaria dúbia</i> (Krunth)	Myrtaceae	<i>Camu-camu</i>
<i>Annona squamosa</i>	Annonaceae	<i>Fruta-do-conde</i>
<i>Annona muricata</i>	Annonaceae	<i>Graviola</i>
<i>Spondias mombin</i> L.	Anacardiaceae	<i>Taperebá</i>

The samples were taken to the laboratory of Environmental Chemistry of the Federal University of Roraima (Brazil), where they were selected those that had an excellent state of conservation, washed previously with distilled water, then with 1% sodium hypochlorite solution and again with distilled water.

After separating the material in pulp, bark and seed, they were taken to the laboratory of the Agronomic Research Center (NUPAGRI), at the Agricultural Sciences Center, Campus de Cauamé, UFRR, where they were lyophilized in LÍOTOP liquefaction model L 101 for 48 hours until drying the material. Subsequently, the material was dried, milled with LABOR model SP31 knife mill and placed in hermetically sealed bags and stored away from the light until the analyzes were carried out.

2.2 PREPARATION OF THE EXTRACTS TO REALIZE THE BIOASSAYS

The oil and extracts were obtained by extraction from hexane solvent in a Soxhlet apparatus for 6 h. The hexane was evaporated on a rotaevaporator and the oil and extracts were properly packaged in amber vials under nitrogen atmosphere and stored in a freezer (Jorge and Luzia, 2012).

2.2.1 Bioassays of fungi and yeasts

The extracts of pulps, barks and seeds fruit studied were tested against the following microorganisms: yeast *Candida albicans* (ATCC 18804), Gram-positive bacteria *Staphylococcus aureus* (ATCC 29212), and *Bacillus cereus* (ATCC 11778), Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028).

A pre-inoculum was prepared in which the microorganisms were transferred from the culture medium where they were stored into test tubes containing 3.0 mL of culture medium (BHI for bacteria and Sabouraud Broth for yeast). The tubes were then incubated in an oven at 37.5 °C for 24-48 h. With the aid of a micropipette, 500 µL of this pre-inoculum were transferred to test tubes containing sterile distilled water. The tubes were homogenized and the concentration adjusted to 600 nm (bacteria) and 530 nm (yeast), until obtaining transmittance between 74-75% (bacteria) and 75-76% (yeast), corresponding to the 0.5 McFarland standard turbidity, thus obtaining the suspensions of the inocula used in the bioassay. To prepare the working solution the samples were previously solubilized in 12.5 mg.mL⁻¹ dimethylsulfoxide (DMSO). From this solution, an aliquot of 40 µL was added to 960 µL of the culture medium used in the bioassay, obtaining a solution with concentration of 500 µg.mL⁻¹. The bioassays were run in 96-well plates in triplicate,

adding 100 μL of the working solution at the concentration of $500 \mu\text{g.mL}^{-1}$ in three wells. Then, 100 μL of standardized microorganism inoculum was added to each well. Four controls were performed: growth control of the microorganism (to verify cell viability); the blank, which consists of the sample solution at the same concentrations evaluated, replacing the inoculum with sterile distilled water; positive control (the working solution is replaced by a commercial antibiotic) and the sterility control of the culture medium containing 100 μL of culture medium and 100 μL of sterile distilled water. The microplates were incubated in an oven at 37.5°C and after 24 h the plate reader was read at 490 nm. The antibiotics used for the quality control of the assays were: ampicillin, for bacteria and miconazole, for yeast, previously prepared as described for the samples tested (CLSI, 2012).

2.2.2 Inhibition bioassay of the enzyme Acetylcholinesterase (AChE)

The extracts of the different parts of Amazonian fruits were tested against the AChE enzyme inhibitory activity bioassay by the UV-visible molecular spectrophotometry method in 96-well microplates. Enserin (10 mg mL^{-1}) was used as the standard inhibitor and as negative control the assay was performed without the presence of inhibitors. The tests were performed in five-fold. 25 μL of acetylcholine iodide (15 mM) was pipetted into each well; 125 μL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB); 50 μL of 0.1% w/v tris-HCl pH 8 bovine serum albumin and 25 μL of the samples (10 mg mL^{-1}) solubilized in DMSO with 10% v/v tween. The plates were read at 405 nm for 9 times over a period of 8 minutes. Immediately after the first reading, 25 μL of the enzyme acetylcholinesterase (*Electrophorus electricus*, Sigma Aldrich) (0.222 U mL^{-1}) was added and 9 readings were performed over a period of 8 min at 405 nm, the percentage inhibition of the enzyme from equation 1.

$$\% \text{ inhibition} = ((C-A) \times 100) / C \quad (1)$$

At where:

C = Control containing enzyme and substrate.

A = Assay containing sample, enzyme and substrate.

2.3 STATISTICAL ANALYSIS

Principal component analysis (PSA) and component hierarchy analysis (HSA) for total phenolic compounds and antioxidant activity by the two methods for the different parts of the fruit were evaluated using InfoStat software version 2016 (Rienzo et al., 2016)..

3 RESULTS AND DISCUSSION

Results of the activity of pulps, barkss and seeds of the different fruits studied in the microbiological inhibition and AChE inhibition tests are respectively are presented in Tables 2-4.

The inhibitory potential of pulp oils and extracts against *S. aureus* was relatively low for the oils tested, reaching only 28.80% for camu-camu pulp oil, and 25% inhibition for abiu and araçá, compared to ampicillin, the standard utilized, which presented 98.8% inhibition. The other Gram-positive bacterium tested, *B. cereus*, was less inhibited than *S. aureus* bacterium by the oils and pulps extracts from the Amazonian fruits. The highest percentages of inhibition were observed for abiu oil with 16.75% inhibition and araçá (15.97%), being very low values in relation to the standard ampicillin that showed 96.69% inhibition. According to Cordeiro (2011) *S. aureus* is present in both barks and mucous membranes, being considered as a causal agent of 7.7% of outbreaks of food poisoning in Brazil (Brazil, 2015). Morais (2018) developed a study of biological activity for peppers from species *Capsium* spp. from the Amazonian region finding percent inhibition of *S. aureus* bacteria (13.71%) in line with the values found in this work.

B. cereus is present in food since it is resistant to the process of pasteurization. This bacterium reaches the environment easily contaminating food when the appropriate processing conditions are not used. It produces different toxins that shave human health when consumed in foods contaminated by this microorganism in concentrations of 105 to 108 CFU per gram of food (GRANUM et al., 1997).

Table 2- Inhibitory potential of pulps oils and extracts against yeast, *bacterium* and AChE.

Samples	<i>C. albicans</i> ATCC 18804	<i>S. aureus</i> ATCC 29212	<i>B. cereus</i> ATCC 11778	<i>E. coli</i> ATCC 25922	<i>S. typhimurium</i> ATCC 14028	AChE
% inhibition						
<i>Abiu</i>	76.71± 5.62	25.85 ± 2.67	16.75 ± 1.80	25.33 ± 2.29	2.28 ± 1.18	29.28 ± 3.03
<i>Acerola</i>	0.00	17.98± 2.96	6.68 ± 2.70	21.65± 10.35	8.64 ± 3.99	52.18 ± 6.30
<i>Araçá</i>	64.28 ± 11.74	25.04 ± 1.55	15.97 ± 2.51	24.09 ± 2.17	13.20 ± 1.45	27.40 ± 4.38
<i>Bacupari</i>	N.D.	N.D.	N.D.	N.D.	N.D.	20.08 ± 4.41
<i>Biribá</i>	N.D.	8.32 ± 0.15	8.22 ± 0.14	15.61 ± 1.27	5.04 ± 0.79	43.42 ± 2.15
<i>Camu-camu</i>	20.52 ± 14.81	28.80 ± 2.49	N.D	30.68 ± 2.48	28.22 ± 4.48	N.D
<i>Fruta-do-conde</i>	16.67 ± 4.27	23.20 ± 3.03	N.D	14.41 ± 1.12	13.56 ± 2.69	25.71 ± 1.19
<i>Graviola</i>	N.D	15.35 ± 4.85	N.D	16.28 ± 0.07	12.79 ± 1.04	44.10 ± 0.62
<i>Taperebá</i>	85.22 ± 19.60	17.36 ± 3.43	13.06 ± 10.87	15.57 ± 3.54	2.73 ± 1.04	40.83 ± 3.60
Standard	Miconazole		Ampicillin			Eserine
	82.82 ±13.65	98.8 ± 1.90	96.69 ± 4.94	96.03 ± 0.59	96.00 ± 0.84	80.04 ± 0.18

N.D.= no detected

For the two Gram-negative bacteria that were tested for the oils, *E. coli* was more inhibited, however in low percentages in relation to the tested standard ampicillin (inhibition = 96.03%). *E. coli* causes different types of diarrheogenic diseases (Kuhnerst et al., 2008). The highest inhibition values were presented for the oil obtained from *camu-camu* pulp with 30.68% and *abiu* pulp with 25.33% inhibition. The other Gram Negative bacterium utilized in this screening was *S. typhimurium* which was also low inhibited by the oils tested. This bacterium is implicated in food poisoning problems causing gastrointestinal problems (Morpeth et al., 2009). Pulp of *camu-camu* was the best inhibitor of *S. typhimurium* (28.22% inhibition).

From the microorganisms tested in this study the yeast *C. albicans* was the most susceptible microorganism to the oils tested. For instance, *taperebá* oil presented 85.22% inhibition, a percentage higher than that obtained for miconazole, the standard tested (82.82%), followed by *abiu* oil (76.71%) and *aracá* oil (64.28%). From the health point of view, it is interesting to look for new natural compounds with the capacity to inhibit this yeast since it causes candidiasis in the human body, an infection that can manifest in both oral and vaginal mucosa. *C. albicans* is the yeast from *Candida* genus predominant in the infection of candidiasis with 50%. *C. glabrata*, *C. parapsilosis* and *C. tropicalis* are the minor yeasts present in that infection. The worldwide mortality rate due to diseases due to yeasts from *Candida* genus is between 15-25% in adults and 10-15% in children (Alangaden, 2011).

As for the potential inhibition of acetylcholinesterase enzyme (AChE) by the oils and fats of the nine pulps, *acerola* pulp was the one with the most potent inhibition of AChE. *Biribá*, *graviola* and *taperebá* presented moderate inhibition potential and the other samples presented weak AChE inhibition potential (Vinutha et al., 2007) since it was considered that, for crude vegetable extracts, values above 50% mean potent inhibitors; between 30 and 50% are the moderate inhibitors and below 30% are weak inhibitors on AChE.

Table 3- Inhibitory potential of barks oils and extracts against yeasts, *bacterium* and AChE

Samples	<i>C. albicans</i> ATCC 18804	<i>S. aureus</i> ATCC 29212	<i>B. cereus</i> ATCC 11778	<i>E. coli</i> ATCC 25922	<i>S. typhimurium</i> ATCC 14028	AChE
	% inhibition					
Abiu	48.13 ± 16.22	20.53 ± 1.17	12.69 ± 1.90	21.53 ± 2.67	8.62 ± 1.10	35.31 ± 4.22
Acerola	87.12 ± 25.70	12.91 ± 2.20	5.88 ± 2.13	24.65 ± 3.84	19.01 ± 0.11	N.D
Araçá	N.D.	N.D	N.D	N.D	N.D	N.D
Bacupari	31.85 ± 11.79	11.84 ± 2.71	38.70 ± 3.35	17.7 ± 5.21	8.12 ± 2.45	N.D
Biribá	39.14 ± 9.56	18.77 ± 2.88	N.D	13.42 ± 1.77	15.09 ± 2.55	86.39 ± 8.76
Camu-camu	71.35 ± 16.35	35.72 ± 1.95	14.06 ± 5.17	13.12 ± 5.67	28.04 ± 4.40	N.D
Fruta-do-conde	N.D	15.28 ± 2.05	N.D	11.70 ± 1.90	N.D	43.22 ± 3.06
Graviola	30.75 ± 1.96	14.6 ± 4.90	N.D	16.28 ± 0.07	11.53 ± 2.28	29.45 ± 3.18
Taperebá	94.46 ± 7.82	23.26 ± 1.39	19.60 ± 5.93	18.91 ± 4.55	5.85 ± 2.00	56.88 ± 2.32
Standard	Miconazole		Ampicillin			Eserine
	82.82 ±13.65	98.8 ± 1.90	96.69 ± 4.94	96.03 ± 0.59	96.00 ± 0.84	80.04 ± 0.18

N.D. = not detected.

The inhibitory potential of the oils and extracts of the barks against *S. aureus* was lower than for the oils extracted from the corresponding pulps, since the samples tested did not reach 25% inhibition. *Taperebá* presented the highest percentage of inhibition against *S. aureus* (23.26%) while the tested standard ampicillin presented 98.8%. For the other Gram-positive bacterium tested (*B. cereus*), the oils from barks also presented low inhibition, the major percentage of inhibition found for the oil extracted from *bacuparí* barks with 38.70% of inhibition.

Form the two Gram-negative bacteria tested, *E. coli* presented the highest percentage of inhibition, but the values found are still low in relation to the tested standard ampicillin. The better inhibition was presented by *acerola* extract with 24.65% of inhibition followed by *abiu* barks oil with 21.53% inhibition. For *S. typhimurium*, the highest percentage of inhibition was for the oil from *camu-camu* barks (28.04%) followed by oil from *acerola* barks oil (19.01% inhibition). Among the microbiological inhibition tests for fruits barks, the best results were obtained towards the yeast *C. albicans*. The oil from barks of *taperebá* presented a potent antimicrobial effect with 94.46% inhibition. This value is even higher than that obtained for miconazole, the standard tested that showed 82.82% inhibition. The oil from barks of *acerola* inhibited 87.12% of *C. albicans* growth, which was also superior to the inhibition presented by the standard tested. Another extract very active against this yeast was that from *camu-camu* barks with 71.35% inhibition.

Concerning the barks of the studied fruits, the oil extracted from *biribá* barks presented good inhibition of AChE (86.39%), even higher than the value found for the positive standard serine, in the conditions utilized in this test. The oil of *taperebá* barks also presented potent inhibition while *abiu* barks and *fruta-do-conde* presented moderate inhibition. Mota et al. (2012) tested different ethanolic extracts of medicinal plants in Brazil for the inhibitory capacity towards the AChE enzyme. In that study, the potent inhibition of AChE by the aqueous extract of *Vitex agnus-castus* L. was highlighted (74%), a value slightly lower than that found in this work for *biribá* barks oil.

Table 4- Inhibitory potential of oils and extracts of the seeds against yeasts, bacterium and AChE

Samples	<i>C. albicans</i> ATCC 18804	<i>S. aureus</i> ATCC 29212	<i>B. cereus</i> ATCC 11778	<i>E. coli</i> ATCC 25922	<i>S. typhimurium</i> ATCC 14028	AChE
	% inhibition					
<i>Abiu</i>	59.87 ± 10.33	22.13 ± 2.19	12.07 ± 3.71	22.76 ± 2.04	2.96 ± 0.94	N.D.
<i>Acerola</i>	N.D.	26.78 ± 2.39	17.28 ± 1.72	27.99 ± 2.28	13.21 ± 0.31	30.19 ± 6.04
<i>Araçá</i>	85.23 ± 13.11	25.30 ± 3.43	13.07 ± 1.85	25.55 ± 3.40	16.94 ± 2.46	22.71 ± 4.97
<i>Bacupari</i>	N.D.	16.47 ± 1.37	26.73 ± 3.79	17.51 ± 3.05	14.29 ± 1.93	N.D.
<i>Biribá</i>	N.D.	7.95 ± 4.08	7.85 ± 2.27	13.04 ± 2.90	13.82 ± 0.24	59.34 ± 7.48
<i>Camu-camu</i>	39.26 ± 14.61	27.11 ± 3.74	N.D.	18.76 ± 3.86	19.13 ± 1.57	33.10 ± 6.10
<i>Fruta-do-conde</i>	N.D.	24.89 ± 4.34	N.D.	12.71 ± 3.39	17.50 ± 2.80	54.49 ± 4.93
<i>Graviola</i>	N.D.	15.18 ± 0.90	N.D.	13.50 ± 4.05	10.00 ± 0.95	48.88 ± 3.29
<i>Taperebá</i>	7.44 ± 0.32	17.07 ± 3.21	N.D.	13.63 ± 4.36	15.35 ± 2.31	62.17 ± 5.14
Standard	Miconazole			Ampicillin		Eserine
	82.82 ± 13.65	98.8 ± 1.90	96.69 ± 4.94	96.03 ± 0.59	96.00 ± 0.84	80.04 ± 0.18

N.D. = not detected.

The inhibitory potential of the oils and extracts prepared from the seeds (Table 4) was low. The oil of *camu-camu* seeds presented the best behavior but inhibition of *S. aureus* was only 27.11% while ampicillin achieved 98.8% inhibition, while for *S. cereus*, *bacuparí* seeds inhibited 26.73% followed by the *acerola* seed with 17.28% (ampicillin with 96.69% inhibition).

The Gram Negative *bacteria* were again, only slightly inhibited by the extracts. *E. coli* was more susceptible to the oil of *acerola* seeds (27.99% inhibition) and *aracá* seeds (25.55%), values very low compared to positive control (96.63%). For *S. typhmuri*, inhibition by seeds extracts and oils were still lower (19.13% inhibition for *camu-camu* oil). Only four of the nine oils and extracts from seeds inhibited *C. albicans*. *Aracá* oil was slightly more active (85.23% inhibition) than miconazole (82.82% inhibition).

Biribá, *taperebá* and *fruta-do-conde* oils presented potent inhibitory potential towards AChE according to the classification proposed by Vinutha et al. (2007). *Acerola*, *graviola* and *camu-camu* seeds presented moderate potential and the remaining seed oils presented weak inhibitory potential or did not present any inhibition of enzyme AChE.

Dos Santos et al. (2015) studied the bioactive potential of *Annona hypoglauca* seeds, another species of *Annona*, finding 79.55% of AChE inhibition, which is higher than the results found for the Annonaceae seeds studied in the present work. The percentage of *C. albicans* inhibition reported for *A. hypoglauca* (90.11%) while for the bacteria *E.coli* and *S. aureus*, literature results agree with those presented in this work.

3.1 STATISTICAL ANALYSIS

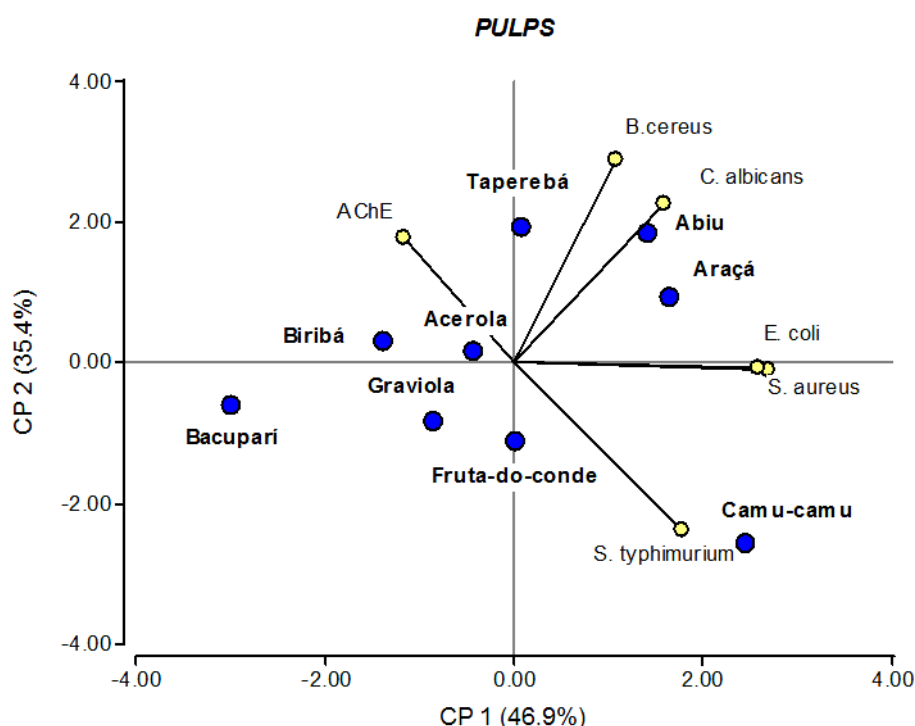
Firstly, the coefficient was calculated to evaluate the consistency of the hierarchical groupings, obtaining a value of 0.973, and values close to the unit indicate that a better representation (Ferreira et al., 2002; Cruz et al., 2003; Moura et al., 2006).

3.2.1 Principal component analysis (PCA)

The main components analyses were carried out jointly for the evaluated systems (*abiu*, *bacuparí*, *acerola*, *graviola*, *camu-camu*, *fruta-do-conde*, *araçá*, *biribá*

and taperebá) independently for each part of the fruits for *C. albicans*, *S. aureus*, *B. cereus*, *E.coli*, *S. typhimurium* and AChE in the different parts of the fruit) in order to find variables (main components), which are not correlated to explain the structure of the variation. The weight of each variable analyzed in each component (axes) is represented. The main components biplot for the different parts of the evaluated fruit are shown in Figures 1-3.

Figure1- Distribution of the original variables among the different fruits pulp on the first and second main component (CP1 and CP2).



In Figure 1, the correlation of the two main components PC1 and PC2 is shown for the percentage of inhibition of the different oils and extracted from the pulps of the nine fruits compared to the different microorganisms tested (yeasts, bacteria) and the inhibition of the AChE enzyme where PC1 shows more information, with a greater variance value (46.9%) and PC2 carries the maximum part of residual information with a value of 35.4% for variance, with 82.3% of the total variance between the different oils and extracts tested for the different microorganisms and AChE in the different fruit pulps studied.

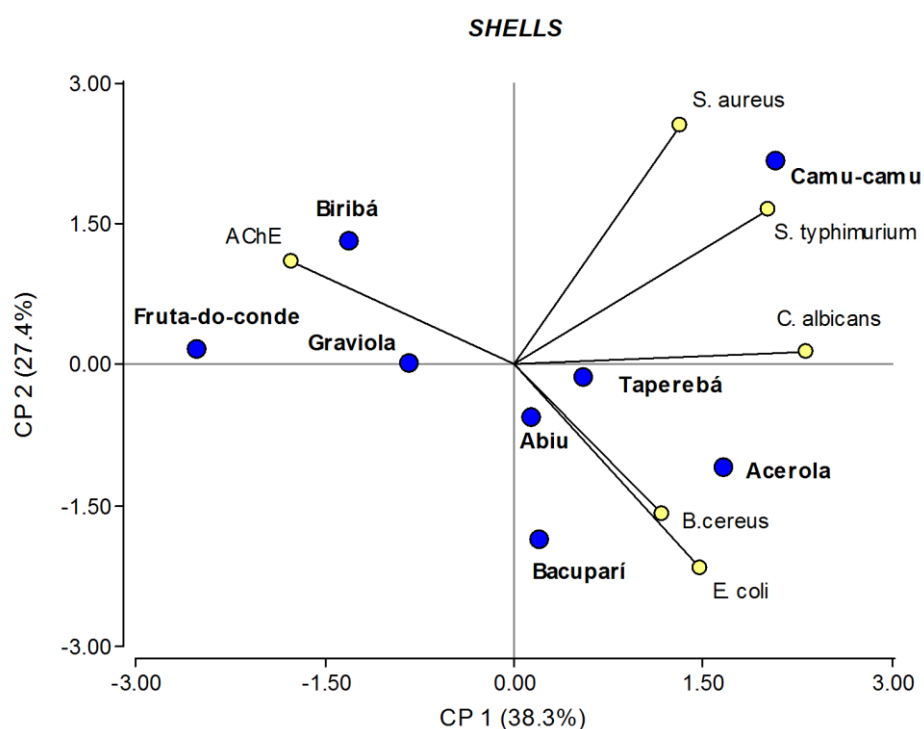
The variables *B. cereus*, *C. albicans*, *E. coli*, *S. aureus* and *S. typhimurium* are those that contribute in a more significant way to the first main component, presenting a positive score for the first main component and therefore presenting

higher variations to the average. The oils and extracts from the *abiu* pulps and *araçá* pulps present a positive and similar contribution for *B. aureus* and *C. albicans*, as opposed to the percentage of AChE inhibition, which has a negative contribution for the first principal but positive component for the second main component, being related to the value of AChE the *acerola* and *biribá* pulp oil.

In the second main component (CP2), the oil that contributes most positively to that component, is the *taperebá*, and in contrast, who contributes with less value to this component, with a highly negative contribution is the *fruta-do-conde* oil. In contrast to the variable AChE that presents a positive contribution for the second main component, *S. typhimurium*, presents a highly negative contribution for the second main component, being related to that parameter in the *camu-camu* oil and to a lesser extent the *fruta-do-conde* oil.

In the CP1 and CP2 planes we have *E. coli* and *S. aureus*, so the covariance is zero for these two variables, not contributing to the main components. In the fourth quadrant, *graviola* and *bacupari* pulps oil, present a similar behavior but are not characterized by any of these variables.

Figure 2- Distribution of the original variables between the different barks fruits on the first and second main components (CP1 and CP2).

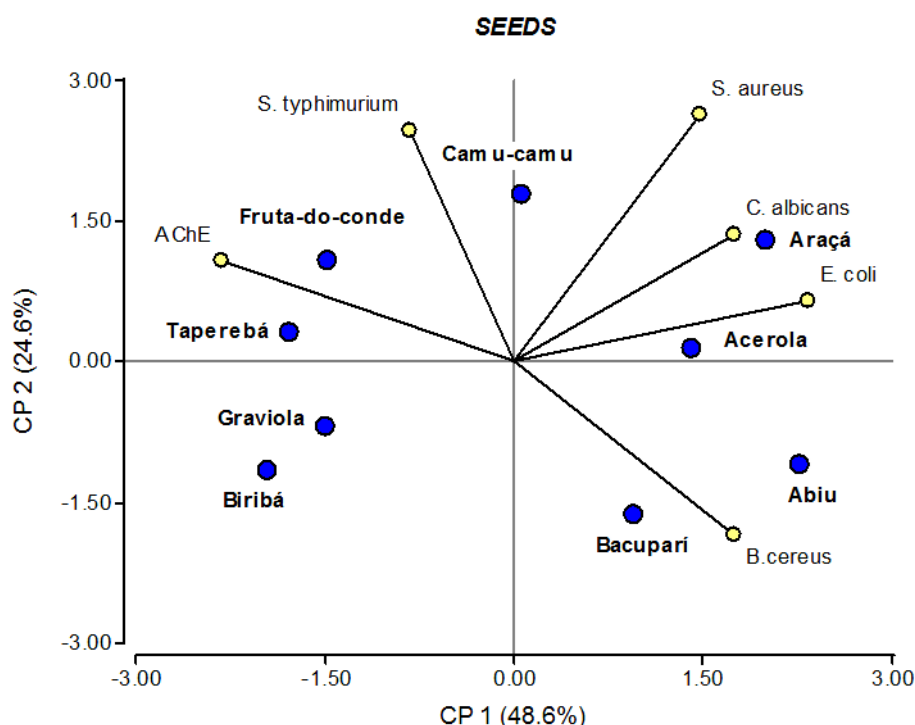


In Figure 2, the correlation of the two main components PC1 and PC2 is shown for the percentage of inhibition of the different oils and extracted from the barks of the nine fruits compared to the different microorganisms tested (yeasts, bacteria) and the inhibition of the enzyme AChE, where PC1 shows more information, with a greater variance value (38.3%) and PC2 carries the maximum part of residual information with a value of 65.7% for variance, with 67.7% of the total the variance between the different correlated parameters for oils and extracts obtained from the fruit barks studied.

Unlike those extracted from pulps, for the barks, the variables *S. aureus*, *S. typhimurium*, *C. albicans*, *B. cereus* and *E. coli* contribute the most to the first main component, and therefore, present variations above the average, where there is a strong correlation of the percentage of inhibition of the *camu-camu* extract barks for the microorganisms *S. aureus*, *S. typhimurium* and *C. albicans*. On the other hand, inhibition against bacteria such as *B. cereus* and *E. coli*, have a strong contribution for the first main component for oils and acerola, abiu, taperebá and bacuparí extract barks, have a strong positive correlation for the first component main and opposed with AChE where there is a strong negative correlation for the first main component.

For the second main component, the oils and extracts that contribute in a more positive way are the *camu-camu* barks, followed by the *biribá* barks. For the percentage of inhibition of the enzyme AChE, the extracts of *biribá*, *fruta-do-conde* and *graviola* present similarity in terms of the percentage of inhibition, unlike *E. coli* and *B. cereus*, which contribute in a negative and contradictory way for said main component.

Figure 3. Distribution of the original variables among the different fruits for the seeds on the first and second main component (CP1 and CP2).



In figure 3, the correlation of the two main components PC1 and PC2 is shown for the percentage of inhibition of the different oils and extracted from the seeds of the nine fruits against the different microorganisms tested (yeast, bacteria) and the inhibition of AChE, where PC1 shows more information, with a higher variance value (48.6%) and PC2 carries the maximum part of residual information with a value of 24.6% for variance, with 73.2% of the total variance between different percentages of microbial inactivation and AChE for the oils and extracts tested for the seeds of the nine fruits studied.

In the first main component, the microorganisms *S. aureus*, *C. albicans*, *E. coli* and *B. cereus* are those that contribute in a more significant way to the first main component, presenting the araçá and acerola seeds extract values close to the inhibition of those microorganisms. *B. cereus*, who presented a high value for the first main component, shows that the abiu and bacuparí extract seeds have similarity in terms of the inhibition of that microorganism.

In the second main component, the sample that presents a greater contribution for that component is the *camu-camu* oil extract, thus not contributing to the first main component since it is at the intersection of the two main components

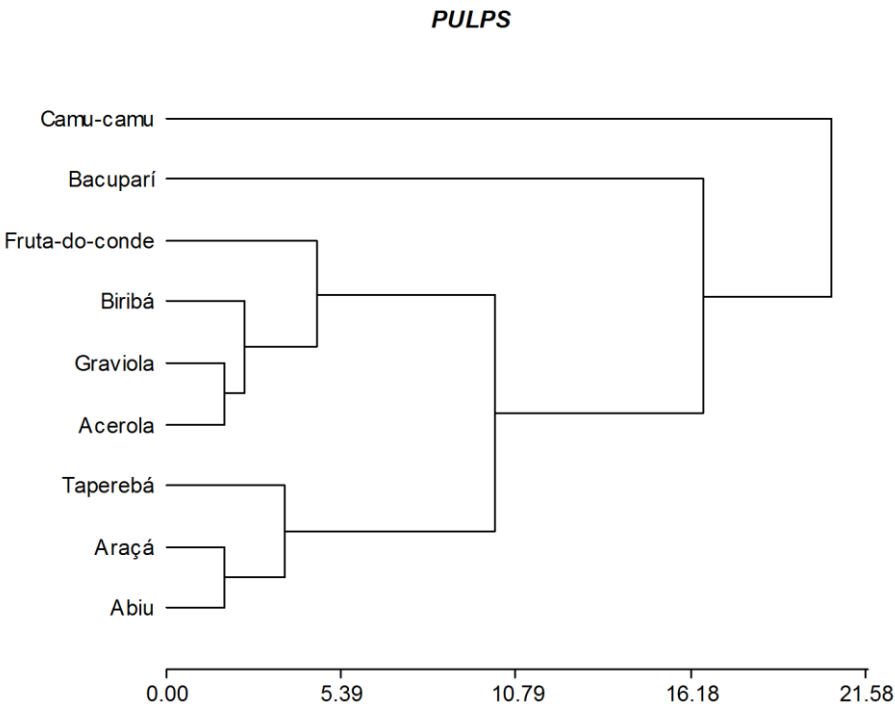
CP1 and CP2. The variables that have a strong positive contribution for the second main component is the fruta-do-conde and taperebá seeds oil, presenting values close to this variable the fruta-do-conde and taperebá seeds oil and opposed with *B. cereus* where the contribution that presents for this second main component is negative.

3.3 ANALYSIS OF HIERARCHICAL GROUPINGS (HCA)

Through HCA, data can be displayed in a two-dimensional space in order to emphasize their natural groupings and patterns, relating the samples so that the most similar are related to each other presenting the samples in dendrogram, grouping the samples and variables according to with their similarity. Figures 4-6 show the dendrograms for HCA analysis of inhibition of the different strata studied.

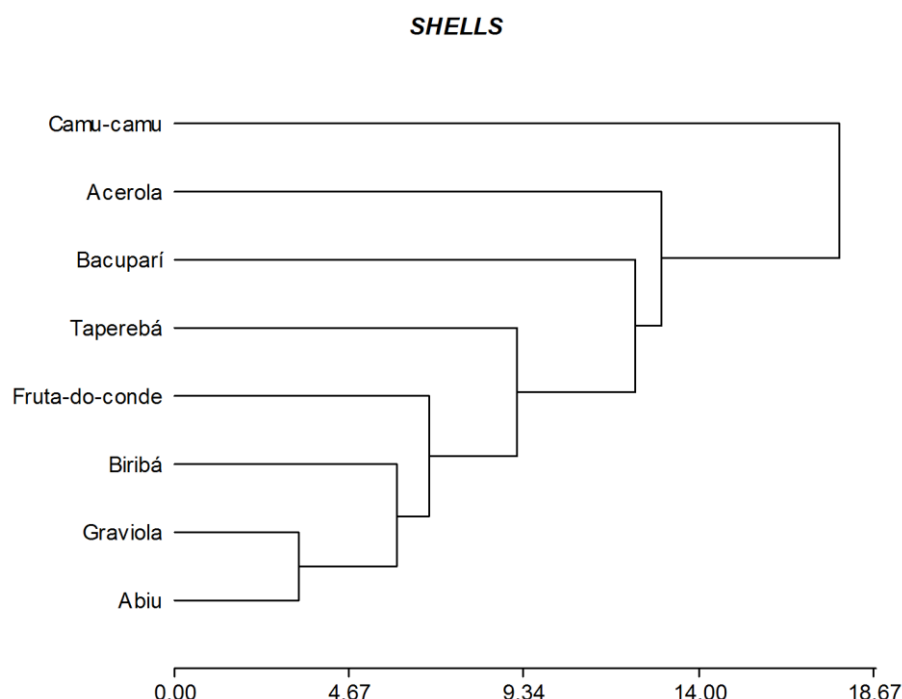
Figures 4-6 show the dendrograms for HCA analysis of the inhibition of the different strata studied.

Figure 4- Dendrogram by HCA, Euclidean distance and incremental connection technique for the percentage of inhibition present in fruits pulp extracts studied.



For the percentages of inhibition of oils and extracts of the fruits studied, the trends observed through the analysis of PCA main components were observed through the HCA, mainly observing two large groups: one of them formed by the association of *araçá* with *abiu* that present a major contribution smaller euclidean distance and they are grouped together with *taperebá* for a major euclidean distance, but are still associated. On the other hand, the other existing grouping just as happens in the HCA it is for *acerola* and *graviola* that they are strongly associated and increasing the euclidean distance they are associated with the *fruta-do-conde*. Finally, the two fruits whose extracts have opposing antimicrobial properties. which are the *bacuparí* pulp with the *camu-camu* pulp. are not related in the HCA joining the elevated euclidean distance of 21.58%.

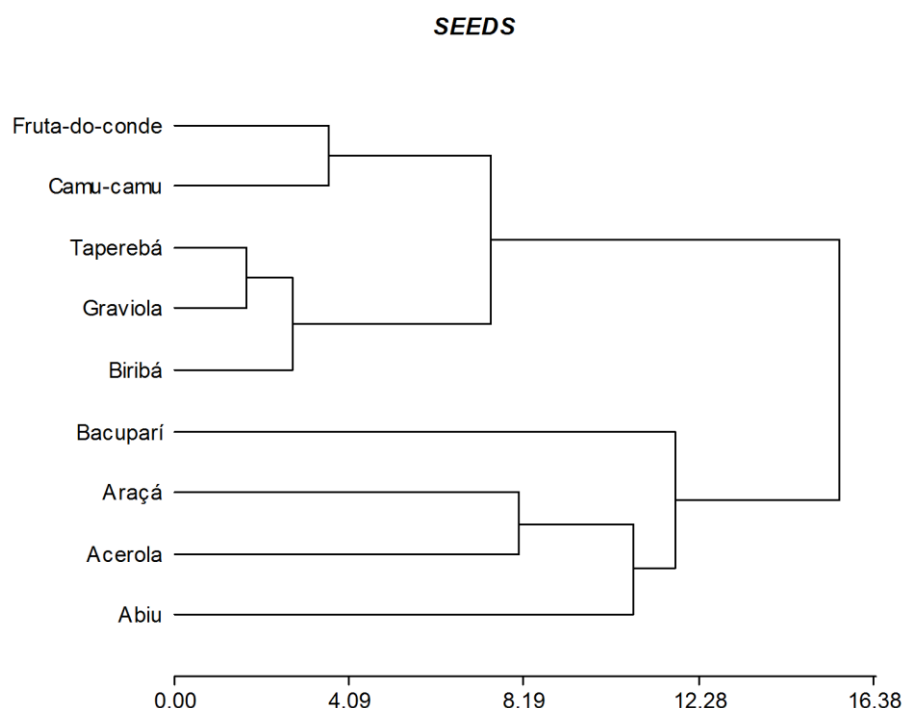
Figure 5- Dendrogram by HCA, Euclidean distance and incremental connection technique for the percentage of inhibition present in extracts of barks fruits studied.



For the percentages of inhibition of fruits oils and extracts studied the trends observed through the analysis of PCA main components of the barks were observed by HCA where there is great dispersion between the grouping of the studied fruits

where the main grouping to be highlighted with smaller euclidean distance is for *graviola* with *abiu*.

Figure 6-.Dendrogram by HCA, Euclidean distance and incremental connection technique for the percentage of inhibition present in seeds fruit extracts studied.



For the seeds two large clusters are observed being in agreement with the results presented by PCA. The first association is that between *taperebá* and *graviola* with smaller euclidean distance that are later associated with *biribá*. The other association with Euclidean distance of 4.09 is for the strata of the *fruta-do-conde* and *camu-camu*. All these fruits are grouped together and the HCA shows that they do not have a relation with or another group of fruits that contribute in an opposite way (*bacuparí*, *araçá*, *acerola* and *abiu*) whose connection happens with elevated euclidean distance of 16.38..

4 CONCLUSIONS

Given the potent results of the oils and extracts tested in this article against the potential inhibition of *C. albicans* yeasts such as the *barks* and *pulps* of *taperebá*. *acerola* barks or *araçá* seed, this can be a starting point for the development of new

drugs with specific action to minimize virulent factors making difficult the development of the infectious process of candidiasis. caused by the yeast *C. albicans*.

On the other hand, the results found for the antibacterial action of the oils and extracts did not present high inhibition percentage to inhibit the bacteria of pathogenic action.

Finally, in relation to inhibition of AChE, several extracts demonstrate a strong inhibitory capacity of the enzyme such as *biribá* barks, *taperebá* seed or *acerola* pulp, but in most of the studies, the isolated compounds responsible for the AChE inhibitory activity have not been identified or characterized.

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7 CONSIDERAÇÕES FINAIS

De acordo com os resultados obtidos nos diferentes capítulos desta tese de doutorado, podemos afirmar a importância do estudo das frutas amazônicas avaliadas nesse trabalho, já que existem poucos dados na literatura sobre a composição química nas diferentes partes das frutas, seus compostos bioativos e suas propriedades biológicas para serem utilizadas para fins biotecnológicos.

O conhecimento da composição química e aplicações biotecnológicas das frutas nativas da Amazônia e aquelas que tem sido introduzida, serve para valorizar esses produtos e aproveitar seu potencial como fonte de renda para as populações locais, já que são frutas de grande atrativo no exterior, podendo assim, abrir um grande mercado de produtos de qualidade com interesse funcional devido às propriedades que apresentam. Porém, existem poucos estudos completos sobre estas fruteiras em quanto a sua composição química e nutricional e seus benefícios para a saúde já que essas frutas, ainda não ocupam um papel relevante do seu potencial.

Ao mesmo tempo, a composição químicas de alimentos em geral e frutas em particular, a análise bromatológica é muito importante, tanto para saber seu aporte alimentício e calórico como sua ação no organismo. As sementes têm um aporte energético maior para o organismo seguido das peles onde os valores energéticos são maiores em relação à polpa. O valor é superior nas sementes devido principalmente à elevadas concentrações de óleo nas sementes. Dado que as sementes dessas frutas não são parte comestível com a exceção das sementes de araçá, esses resíduos podem ser utilizados na preparação de outros produtos com interesse biotecnológico.

Essas elevadas concentrações de ácidos graxos são destacadas principalmente nas sementes da família das *Annonaceae*. Uma característica de todas as frutas estudadas neste trabalho, é a presença de concentrações elevadas de ácidos graxos insaturados em relação aos ácidos graxos saturados, sendo eles essenciais para o bom funcionamento do organismo, já que sua deficiência provoca doenças cardiovasculares e aumentam os níveis de concentração no sangue, sendo mais uma justificativa para seu aproveitamento na elaboração de bioproductos.

Além disso, as frutas são fonte principais de minerais tanto de macronutrientes como micronutrientes, sendo substâncias essenciais para nosso

organismo e para promover o bom funcionamento dos diferentes sistemas fisiológicos. Foram encontradas concentrações significativas dos diferentes nutrientes em todas as partes das frutas, destacando-se entre os macronutrientes, elevadas concentrações de potássio nas polpas e entre as micronutrientes altas concentrações de manganês e zinco na polpa do abiu. Na pele e cascas das diferentes frutas estudadas destacam novamente altas concentrações do potássio seguidas do cálcio e entre os micronutrientes destaca a elevada concentração de zinco, especialmente na casca do araçá. Por último, nas sementes das diferentes frutas novamente destacam as elevadas concentrações de potássio entre os macronutrientes seguidas do fósforo, sendo as sementes por outro lado, ricas em micronutrientes como ferro, zinco e cobalto em certas sementes em concentrações de traços.

Entre as moléculas com maior interesse como potencial biotecnológico, estão os compostos fenólicos os quais apresentam atividade antioxidante, sendo um ponto a favor das frutas, acrescentando assim seu valor, já que esses compostos ajudam na prevenção de doenças degenerativas que possuem alta ocorrência na população como câncer, aterosclerose, diabetes, artrite, malária e doenças cardiovasculares. Esses compostos fenólicos que apresentam atividade antioxidante, também estão relacionados com a prevenção do envelhecimento. As concentrações de compostos fenólicos determinadas nesta tese são elevadas, especialmente em frutas como o camu-camu, acerola, casca do abiu e polpa do araçá. Essas frutas que apresentam uma maior concentração de compostos fenólicos são ao mesmo tempo as que apresentam uma melhor atividade antioxidante.

Outra característica das frutas amazônicas estudadas, é a presença de moléculas bioativas como carotenoides e vitamina C. Os carotenoides apresentam funções indispensáveis para o desenvolvimento da vida como captação de luz e fotoprotetora. Por outro lado, eles entram dentro do grupo dos antioxidantes, atuando na neutralização de espécies reativas de oxigênio e nitrogênio produzidas no metabolismo celular assim como muitos de eles apresentam atividade provitamina A. Eles são encontrados em concentrações mais elevadas na pele que nas polpas e quase insignificante sua presença nas sementes, sendo justificados os maiores valores na pele pela função protetora que esses compostos apresentam. A vitamina C é outra das biomoléculas estudadas nesta tese, onde apresenta um

importante papel em certas frutas como o camu-camu e acerola, especialmente na polpa e na casca, mais também é também encontradas nas sementes.

Por último, neste trabalho foram avaliadas as propriedades biotecnológicas de óleos e extratos hexânicos para inibir a enzima acetilcolinesterasa, leveduras e bactérias, apresentando potente potencial inibitório da enzima acetilcolinesterasa para certas partes das frutas como a casca do biribá e a semente do araçá podendo ser utilizados esses óleos para desenvolver ensaios *in vivo*. Os outros testes que apresentaram resultados inibitórios bons, foram para a levedura *Candida albicans*, podendo ser aproveitados esses óleos e extratos como alvo para a fabricação de cremes contra as infecções dessas leveduras.

APÊNDICE

APÊNDICE A – Cadastro das amostras estudadas nesta tese no sistema nacional de gestão do patrimônio genético e do conhecimento tradicional associado SISGEN.



Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº A9DC64A

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	A9DC64A
Usuário:	ISMAEL MONTERO FERNÁNDEZ
CPF/CNPJ:	
Objeto do Acesso:	Patrimônio Genético
Finalidade do Acesso:	Pesquisa

Espécie

Spondias Mombin L.
Annona muricata L. Annonaceae
Pouteria oaimito (Ruiz & Pavón) Radlk.
Malpighia emarginata D.C
Psidium cattleianum Myrtaceae
Rheedia gardneriana Tr. & Planch.
Rollinia mucosa Jacq. Ball
Myrtilaria dubia (Kunth) Mo Vaugh, Myrtaceae
Annona squamosa L.

Título da Atividade: BIOPROSPECÇÃO DE FRUTAS NATIVAS AMAZÔNICAS COM POTENCIAL DE COMPOSTOS BIOATIVOS, CAPACIDADE ANTIOXIDANTE E ESTUDOS BIOLÓGICOS

Equipe

ISMAEL MONTERO FERNÁNDEZ	UFRR
Edvan Alves Chagas	EMBRAPA-RR
Antonio Alves de Melo Filho	Universidade Federal de Roraima
Rioardo Carvalho dos Santos	Universidade Federal de Roraima
Selvin Antonio Saravia Maldonado	Universidade Nacional de Agricultura (Honduras)

Data do Cadastro: 04/11/2018 16:48:57

Situação do Cadastro: Concluído



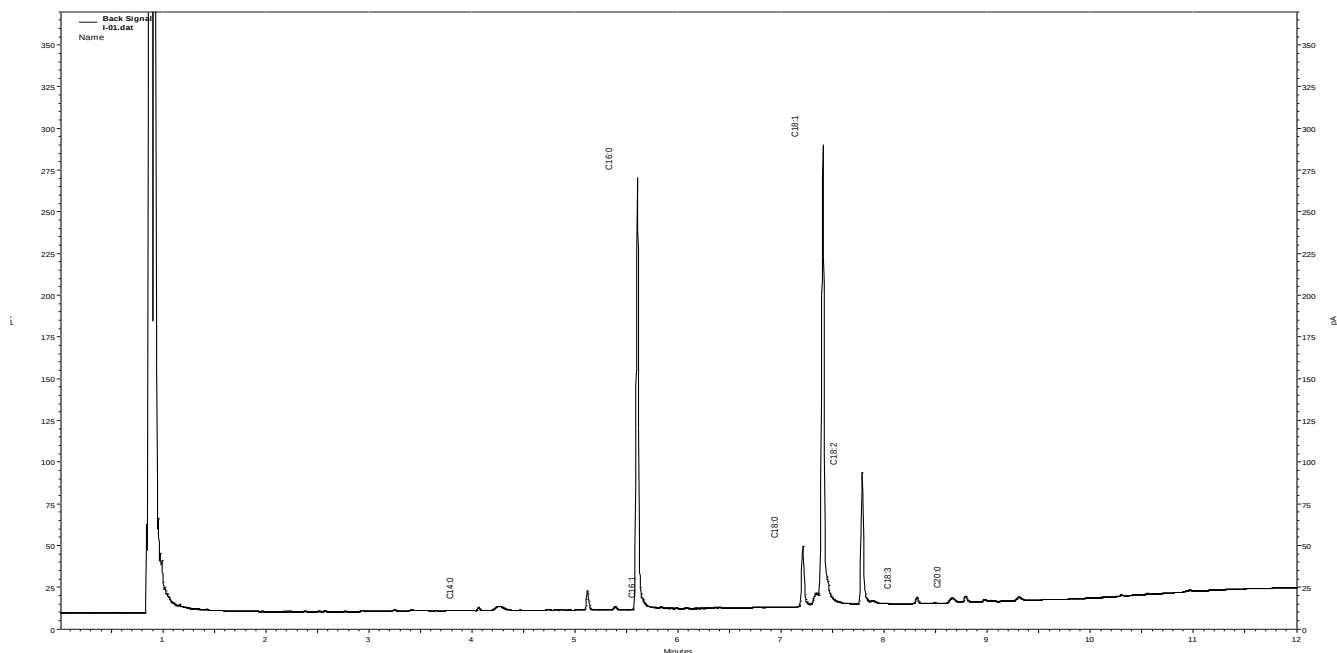
Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em 16:47 de 04/11/2018.



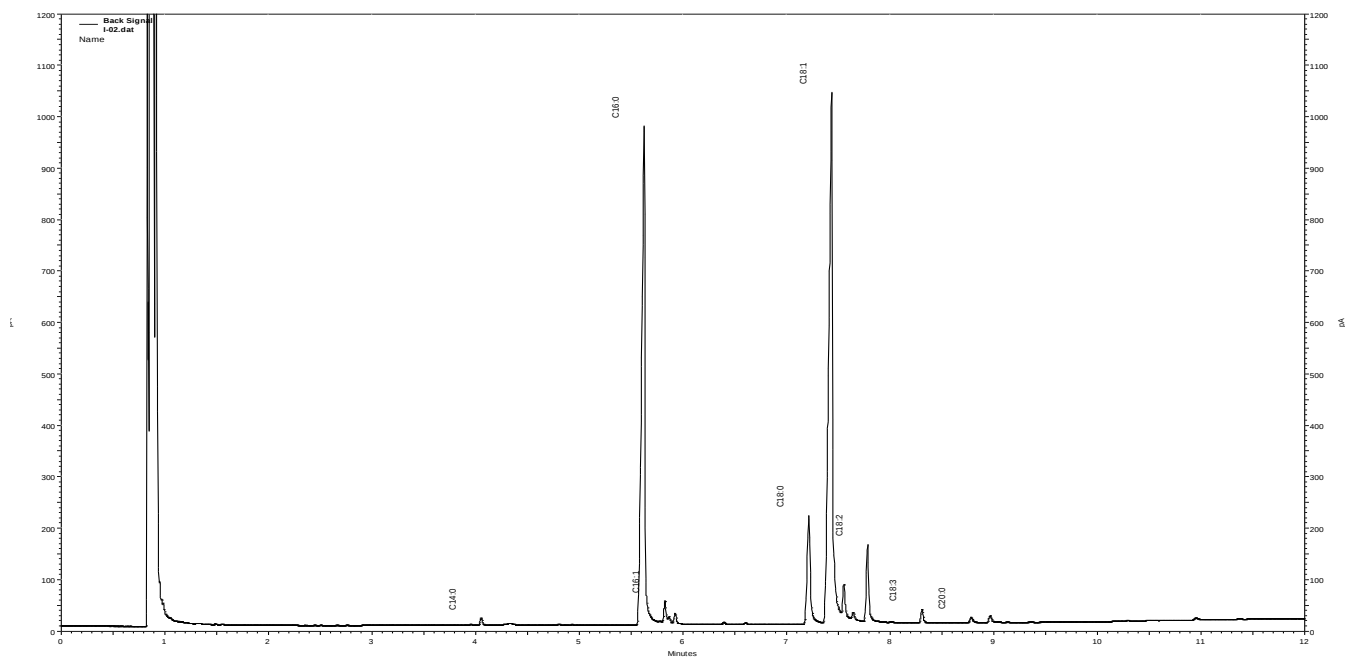
SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - SISGEN

APÊNDICE B – Cromatogramas por GC-FID de óleos e gorduras brutos de sementes de frutas amazônicas estudadas nesta tesis.

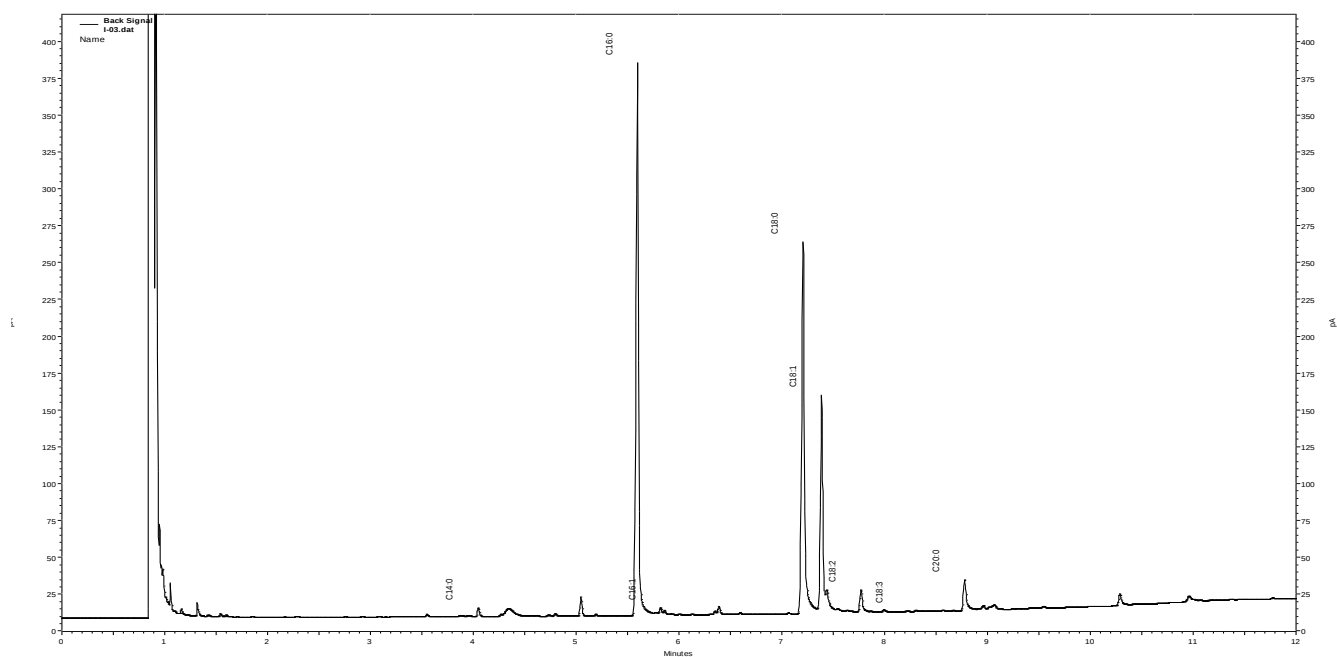
Abiu (*Pouteria caimito* (Ruiz & Pavón) Radlk.)



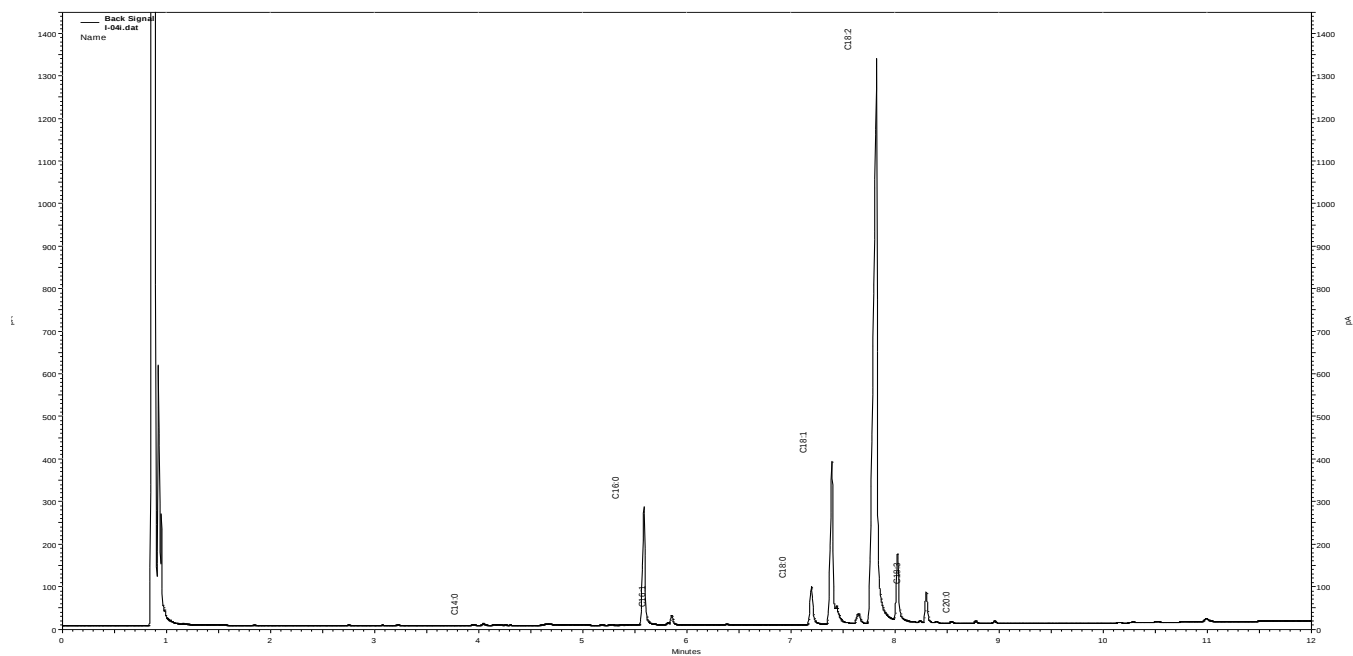
Bacuparí (*Rheedia gardneriana* Tr. & Planch) (Clusiaceae)



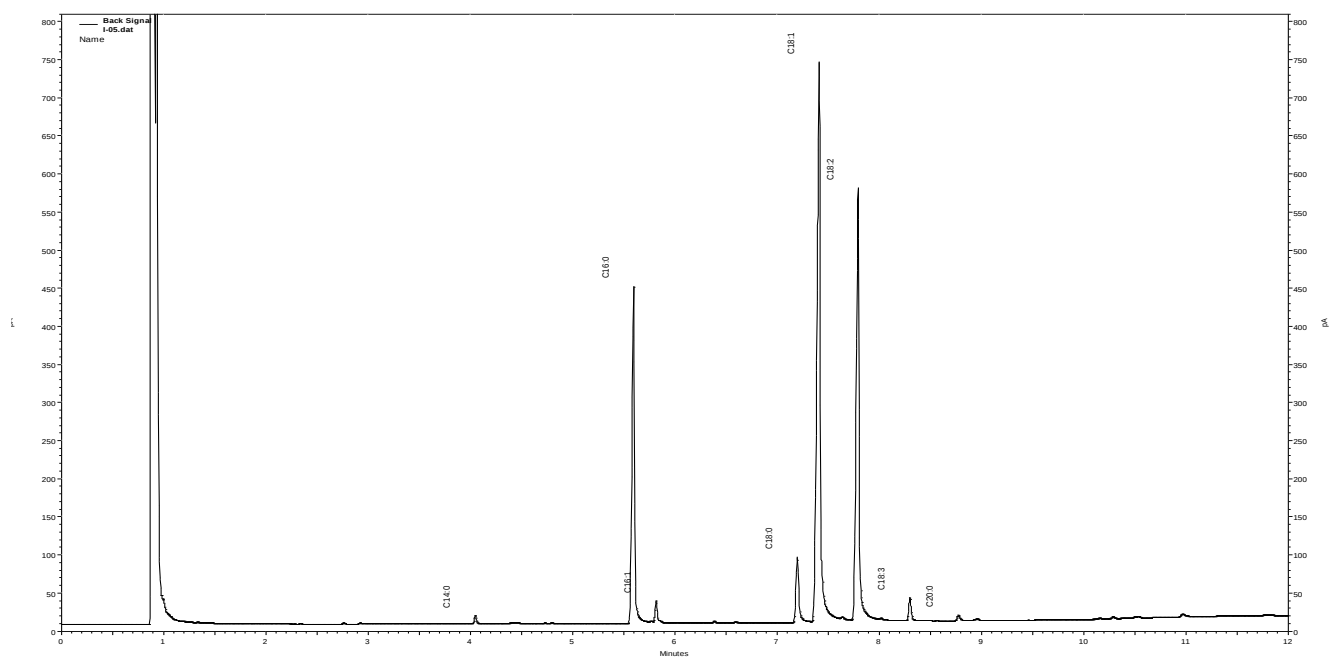
Taperebá (Spondias. Mombin L.)



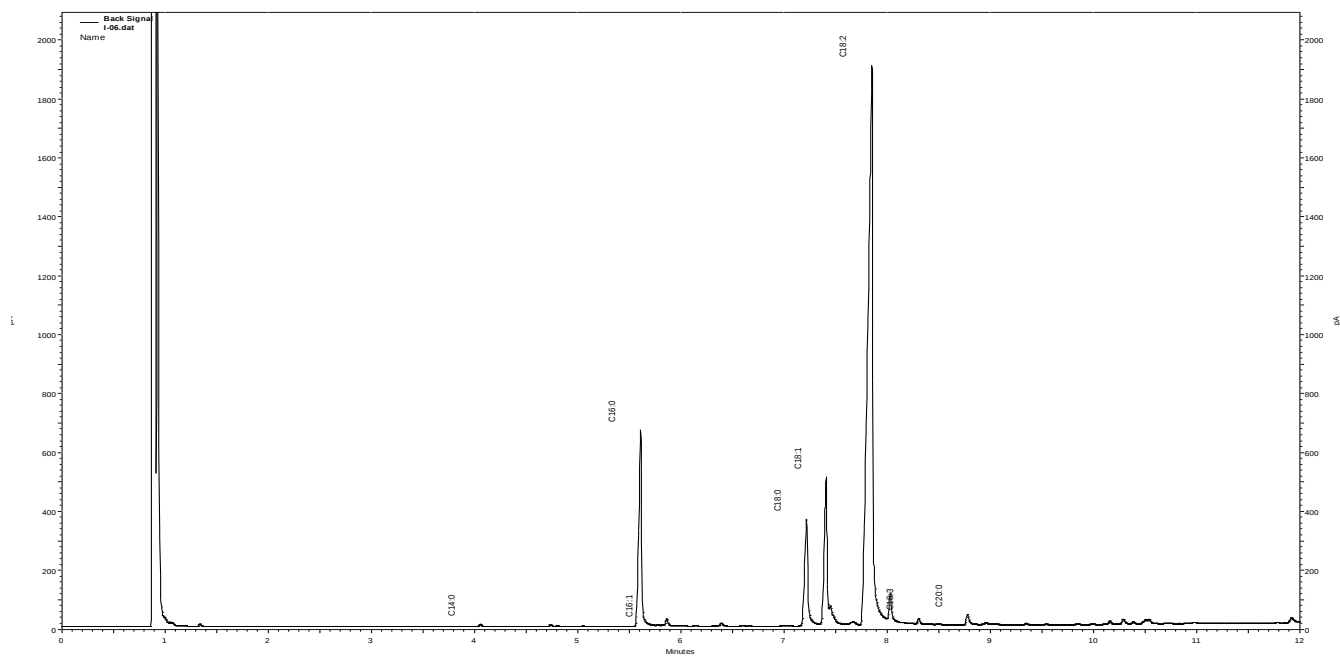
Araçá amarelo (Psidium catteaianum Myrtaceae).



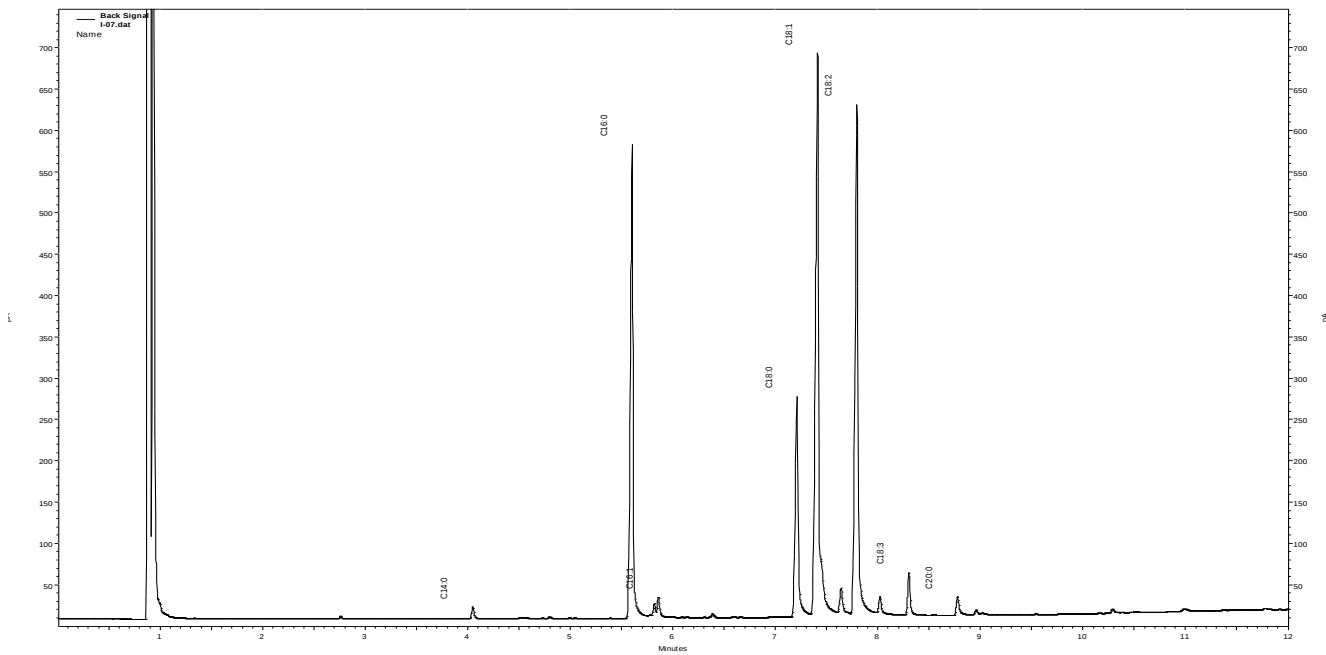
Graviola (*Annona muricata* L.) (Annonaceae)



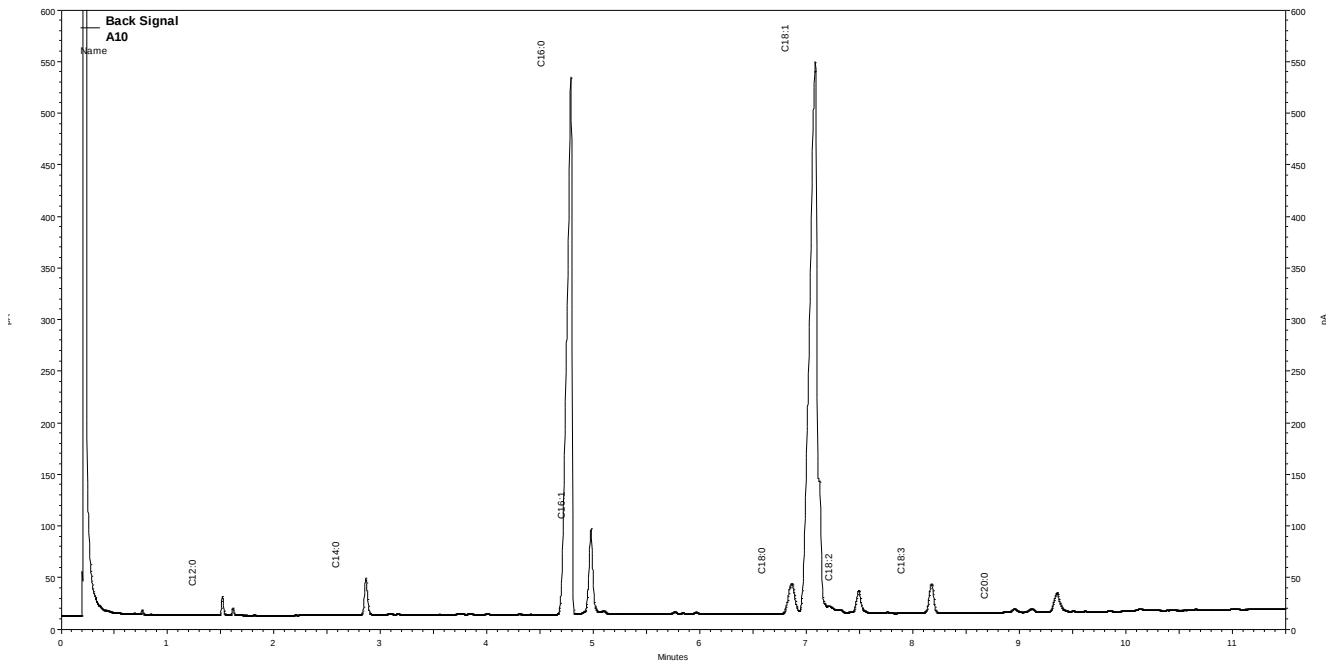
Camu-camu (*Myrciaria dúbia* (Krunth) Mc Vaugh), (Myrtaceae)



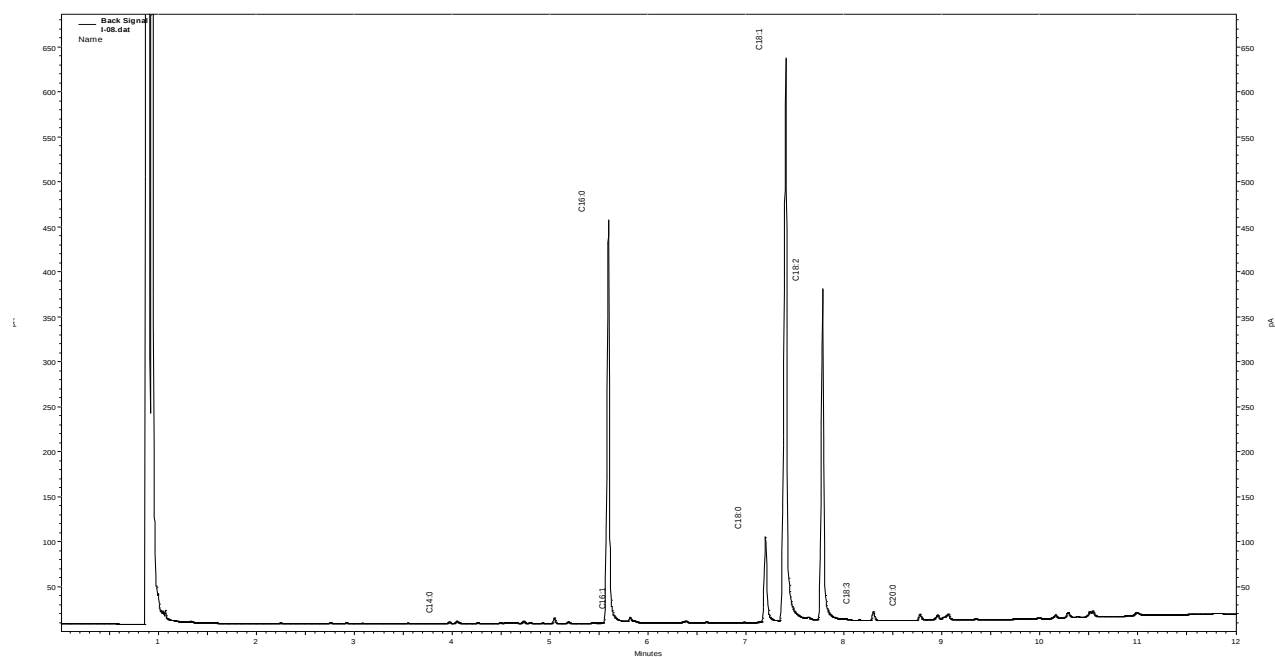
Acerola (*Malpighia emarginata* D.C.)



Biribá (*Rollinia mucosa* Jacq. Baill).



Fruta-do-conde (*Annona squamosa* L.).



APÊNDICE C – Trabalhos publicados em congressos derivados deste trabalho.

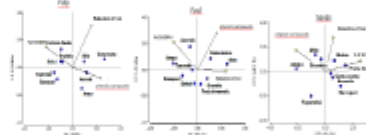


6th INTERNATIONAL CONFERENCE ON INDUSTRIAL BIOTECHNOLOGY 15 - 18 April, 2018 - Venezia, Italy



Evaluation of total phenolic compounds and antioxidant activity in Amazon fruit

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INTRODUCTION	RESULTS AND DISCUSSION
Phenolic compounds are part of secondary metabolites of plants, mainly showing the function of protecting the plant against organisms and pests, consequently, influencing the nutritional value of food, and sensorial quality, besides conferring physical-chemical attributes such as color, texture, bitterness and astringency (Everts et al., 2010). The phenolic compounds present bioactive potential as anti-inflammatory, antioxidant and antitumor (BOGANI et al., 2007; WENG AND YEN, 2012). In this way, the total phenolic compounds and the antioxidant potential will be evaluated and for nine fruits grown in the northern Amazon (abiu, acerola, camu-camu, bacupari, fruit of the earl, graviola, ananás, biribá and taperebá). Iron reduction method, and DPPH, as well as total phenolic compounds by the Folin-Ciocalteu method, and statistically correlated using multivariate analysis analysis technique PCA with Infostat program version 2016.	Taperebá seed with values of 7.31 ± 0.21 mg 100 g⁻¹ EGA, the bacupari with low values of phenolic compounds in all parts of the fruit, presenting 31.2 ± 1.1 mg 100 g⁻¹ EGA for the pulp, 85.4 ± 1.1 mg 100 g⁻¹ EGA for the seed and 70.21 ± 2.1 mg 100 g⁻¹ EGA for the waterfall. Camu-camu seed with 241.1 ± 7.4 mg 100 g⁻¹ EGA, the shell and seed of graviola with 427.3 ± 11.2 mg 100 g⁻¹ EGA for the waterfall and 632.3 ± 2.4 mg 100 g⁻¹ EGA for the seed and finally the taperebá pulp with 524.3 ± 11.4 mg 100 g⁻¹ and the taperebá cascade with 550.3 ± 10.1 mg 100 g⁻¹. The remaining samples showed high values of total phenolic compounds, with camu-camu with a concentration of 1241.1 ± 12.4 mg 100 g⁻¹ and pulp with 1741.2 ± 34.3 mg 100 g⁻¹, being the values within the ranges given by Meade et al. (2007).
MATERIAL AND METHODS	
The samples of the different species studied in this work were collected in the markets in several points of the State of Roraima, in the markets of the city of Boa Vista and directly with producers who owned the fruits. The samples were taken to the laboratory of Environmental Chemistry of the Federal University of Roraima, where they were selected those that had an excellent state of conservation. The determination of the total phenolic compounds (CPT) was done according to the methodology proposed by Wolke et al. (2013). The determination of the antioxidant activity in the different extracts was by different methods: the method of extinguishing the absorption of the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH); and the iron reduction method. The DPPH method was developed using visible ultraviolet molecular absorption spectrophotometry, measured at 515 nm (MIRANDA; FRAGA, 2006) in Shimadzu UV-1500.	 Figure 3: Distribution of the original variables between the different fruits on the first and second main components (C1 and C2).
CONCLUSIONS	
 Figure 1: Fruits studied in this work.	It is observed that several fruits such as camu-camu, acerola and bark of abiu, which present a good contribution of phenolic compounds as well as high free antioxidant activity, indicating the presence of bioactive compounds, being a great incentive to revalue these Amazonian fruits and elaboration of herbal products with phytotherapeutic interest or as functional foods.
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