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Metabolomic Approach Reveals the Nutritional Potential of *Brosimum gaudichaudii*, an Underexploited Native Brazilian Fruit

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Brazilian Savanna biodiversity is abundant in native fruits with nutritional and sensory properties with potential technological applications. However, the information available regarding the proprieties of Brazilian native fruits is still limited. This study aimed to analyze the metabolic profile of *Brosimum gaudichaudii* fruits (BGF) using metabolomics approaches, besides its physicochemical and nutritional proprieties. BGF exhibited protein content at least two times higher than other native fruits. Fifty-five metabolites were identified by the metabolomics approaches, mainly fatty acids (n = 22), carbohydrates (n = 13), organic acids (n = 6), and polyphenols (n = 5). The most abundant constituents were D-fructose (42.97%) for sugars, L-asparagine (6.47%) for amino acids, succinic acid (44.11%) for organic acids, and linolenic acid (3.04%) for fatty acids. Highlighted phytochemicals included chlorogenic acid, α-amyrin, and γ-tocopherol. Additionally, this study revealed that BGF is a good source of vitamin C and dietary fiber and an excellent source of vitamin A. Future studies providing nutritional information and suggesting consumption methods and potential industrial applications are essential to familiarize consumers with these foods. Additionally, clarifying the appropriate planting and harvesting seasons can revive traditional practices, especially for native fruits like BGF.

Keywords: *Brosimum gaudichaudii*, unconventional food plant, metabolite profiling, GC-MS, LC-MS, HPLC

Introduction

The Savanna biome encompasses approximately 22% of the Brazilian territory and is characterized by a rich biodiversity of native fruits, which possess nutritional and sensory properties with potential for technological applications. Despite this, only 30% of the Savanna biome's fauna and flora are well-documented. In recent years, there has been a growing interest in studying native fruits and developing new products derived from them. However, research on their physicochemical and nutritional characteristics remains limited.[1,2]

In this context, studies on the metabolite profile of native fruits, particularly *Brosimum gaudichaudii* fruits (BGF), are crucial for regional development, promoting consumption, and reviving traditional food culture.[3] BGF, belonging to the Moraceae family, is a native plant of the Savanna biome, commonly known as "*mama-cadela*", "*arbóreo de cadela*", or "*algodãozinho do campo*".[4] As an unconventional food plant, BGF fruits are not commonly consumed and are often neglected or underutilized by the population.[5] Various unconventional fruits, such as araça, buriti, cagaita, yellow mombin, mangaba, jatobá, jabuticaba, cambuci, pequi, pitanga, gabiroba, and lobeira, are notable for their

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nutritional and technological potential. However, BGFs remain underexplored.[1,3,6]

BGF has a pasty consistency, yellow-orange color, and sweet taste, and is typically consumed *in natura* or used in jams and ice creams. It is rich in soluble (SDF) and insoluble dietary fiber (IDF), as well as bioactive compounds such as phenolic compounds, carotenoids, and vitamin C, which possess antioxidant properties and have been linked to various health benefits. Additionally, BGF shows potential as an ingredient to enhance the nutritional quality of food products. However, it is crucial to characterize the physicochemical and nutritional properties of this native Brazilian fruit to identify its potential applications in food production.[2,7]

Studies on native or wild plants such as BGF have gained importance, as a deeper understanding of their chemical and metabolic composition can aid in species preservation and offer more precise nutritional recommendations for the local population, particularly within the Brazilian Savanna biome. However, the nutritional and physicochemical properties of BGF remain largely unexplored. Further research is needed to elucidate its potential for both direct consumption as a native fruit and its viability for industrial processing applications. Hence, the present work aimed to analyze the physical, physicochemical characteristics and to apply untargeted and targeted metabolomics approaches in order to detect the main nutrients found in BGFs.

Experimental

Sampling

BGFs (n = 5 kg) at mature stage were collected in standard commercial greenhouses in Uberlândia, Minas Gerais, Brazil (18°54'41"S; 48°15'44"W). Mature fruits were harvested when reached their completely yellow-orange coloring peel. All analysis were performed in edible part of BGF (pulp with a thin peel). Samples were frozen in liquid nitrogen and stored at −80 °C for further analysis.

Material and equipment

The standards used in this work were D-glucose, D-fructose, maltose, sucrose, D-galactose, myo-inositol, citric acid, malic acid, tartaric acid, L-alanine, L-serine, L-proline, L-aspartate, and L-glutamate, methyl laurate, methyl tetradecanoate, methyl palmitate, methyl octadecanoate, methyl arachidate, methyl docosanoate, methyl lignocerate, methyl linoleate, (*Z*)-9-oleyl methyl ester, methyl linolenate, methyl palmitoleate, lycopene, lutein, β-carotene, sodium hydroxide, metaphosphoric acid, phosphoric acid, methanol, Folin-Ciocalteau reagent, sodium nitrite, aluminium chloride, ribitol, chloroform, methoxyamine hydrochloride, pyridine, *n*-tridecane, sodium sulfate, hexane, toluene, hydrochloric acid, and *n*-methyl-*n*-(trimethylsilyl) tri-fluoroacetamide. All standards, reagents and solvents used were from Sigma-Aldrich, Saint Louis, MO, USA.

The equipment used in this work was Metrohm pH meter (827 pH Lab, Metrohm, Switzerland), refractometer (Krüss-Optronic GmbH, Germany), high-performance liquid chromatography (HPLC) with diode array detector (DAD) (Thermo Fisher Scientific, Dionex DX 500, USA), liquid chromatography (Prominence model, Shimadzu, Japan)-electrospray ionization-ion trap-mass spectrometry/mass spectrometry (Esquire HCT model, Bruker Daltonics, Germany) (LC-ESI-IT-MS/MS), and gas chromatography-mass spectrometry (GC-MS) (Agilent GC-MS 5977 instrument, Agilent Technologies, USA).

Methods

Physical characterization

BGFs (n = 40) were randomly picked up and the physical parameters were analyzed: total mass (g), seed (g), edible fraction (g), fruit yield (%), transverse diameter (cm) and longitudinal diameter (cm). Fruit yield was calculated as described by Meza *et al.*,[8] using the equation 1.

$$\text{Fruit yield (\%)} = [\text{pulp weight (g)}/\text{total mass (g)}] \times 100 \quad (1)$$

Physicochemical characterization

For the physicochemical parameters, pH, titratable acidity, and total soluble solids (TSS) content were analysed.[8] For pH analysis, 10 g of fresh sample were weighted, diluted in 100 mL distilled water and analyzed by a Metrohm pH meter. Titratable acidity was determined by titration with 0.1 M sodium hydroxide and the result expressed in grams of citric acid *per* 100 g of fresh weight (%). For TSS, fruit sample was filtered to retain solid particles and measured with a refractometer, and the result was expressed in Brix at 20 ºC.

Chemical composition and energy value

Contents of moisture, proteins, lipids, ash, and dietary fiber (DF) of fruits were determined in triplicate according to the standard methods.[9] Available carbohydrates were calculated by difference: 100 − (moisture + proteins + lipids + ash + DF). Results were expressed as g 100 g^{-1} on fresh and dry weights. To calculate energy, 4 kcal g^{-1} of

available carbohydrates, 4 kcal g^{-1} of proteins and 9 kcal g^{-1} of lipids were considered.

Soluble sugars by HPLC analysis

Soluble sugars were extracted with 80% ethanol (v/v) at 80 ºC. Extract was vapored at 45 ºC and the volume suspended with water. They were determined by HPLC coupled with a DAD, with Carbopac PA1 column (4 × 250 mm, 5 µm particle size), isocratic flow of 1 mL min^{-1} of 18 mmol L^{-1} sodium hydroxide for 25 min.[10] The calibration curves were prepared with D-glucose, D-fructose, and sucrose external standards.

Organic acids by HPLC analysis

Organic acids were extracted with 3% metaphosphoric acid in the ratio of 1:8 (m/v). The sample was homogenized, centrifuged and the supernatants were filtered on 0.45 µm membranes. They were determined by HPLC coupled with a DAD, equipped with a µBondpack C18 (300 mm × 3.6 mm internal diameter (i.d.), Waters, USA) and elution (flow rate of 0.5 mL min^{-1}) was carried out in isocratic conditions with 0.1% phosphoric acid, monitored at 210 nm.[10] The calibration curves were prepared with malic, citric and tartaric acids external standards.

Carotenoids by HPLC analysis

Carotenoids were quantified as described in detail by Meza *et al*.[11] Lycopene, lutein, and β-carotene were used as external standards. The results were expressed as mg of carotenoid *per* 100 g of fruit in fresh and dry weight.

Total phenolic compounds by spectrophotometric analysis

Extracts were prepared using 2 g of fruits mixed with 70% methanol and determined by Folin-Ciocalteau colorimetric method.[12] The extracts were mixed with Folin-Ciocalteau reagent and 0.5 mol L^{-1} sodium hydroxide. The absorbance was measured at 760 nm. The results were expressed in mg gallic acid equivalent (GAE) *per* 100 g of fruit in fresh and dry weight.

Total flavonoids by spectrophotometric analysis

Extracts were prepared using 2 g of fruits mixed with 70% methanol. Methanolic extract was mixed with distilled water, 5% sodium nitrite, 10% aluminium chloride, and 1 mol L^{-1} sodium hydroxide.[12] The absorbance was measured at 510 nm. The results were expressed in mg catechin equivalents (CE) *per* 100 g of fruit in fresh and dry weight.

Vitamin C by spectrophotometric analysis

Vitamin C was determined as described by Menezes Filho *et al*.[4] Sample was mixed with distilled water, 20% sulfuric acid, 10% potassium iodide (KI) and 1% starch solution. The solution was titrated with 0.002 mol L^{-1} KI. The result was expressed as mg of vitamin C *per* 100 g of fruit in fresh and dry weight.

Polyphenols by LC-ESI-IT-MS/MS analysis

Flavonoids and phenolic acids were extracted as described by Engelbrecht *et al*.[2] The identification of flavonoids and phenolic acids was performed by LC-ESI-IT-MS/MS, with a C18 column (300 mm × 3.6 mm i.d., Waters, Milford, MA). The ESI was maintained in negative mode and mass analyzer was programmed for full scan between *m/z* 100-1000. The collision energy for negative mode was 3000-3500 V. Polyphenol identification was performed using Data Analysis 4.0 software, comparing mass spectra with available databases: Mass Bank of North America and Mass Bank Europe.

Polar and non-polar metabolite profiling by GC-MS analysis

The analyses of polar and non-polar metabolites were conducted as described by Meza *et al*.[13] For polar metabolites extraction, fruits were mixed with methanol, ribitol, chloroform, and Milli-Q water. The upper hydrophilic phase was collected and dried under nitrogen gas. Methoxyamine hydrochloride (20 mg mL^{-1}) dissolved in pyridine was added to derivatize the samples. For non-polar metabolites extraction, sample was mixed with chloroform, methanol, and *n*-tridecane. Chloroform and 1.5% sodium sulfate were added to the mixture and centrifuged at 1000 rpm for 5 min at 4 °C, and dried with nitrogen gas. Hexane, toluene, methanol and 8% hydrochloric acid were used to reconstitute the samples. The hexane phase was collected and dried with nitrogen gas. Samples was reconstituted with hexane and pyridine. Previously to GC-MS analysis, *N*-methyl-*N*-(trimethylsilyl) tri-fluoroacetamide was added to the derivatized polar and non-polar metabolites samples. Derivatized samples were analyzed using an GC-MS equipped with HP5ms column (30 m × 0.25 m × 0.25 µm) under optimized conditions.[14] A pool of polar metabolite external standards was used: D-glucose, D-fructose, maltose, sucrose, D-galactose, myo-inositol, citric acid, L-alanine, L-serine, L-proline, L-aspartate, and L-glutamate. While, a pool of fatty acid methyl ester external standards was used: methyl laurate, methyl tetradecanoate, methyl palmitate, methyl octadecanoate, methyl arachidate, methyl docosanoate, methyl lignocerate, methyl linoleate, (*Z*)-9-oleyl methyl ester, methyl linolenate, and methyl palmitoleate. Data acquisition and deconvolution were conducted using MassHunter software (Agilent Technologies). NIST20 and Wiley 12th Edition mass spectral libraries were used for metabolite identification.

Contribution of BGF to dietary intake recommendations

The Dietary Guidelines for the Brazilian Population[15] was used as a criterion for calculating the portion of BGF (1 portion of fruit = 70 kcal = 68.14 g). BGF was classified as a "source", "good source" or "excellent source" of a given nutrient when it satisfied 5 to 10%; 10 to 20%; or over 20% of the dietary reference intake (DRI) for adults (both sexes) in the portion, respectively.[15]

Statistical analysis

This work was completely randomized with three biological replicates (each replicate was composed by 40 fruits) for all analysis. The results are presented as mean ± standard deviation and expressed as a descriptive statistic (mean, standard deviation and percentage of the abundance for metabolites) by using Graph Pad Prism 3.0 software.[16]

Results and Discussion

Physical and physicochemical characterization of BGF

The BGF is characterized by its yellow-orange color, round shape, thin skin, soft flesh, and a single seed.[4] The physical characterization of BGF is detailed in Table 1. The whole fruit had a total mass of 11.18 g, with the seed massing 3.63 g, and an edible fraction (pulp and peel) of 7.60 g, yielding 69.09% of the fruit. The transverse and longitudinal diameters of the fruits were 2.68 and 2.37 cm, respectively. As BGF is a wild fruit without standardized production and management, significant variation exists in these parameters. Edaphoclimatic differences, such as soil composition, air humidity, and climate, likely account for the observed physical variations in the fruit.[17]

Moreover, the physicochemical parameters of BGF were analyzed and reported in Table 1. BGF showed medium pH of 6.07, acidity of 5.14% and TSS of 20.07 ºBrix. Similar results of pH (5.96) and acidity (5.85%) were reported by Land *et al*.[18] The main soluble solids found in BGF are D-fructose (48.87%), D-glucose (30.30%) and sucrose (20.83%), which are parameters related to desired sensory characteristics such as taste and flavor, and consequent consumer acceptance.[8] Determining the physicochemical parameters of native fruits, particularly BGF, is crucial for the food industry. The balance between soluble solids and acidity levels directly impacts the quality of the fruit and its suitability for processing.[4]

Table 1. Physical, physicochemical, and nutritional parameters of *Brosimum gaudichaudii* fruits

Parameter	Fresh weight	Dry weight
Physical parameters		
Fruit (total mass) / g	11.18 ± 1.41	NA
Seed / g	3.63 ± 0.77	NA
Edible fraction (pulp + peel) / g	7.60 ± 1.01	NA
Fruit yield / %	69.09 ± 12.59	NA
Transverse diameter / cm	2.68 ± 0.17	NA
Longitudinal diameter / cm	2.37 ± 0.13	NA
Physicochemical parameters		
pH	6.07 ± 0.10	NA
Titratable acidity / (% citric acid)	5.14 ± 0.18	NA
Soluble solids / ºBrix	20.07 ± 0.29	NA
Chemical composition and energy		
Moisture / (g 100 g^{-1})	69.69 ± 0.43	NA
Proteins / (g 100 g^{-1})	3.25 ± 0.18	10.47 ± 0.70
Lipids / (g 100 g^{-1})	2.03 ± 0.02	6.71 ± 0.05
Ash / (g 100 g^{-1})	0.45 ± 0.09	1.49 ± 0.29
Soluble dietary fiber / (g 100 g^{-1})	1.23 ± 0.55	4.06 ± 1.82
Insoluble dietary fiber / (g 100 g^{-1})	5.71 ± 1.15	18.82 ± 3.80
Total dietary fiber[a] / (g 100 g^{-1})	6.94 ± 0.60	22.88 ± 1.98
Available carbohydrates[b] / (g 100 g^{-1})	18.02 ± 0.23	59.44 ± 0.76
Energy / (kcal 100 g^{-1})	102.73 ± 0.38	338.93 ± 1.24
Primary metabolites / (mg 100 g^{-1})		
Glucose	309.42 ± 27.39	1020.84 ± 90.38
Fructose	499.07 ± 20.39	1646.57 ± 67.27
Sucrose	212.68 ± 11.67	701.68 ± 38.51
Malic acid	1119.21 ± 73.62	3692.55 ± 242.91
Citric acid	378.71 ± 22.43	1249.46 ± 73.99
Tartaric acid	48.19 ± 2.49	158.99 ± 25.33
Secondary metabolites / (mg 100 g^{-1})		
Total phenolic compounds	74.04 ± 2.49	244.27 ± 8.22
Total flavonoids	1.15 ± 0.10	3.79 ± 0.32
Lutein	1.25 ± 0.17	4.14 ± 0.56
Lycopene	6.81 ± 3.93	22.48 ± 12.98
β-Carotene	10.15 ± 1.85	33.48 ± 6.09
Vitamin C	20.02 ± 0.97	66.06 ± 3.19

[a]Total dietary fiber calculation: soluble dietary fiber + insoluble dietary fiber. [b]Available carbohydrates calculation: 100 – (moisture + proteins + lipids + ash + total dietary fiber). Results expressed as mean ± standard deviation. n = 3 (each replicate was composed by 40 fruits). NA: not applicable.

Nutritional characterization of BGF

Chemical composition

The chemical composition of BGF was presented in both fresh and dry weight (Table 1). BGF had a high

moisture content (69.69 g 100 g^{-1}), which demonstrated the potential for the development of various products, such as jams, ice creams, and beverages as previously demonstrated Land *et al*.[18] The content of protein in BGF was 3.25 g 100 g^{-1}, while in dry weight was 10.47 g 100 g^{-1}. The protein recommendation for adults (both sexes) is 0.80 g of good quality protein *per* kg body weight *per* day, value obtained from available nitrogen balance studies. BGF had 2.21 g of protein *per* portion, so it is not considered a source of protein as it would only provide 3.94% of the daily recommendations for a 70 kg adult.[15] This result was expected, since fruits are not generally considered sources of protein. The lipid contents of these native fruits were 2.03 and 6.71 g 100 g^{-1} in fresh weight (f.w.) and dry weight (d.w.), respectively. The ash contents of BGF were 0.45 and 1.49 g 100 g^{-1} in f.w. and d.w., respectively.

The available carbohydrate contents (calculated by difference) of the BGF were 18.02 and 59.44 g 100 g^{-1} in f.w. and d.w., respectively (Table 1). The BGF can be considered a source of available carbohydrates as it provided 9.44% of the daily recommendation for adults in one portion of fruit (Table 2). In addition, the total dietary fiber (TDF) found in BGF was 6.97 and 22.88 g 100 g^{-1} in f.w. and d.w., respectively. From this total, IDF (5.71 and 18.82 g 100 g^{-1} in f.w. and d.w., respectively) was the fraction that contributed most when compared to SDF (1.23 and 4.06 g 100 g^{-1} in f.w. and d.w., respectively) (Table 1). The BGF were considered a good source of DF, as it provided between 12.44-15.73% and 18.88-22.48% of the daily recommendation for man and female adults in one portion of fruit, respectively (Table 2). In terms of energy, BGF had an energy density of 102.73 and 338.93 kcal 100 g^{-1} in f.w. and d.w., respectively (Table 1). In this context, BGF showed low energy value, since fresh fruits with 70 to 150 kcal 100 g^{-1} are considered low energy density food.[15]

In the context of the biodiversity of the Brazilian Savanna, BGF can be compared with other native fruits such as araçá, buriti, cagaita, yellow mombin, mangaba, and marolo. Buriti (68.86 g 100 g^{-1}) and marolo (70.56 g 100 g^{-1}) have moisture contents similar to BGF. However, araçá, cagaita, yellow mombin, and mangaba have higher moisture values, ranging from 80.41 to 92.8 g 100 g^{-1}. The lipid content of yellow mombin and buriti is 0.48 and 7.72 g 100 g^{-1}, respectively, while the protein content of araçá and buriti is 0.42 and 1.73 g 100 g^{-1}. The ash content of cagaita and buriti is 0.30 and 1.01 g 100 g^{-1}, respectively. BGF stands out for its protein content, which is at least twice as high as that of other native fruits, while its lipid and ash contents fall within the ranges reported in the literature for other native fruits.[3]

Primary metabolites in BGF

Primary metabolites such as carbohydrates (n = 13), organic acids (n = 6) and fatty acids (n = 22) were identified by untargeted metabolomics approach (Table 3). The most abundant sugar identified was D-fructose (42.97%) and sucrose (16.31%), representing 80.83% of total carbohydrates. While succinic acid (4.57%) and

Table 2. Contribution of *Brosimum gaudichaudii* fruit to meet the dietary reference intakes of available carbohydrates, dietary fibre, and vitamins A and C for adults

Age group / years		Available carbohydrates[a] / (g *per* day)	Dietary fibre[b] / (g *per* day)	Vitamin A / (μg RAE *per* day)	Vitamin C / (mg *per* day)
			Male		
19-50	DRI	130	38	900	90
	daily value / %	9.44	12.44	64.03	15.14
	classification	source	good source	excellent source	good source
51- >70	DRI	130	30	900	90
	daily value / %	9.44	15.73	64.03	15.14
	classification	source	good source	excellent source	good source
			Female		
19-50	DRI	130	25	700	75
	daily value / %	9.44	18.88	82.34	18.17
	classification	source	good source	excellent source	good source
51- >70	DRI	130	21	700	75
	daily value / %	9.44	22.48	82.34	18.17
	classification	source	excellent source	excellent source	good source

One portion of *B. gaudichaudii* provides 70 kcal and corresponds to 6 fruits or 68.14g (11.18 g *per* fruit). [a]Available carbohydrates calculation: 100 – (moisture + proteins + lipids + ash + total dietary fibre). [b]Dietary fiber calculation: soluble dietary fiber + insoluble dietary fiber. DRI: dietary reference intakes considered RDA (Recommended Dietary Allowances) values previously described by the literature;[15,19] RAE: retinol activity equivalents.

Table 3. Metabolic profiling of *Brosimum gaudichaudii* fruits by GC-MS approach

Metabolite	Molecular formula	CAS	Retention time / min	Mass-to-charge ratio (*m/z*)	Relative abundance / %
Carbohydrates (n = 13)					
D-Fructose	$C_6H_{12}O_6$	7776-48-9	22; 27; 42	73; 307; 217	42.97
Sucrose	$C_{12}H_{22}O_{11}$	25702-74-3	50; 40	217; 361	16.31
D-Galactose	$C_6H_{12}O_6$	10257-28-0	23; 27; 29; 31; 34	73; 319; 204; 319; 73	3.71
Inositol	$C_6H_{12}O_6$	173524-45-3	29; 30; 31; 36	73; 73; 73; 73	3.10
Galactaric acid	$C_6H_{10}O_8$	526-99-8	28	333	2.82
D-Glucose	$C_6H_{12}O_6$	2280-44-6	28	204	1.70
Maltose	$C_{12}H_{22}O_{11}$	4482-75-1	37; 38; 39; 41	204; 204; 191; 217	0.66
D-Cellobiose	$C_{12}H_{22}O_{11}$	16462-44-5	38; 42; 43	204; 204; 204	0.62
Arabinofuranose	$C_5H_{10}O_5$	41546-26-3	24	217	0.50
2-α-Mannobiose	$C_{12}H_{22}O_{11}$	15548-39-7	41	361	0.40
β-D-Glucopyranuronic acid	$C_6H_{10}O_7$	23018-83-9	35	73	0.33
D-Mannose	$C_6H_{12}O_6$	530-26-7	29; 35; 41	204; 387; 204	0.17
Sedoheptulose	$C_7H_{14}O_7$	3019-74-7	32	73	0.04
Total carbohydrates	–	–	–	–	73.34
Amino acids (n = 6)					
L-Asparagine	$C_4H_8N2O_3$	3130-87-8	20; 22; 26	159; 73; 73	6.47
L-Threonine	$C_4H_9NO_3$	13095-55-1	13; 15	73; 73	0.42
L-Proline	$C_5H_9NO_2$	1150316-19-0	18; 26	156; 216	0.35
L-Alanine	$C_3H_7NO_2$	115967-49-2	12; 15; 27; 33	73; 160; 160; 116	0.12
L-Serine	$C_3H_7NO_3$	302-84-1	12; 14	132; 204	0.09
L-Leucine	$C_6H_{13}NO_2$	61-90-5	16; 32	172; 204	0.03
Total amino acids	–	–	–	–	7.47
Organic acids (n = 6)					
Succinic acid	$C_4H_6O_4$	110-15-6	13; 14; 18	147; 147; 73	4.57
2-Thiobarbituric acid	$C_4H_4N_2O_2S$	504-17-6	26	345	4.52
D-Gluconic acid	$C_6H_{12}O_7$	133-42-6	29	73	0.73
Shikimic acid	$C_7H_{10}O_5$	138-59-0	25	204	0.36
Glucaric acid	$C_6H_{10}O_8$	25525-21-7	30	73	0.13
Benzoic acid	$C_7H_6O_2$	1079-02-3	11; 28	179; 281	0.06
Total organic acids	–	–	–	–	10.36
Saturated fatty acids (n = 15)					
Palmitic acid	$C_{16}H_{32}O_2$	408-35-5	29; 30.9; 31.3; 33; 36; 41	74; 31; 31; 299; 311; 371	1.02
Eicosanoic acid	$C_{20}H_{40}O_2$	506-30-9	36; 38	74; 369	0.72
Stearic acid	$C_{18}H_{36}O_2$	57-11-4	33	74	0.57
Myristic acid	$C_{14}H_{28}O_2$	32112-52-0	27	285	0.002
Tetracosanoic acid	$C_{24}H_{48}O_2$	557-59-5	45; 45.2	411; 425	0.07
Tricosanoic acid	$C_{23}H_{46}O_2$	2433-96-7	41; 44	74; 397	0.04
Docosanoic acid	$C_{22}H_{44}O_2$	112-85-6	41; 42	397; 383	0.04
Heneicosanoic acid	$C_{21}H_{42}O_2$	2363-71-5	38; 40	74; 74	1.87
Lauric acid	$C_{12}H_{24}O_2$	143-07-7	39	179	0.02
Pentacosanoic acid	$C_{25}H_{50}O_2$	506-38-7	44	74	0.02
13-Methyltetradecanoic acid	$C_{15}H_{30}O_2$	2485-71-4	27	74	0.02
Undecanoic acid	$C_{11}H_{22}O_2$	112-37-8	20	74	0.02
Nonadecanoic acid	$C_{19}H_{38}O_2$	12707-74-3	35	74	0.01
Hexacosanoic acid	$C_{26}H_{52}O_2$	506-46-7	45; 48	74; 439	0.01

Table 3. Metabolic profiling of *Brosimum gaudichaudii* fruits by GC-MS approach (cont.)

Metabolite	Molecular formula	CAS	Retention time / min	Mass-to-charge ratio (*m/z*)	Relative abundance / %
Octanoic acid	$C_8H_{16}O_2$	124-07-2	33	165	0.004
Total saturated fatty acids	–	–	–	–	4.44
Unsaturated fatty acids (n = 7)					
Linolenic acid	$C_{18}H_{30}O_2$	463-40-1	33	79	3.04
cis-13,16-Docosadienoic acid	$C_{22}H_{40}O_2$	7370-49-2	39; 50	55; 393	0.04
Linoleic acid	$C_{18}H_{32}O_2$	121250-47-3	34	73	0.03
Methyl 13-eicosenoate	$C_{21}H_{40}O_2$	69120-02-1	36	55	0.02
Oleic acid	$C_{18}H_{34}O_2$	112-80-1	34	129	0.01
Myristoleic acid	$C_{14}H_{26}O_2$	544-64-9	27	74	0.01
cis-11,14-Eicosadienoic acid	$C_{20}H_{36}O_2$	5598-38-9	36	67	0.01
Total unsaturated fatty acids	–	–	–	–	3.16
Phytosterols / tocopherols (n = 5)					
α-Amyrin	$C_{30}H_{50}O$	638-95-9	51	218	0.66
Stigmasterol	$C_{29}H_{48}O$	83-48-7	50	129	0.06
γ-Tocopherol	$C_{28}H_{48}O_2$	54-28-4	46; 47	488; 416	0.09
δ-Tocopherol	$C_{28}H_{48}O_2$	119-13-1	45	474	0.01
β-Tocopherol	$C_{28}H_{48}O_2$	148-03-8	46	488	0.003
Total phytosterols / tocopherols	–	–	–	–	0.82
Other metabolites (n = 6)					
1,2,3-Propanetricarboxylic acid	$C_6H_8O_6$	850848-65-6	25	273	0.10
Dodecanal	$C_{12}H_{24}O$	112-54-9	30	73	0.01
2-Piperidinecarboxylic acid	$C_6H_{11}NO_2$	119678-10-3	9; 18; 20	84; 156; 73	0.01
2-Hydroxyundecanoic acid	$C_{11}H_{22}O_3$	19790-86-4	21	229	0.01
Tetracosan-1-ol	$C_{24}H_{50}O$	506-51-4	43	411	0.06
Adenosine	$C_{10}H_{13}N_5O_4$	30143-02-3	39	73	0.002
Total other metabolites	–	–	–	–	0.19
Total metabolites	–	–	–	–	100

GC-MS: gas chromatography-mass spectrometry; CAS: chemical abstracts service registry number.

2-thiobarbituric acid (4.52%) accounted for most of the organic acid content identified, representing 87.74% of the total organic acids. Moreover, sugars such as D-glucose, D-fructose and sucrose, and organic acids such as malic, citric, and tartaric acids were quantified in the native fruits (Table 1). D-Fructose (1646.57 g 100 g^{-1} d.w.) had the highest content among the soluble sugars, followed by D-glucose (1020.84 g 100 g^{-1} d.w.) and sucrose (701.68 g 100 g^{-1} d.w.). Among the organic acids quantified, malic acid (3692.55 g 100 g^{-1} d.w.) had the highest content, followed by citric acid (1249.46 g 100 g^{-1} d.w.) and tartaric acid (158.99 g 100 g^{-1} d.w.). The composition of soluble sugars and organic acids in the fruit, along with the ratio between them, plays a crucial role in the sensory properties of BGF. This balance between sweetness and acidity is likely responsible for the desirable flavor of the fruit.[8]

The most abundant amino acid found in BGF was L-asparagine (6.47%), representing 86.6% of total amino acids. Asparagine contributes to the storage and transport of nitrogen in plants and its accumulation in BGF may be associated with stress conditions, particularly in situations where the plant cannot support the normal level of protein synthesis.[20] The seasonal climate of the Savanna biome, characterized by a rainy season in spring and summer and a dry season in fall and winter, along with high temperatures, can create stressful conditions that lead to changes in the metabolism of the fruit. Additionally, other amino acids present in smaller quantities, such as proline (4.68%) and serine (1.20%), contribute to the sweetness of BGF.

Regarding the fatty acid profile, heneicosanoic acid (1.87%) and palmitic acid (1.02%) accounted for 65.09% of the total saturated fatty acids, while linolenic acid (3.04%) contributed 96.20% of the total unsaturated fatty acids. The fatty acid composition is critical for the quality of ripe fruit, as it is associated with the fruit's taste and the biosynthesis of aroma compounds.[11] However,

studies on the volatile compound profile of BGF are essential to better understand its aromatic composition.

Secondary metabolites in BGF

Secondary metabolites are crucial nutrients for humans, as they play significant roles in providing health benefits. The bioactive compounds, including total phenolic compounds, total flavonoids, carotenoids, and vitamin C, of BGF were analyzed in both fresh and dry weights (Table 1). The content of total phenolic compounds in BGF was 74.04 mg 100 g^{-1} (f.w.), while the total flavonoids was 1.15 mg 100 g^{-1} (f.w.) (Table 1). The following phenolic compounds (n = 2) and flavonoids (n = 3) were identified in the BGF samples by LC-ESI-IT-MS/MS approach in this study: chlorogenic acid, 4-*O*-caffeoylquinic acid, hesperidin, quercetin-4'-*O*-glucoside (spiraeoside) and quercetin-3-*O*-glucuronide (Table 4). Polyphenols are pivotal as antioxidant agents, with chlorogenic acid particularly noteworthy for its potential to mitigate health risks, including inflammation, diabetes, and cardiovascular diseases, owing to its antioxidant properties.[21]

The carotenoids quantified were lutein (1.25 mg 100 g^{-1} f.w.), lycopene (6.81 mg 100 g^{-1} f.w.) and β-carotene (10.15 mg 100 g^{-1} f.w.) (Table 1). Carotenoids are significant phytochemicals for human health, serving as precursors to vitamin A and exhibiting antioxidant activities. Within the plant, carotenoids play a crucial role as aroma precursors and contribute to the color transition during fruit ripening, thereby influencing the flavor and visual perception of ripe fruit.[22] The fruits of BGF had 18.21 mg 100 g^{-1} of carotenoids or 845 μg RAE 100 g^{-1}, classified as an excellent source of vitamin A, satisfying 64.03 and 82.34% of the needs for this nutrient for adult men and women, respectively (Table 2). Other study found 25.26 mg of carotenoids *per* 100 g of BGF from Goiás (Brazil) and identified zeaxanthin, β-cryptoxanthin and β-carotene by HPLC analysis.[23]

The level of vitamin C was 20.02 mg 100 g^{-1} (f.w.) in BGF (Table 1). Vitamin C is crucial as both an antioxidant and a cofactor in redox reactions. It also plays a regulatory role in controlling cell differentiation, which is linked to the development of certain cancers. Recent studies[26] have highlighted the significance of ascorbate in activating epigenetic mechanisms that govern cell differentiation, the dysregulation of which can contribute to the onset of specific cancer types. The BGF were considered a good source of vitamin C as it meets 15.14 and 18.17% of the daily recommendations for male and female adults, respectively, in one portion (Table 2).

Moreover, phytosterols and tocopherols were identified

Table 4. Identification of polyphenols (phenolic acids and flavonoids) in *Brosimum gaudichaudii* fruits by LC-ESI-IT-MS/MS approach

Identified compound	Retention time / min	*m/z* of ion molecular [M – H]	*m/z* of fragment ions (MS/MS) (relative abundance / %)	Score of similarity / %
Phenolic acids				
Chlorogenic acid (3-*O*-caffeoylquinic acid)	10.6	353.27	191.07 (100); 179.05 (49.5); 192.07 (13.9); 191.47 (12.2); 179.39 (9.9)	98.30
4-*O*-Caffeoylquinic acid isomer 1	13.4	353.32	173.08 (100); 179.07 (45.4); 191.08 (22.60); 173.45 (13.80); 180.07 (11.7)	86.90
Chlorogenic acid isomer 1	14.1	353.26	191.07 (100); 191.52 (13.2); 192.04 (5.9); 179.08 (3.7); 173.00 (0.9)	99.90
4-*O*-Caffeoylquinic acid isomer 2	14.8	353.24	173.09 (100); 179.06 (37.6); 191.11 (14.9); 173.53 (10.3); 180.01 (4.9)	88.41
Chlorogenic acid isomer 2	17.2	353.29	191.06 (100); 192.06 (12.8); 191.46 (12.4); 179.04 (5.0); 207.08 (4.5)	99.50
Flavanones				
Hesperetin 7-*O*- Rutinoside (hesperidin)	23.2	609.29	301.15 (100); 300.19 (20.5); 301.57 (12.0); 302.19 (11.9); 343.19 (10.6)	98.10
Flavonols				
Quercetin-4'-*O*-glucoside (spiraeoside) isomer 1	24.6	463.30	301.16 (100); 300.17 (27.2); 302.13 (14.0); 301.58 (10.7); 299.20 (4.4)	95.50
Quercetin-4'-*O*-glucoside (spiraeoside) isomer 2	24.9	463.29	301.17 (100); 302.16 (23.9); 300.17 (17.6); 301.59 (12.1); 303.19 (6.0)	93.10
Quercetin-3-*O*-glucuronide	25.2	477.22	301.17 (100); 301.61 (9.6); 302.18 (8.5); 303.14 (5.0); 255.20 (3.7)	99.00

All mass spectra were compared to the databases available on the internet.[24,25] Only those compounds shown as first choice in the databases, with the highest score and above 70% of similarity were placed in this table. LC-ESI-IT-MS/MS: liquid chromatography-electrospray ionization-ion trap-mass spectrometry/mass spectrometry.

by GC-MS approach (Table 3). The phytosterols were the more abundant in BGF than the tocopherols. The phytosterols identified were α-amyrin (0.66%) and stigmasterol (0.06%), while tocopherols were γ-tocopherol (0.09%), δ-tocopherol (0.01%) and β-tocopherol (0.003%). Phytosterols and tocopherols are vital compounds for health. Phytosterols are linked to a decrease in LDL-cholesterol and total cholesterol levels, while tocopherols exhibit antioxidant properties, effectively scavenging lipid peroxyl radicals and reactive oxygen species.[12,13]

Conclusions

Brazilian BGF exhibited abundant primary metabolites, including protein, D-fructose, L-asparagine, succinic acid, heneicosanoic acid, and linolenic acid, as well as secondary metabolites such as β-carotene, chlorogenic acid, α-amyrin, and γ-tocopherol. These compounds contribute to the fruit's quality and its nutritional attributes related to health. BGF has been highlighted as an excellent source of vitamin A and a good source of vitamin C and dietary fiber. This study addressed the important aspect of researching unconventional native Brazilian plants. However, further studies providing nutritional information and suggesting consumption methods and potential industrial applications are crucial to increase consumer acceptance of these foods. Additionally, information regarding the correct planting and harvesting seasons can help revive traditional practices, especially for native fruits like BGF.

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Author Contributions

Grazieli B. Pascoal was responsible for conceptualization, formal analysis, investigation, and writing original draft; Silvia L. R. Meza for investigation, validation, writing original draft, review and editing; Eric C. Tobaruela for data curation, methodology, validation, and writing review and editing; Isabel L. Massaretto for formal analysis, investigation, software, and validation; Jéssica A. Giacomolli for formal analysis and methodology; Juliana F. Chiareto for formal analysis and methodology; Florença M. Borges for formal analysis, investigation, and methodology; Danielle O. Borges for formal analysis, investigation, and methodology; Eduardo Purgatto for project administration, resources, supervision, visualization, and writing review and editing. All authors discussed the results and contributed to the final manuscript.

References

1. Arruda, H. S.; Araújo, M. V. L.; Marostica Jr., M. R.; *Food Chem.: Mol. Sci*. **2022**, *5*, 100148. [Crossref]
2. Engelbrecht, L. M. W.; Ribeiro, R.V.; Yoshida, N. C.; Gonçalves, V. S.; Pavan, E.; Martins, D. T. O.; Santos, E. L.; *Chem. Biodiversity* **2021**, *18*, e2001068. [Crossref]
3. Schiassi, M. C. E. V.; Souza, V. R.; Lago, A. M. T.; Campos, L.G.; Queiroz, F.; *Food Chem*. **2018**, *245*, 305. [Crossref]
4. de Menezes Filho, A. C. P.; Oliveira Filho, J. G.; Castro, C. F. S.; *Sci. Elec. Arch*. **2021**, *14*, 74. [Crossref]
5. Kinupp, V. F.; Lorenzi, H.; *Plantas Alimentícias Não Convencionais (PANC) no Brasil: Guia de Identificação, Aspectos Nutricionais e Receitas Ilustradas*, vol. 1, 1st ed.; Plantarum: Nova Odessa, Brazil, 2014.
6. Biazotto, K. R.; Mesquita, L. M. S.; Neves, B. V.; Braga, A. R. C.; Tangerina, M. M. P.; Vilegas, W.; Mercadante, A. Z.; De Rosso, V. V.; *J. Agric. Food Chem*. **2019**, *67*, 1860. [Crossref]
7. Coutinho, A. M.; Santos, L. G. J.; Damasceno, E. M. A.; Soares, N. Z. D.; *Braz. J. Healthy Pharmacy* **2019**, *1*, 28. [Link] accessed in July 2024
8. Meza, S. L. R.; Egea, I.; Massaretto, I. L.; Morales, B.; Purgatto, E.; Egea-Fernández, J. M.; Bolarin, M. C.; Flores, F. B.; *Front. Plant Sci*. **2020**, *11*, 587754. [Crossref]
9. Latimer Jr., G. W.; *Official Methods of Analysis of AOAC International*, vol. 1, 22nd ed.; Association of Official Analytical Collaboration (AOAC) Publications: New York, USA, 2023.
10. Agius, C.; von Tucher, S.; Poppenberger, B.; Rozhon, W.; *MethodsX* **2018**, *5*, 537. [Crossref]
11. Meza, S. L. R.; Tobaruela, E. C.; Pascoal, G. B.; Magalhaes, H. C. R.; Massaretto, I. L.; Purgatto, E.; *Plants* **2022**, *11*, 366. [Crossref]
12. Meza, S. L. R.; Massaretto, I. L.; Sinnecker, P.; Schmiele, M.; Chang, Y. K.; Noldin, J. A.; Marquez, U. M. L.; *Int. J. Food Sci. Technol*. **2021**, *56*, 3218. [Crossref]
13. Meza, S. L. R.; Tobaruela, E. C.; Pascoal, G. B.; Massaretto, I. L.; Purgatto, E.; *Foods* **2021**, *10*, 877. [Crossref]
14. Kind, T.; Wohlgemuth, G.; Lee, D.Y.; Lu, Y.; Palazoglu, M.; Shahbaz, S.; Fiehn, O.; *Anal. Chem*. **2009**, *81*, 10038. [Crossref]
15. Institute of Medicine (IOM).; *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*, vol. 1, 1st ed.; National Academy Press: Washington, USA, 2005.

16. Motulsky, H.; *Graph Pad Prism*, 3.0; University of California, USA, 1989.
17. Jorge, L. W. V. S.; Rocha, N. A.; Silva, M. K.; Ramos, C. M. V. P.; Mourão, P. S.; Oliveira, C. G.; Rodrigues Filho, M. G.; Oliveira, M. L.; Moraes, B. C.; Uchôa, V. T.; *Res. Soc. Dev.* **2022**, *11*, e498111436530. [Crossref]
18. Land, L. R. B.; Borges, F. M.; Borges, D. O.; Pascoal, G. B.; *Demetra* **2017**, *12*, 509. [Crossref]
19. Philippi, S. T.; *Pirâmide dos Alimentos: Fundamentos Básicos da Nutrição*, vol. 1, 3rd ed.; Manole: São Paulo, Brazil, 2018.
20. Lea, P. J.; Sodek, L.; Parry, M. A. J.; Shewry, P. R.; Halford, N. G.; *Ann. Appl. Biol*. **2007**, *150*, 1. [Crossref]
21. Upadhyay, R.; Rao, L. J. M.; *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 968. [Crossref]
22. Prasanna, V.; Prabha, T. N.; Tharanathan, R. N.; *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 1. [Crossref]
23. Silva, D. B.; Vieira, R. F.; Martins, F. S.; Conceição, E. C.; Melo, L. A. M. P.; Palhares, D.; *Avaliação Preliminar de Cultivo de Mama-Cadela (Brosimum gaudichaudii Trécul. - Moraceae) em Diferentes Substratos*, vol. 360, 1st ed.; Embrapa Recursos Genéticos e Biotecnologia: Brasília, Brazil, 2020.
24. MassBank of North America, http://mona.fiehnlab.ucdavis.edu, accessed in June 2024.
25. MassBank Europe, https://massbank.eu/MassBank/, accessed in June 2024.
26. Fenech, M.; Amaya, I.; Valpuesta, V.; Botella, M. A.; *Front. Plant Sci.* **2019**, *9*, 2006. [Crossref]

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