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Physicochemical composition, centesimal, and phytochemical profile of umbu seed flour (*Spondias tuberosa* Arruda)

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Abstract

This study aimed to investigate the nutritional potential of *umbu* (*Spondias tuberosa Arruda*) seed flours. This was an experimental study. The seeds of the *umbu* fruit were subjected to an oven-drying process in order to obtain the flour. Subsequently, the analysis of the centesimal composition, physicochemical, mineral, and fatty acid profile, microbiological investigation, and the screening of bioactive phytochemicals of the *umbu* seed flours in hydroethanolic extracts were carried out. A completely randomized design (CID) was used, with three replications, and the results were expressed as means followed by standard deviation. Analysis of variance (ANOVA) was carried out, and the Tukey's test was applied at a 5% probability level. It was possible to obtain flour from *umbu* seeds. It was observed that they contained significant amounts of insoluble dietary fiber (80.9 ± 0.55a $g.100^{-1}$), protein (6.95 ± 0.10a $g.100^{-1}$), and lipids (5.54 ± 1.13a $g.100^{-1}$). The minerals present included zinc (1.87 ± 0.05b mg.100 g^{-1}), manganese (14.36 ± 0.34c mg.100 g^{-1}), and calcium (104.79 ± 0.92c mg.100 g^{-1}). The flours had high levels of oleic monounsaturated fatty acids (33.10 ± 0.13a $g.100^{-1}$), linoleic polyunsaturated fatty acids (38.06 ± 0.06b $g.100^{-1}$), and palmitic saturated fatty acids (19.1 ± 0.04b $g.100^{-1}$), in addition to considerable levels of phenolic compounds (276.21 ± 14.01a $mg.g^{-1}$ GAE) and flavonoids (891.09 ± 9.01^{a} $mg.g^{-1}$ rutin), with satisfactory sanitary conditions for *Escherichia coli, Salmonella, Bacillus cereus* molds, and yeasts. This study suggests that umbu seed meals can be considered relevant sources of dietary fiber, minerals, and natural antioxidants.

Keywords: *Spondias tuberosa Arruda*; food waste; food and nutrition security; recovery of agricultural waste.

Practical Application: Byproducts from the umbu agroindustry are used in the production of new products.

1 INTRODUCTION

Spondias tuberosa Arruda, popularly known as umbu, is a deciduous, xerophytic fruit plant belonging to the Anacardiaceae family, native to the semi-arid Caatinga biome, and perfectly adaptable to drier climates (de Lima et al., 2018; Dias et al., 2019; Zeraik et al., 2016). The fruit of this plant is widely consumed in Brazil in various formats, "*in nature*," or as juices, mixed drinks, cereal bars, sweets, and jellies, and its leaves are used in popular medicine to treat inflammation (Cangussu et al., 2021; Senes-Lopes et al., 2018).

Due to the presence of bioactive substances with antioxidant, anti-inflammatory, antibacterial, and antitumor effects, interest in research using wild fruits has increased considerably in recent years. However, the umbu fruit industry generates numerous byproducts that are not utilized in a significant way (Carvalho Gualberto et al., 2021; Senes-Lopes et al., 2018).

Previous studies have shown that there are beneficial compounds and physicochemical characteristics in several parts of the plant. High levels of phenolic compounds have been found in bark and seeds (Cangussu et al., 2021; Carvalho Gualberto et al., 2021), vitamin C in the fruit pulp (Gouvêa et al., 2017), organic acids (gallic acid) from the inner bark of the stem (de Moura Barbosa et al., 2018), and fixed oils with antioxidant activities in the leaves and stems (Guimarães et al., 2018). In addition, the byproducts of the seeds have significant amounts of lipids, proteins, and minerals (Dias et al., 2019; Ribeiro et al., 2019; Santos et al., 2019).

In this sense, it is notable that these studies on the use of byproducts of the fruit agroindustry leave a gap in the literature regarding the use of the umbu fruit seed and its physicochemical, centesimal composition, and phytochemical profile. Thus, the determination of the composition of this fruit is important to know its nutritional and functional properties and verify if the chemical properties of this part of the fruit are similar when

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compared with other fractions of the plant already known. Thus, this study aimed to investigate the physicochemical, centesimal composition, and phytochemical profile of umbu (*Spondias tuberosa* Arruda) seed flour.

2 MATERIALS AND METHODS

2.1 Type of study

This is a quantitative and experimental study with formulations of flour from umbu seeds. Physicochemical analysis, centesimal composition, fatty acid profile, phytochemical screening, and microbiological analysis were performed.

2.2 Sample

The umbuzeiro fruits were collected in three samples, at the Farm Quilombola Recruta, near the city of Anagé, Bahia. The samples were collected at three different periods of maturation, approximately 65, 90, and 150 days after the opening of the umbuzeiro flower. Each sample was processed independently to constitute a batch/repeat. After each collection, the fruits were sent to the Laboratory of Forage and Pasture of the Universidade Estadual do Sudoeste da Bahia (UESB), Itapetinga *campus*, BA, Brazil, for processing of umbu seed flour.

For the production of the flour, the fruits were selected and sanitized manually, after which they were pulped until only the seeds remained. The seeds were dried in an oven (Solab—SL-102) with forced air circulation at 55 ± 2°C for 72 h and then ground in a chopper (Trapp TRF-70). After that, the flour was processed in 80-*mesh* sieves for particle size standardization.

The three samples were named: Flour from seeds of fruits collected in period 1 (FP1), Flour from seeds of fruits collected in period 2 (FP2), and Flour from seeds of fruits collected in period 3 (FP3). The flours were packed in sealed polyethylene bags and subjected to freezing (-18°C) until the moment of analysis. All tests of umbu fruit seed flours were performed in triplicate. Due to the lack of resources, the analysis of minerals and fatty acids was performed only in sample FP3.

The yield of flour from umbu seeds was calculated by the ratio between the mass of dried umbu seed flour in grams by the mass of umbu seeds. The physical-chemical analyses (water activity (aw), pH, titratable acidity, and soluble solids) were performed in the Biofactory laboratory of the State University of Southwest Bahia (UESB), Vitória da Conquista *campus*, BA. We adopted the procedures the *Association of Official Analytical Chemists*—AOAC (2010) described.

2.3 Determination of the centesimal composition

The centesimal composition included the determination of moisture content, protein, total lipids, total carbohydrates, total dietary fiber, and ash. These were performed on the three repetitions (FP1, FP2, and FP3). The analyses were performed in the Forage and Pasture Laboratory, the quantification of protein (nitrogen content) was performed by Micro-Kjeldahl (AOAC, 2010) methodology, and the determination of total carbohydrates was performed by the difference of the sum values of moisture, ash, protein, and lipids.

The moisture values were obtained by the oven method until constant weight, according to the AOAC (2010), while the ash content was obtained by incineration in a muffle furnace at a temperature of 550°C. (AOAC, 2010) The lipid content was determined by the intermittent extraction method for oils and fats, Soxhlet type, using the solvent hexane (AOAC, 2010).

For the analysis of soluble (FS), insoluble (IF), and total fiber (TF) content of sample FP3, enzymatic-gravimetric methods were used. (AOAC, 2010) The analysis of fiber content was outsourced and performed at Laboratory Amazile Biagioni Maia (LABM) in Belo Horizonte, Minas Gerais, Brazil.

The determination of minerals was performed according to the method proposed by AOAC (2010), carried out at LABM. Initially, the FP3 was incinerated in a muffle furnace at 550°C to obtain ashes. Then, flame atomic absorption spectrophotometry was used in a spectrophotometer (Micronal b-262) to determine the minerals (e.g., calcium, phosphorus, manganese, potassium, zinc, and selenium).

2.4 Determination of the fatty acid profile

To determine the fatty acid profile, initially, the lipids were extracted from FP3 using the methodology described by Bligh and Dyer (1959), and then the lipid fraction was submitted to transesterification of triacylglycerols into fatty acid methyl esters, as proposed by AOAC-996.06—Fat (total, saturated, and unsaturated) in Foods. The chromatographic analysis was performed in a GC ultra gas chromatograph (Thermo Finnigan Trace), equipped with a flame ionization detector (FID) and BPX-70 fused silica capillary column (120 m, 0.25 mm i.d.). The conditions adopted in the experiment were injector and detector temperatures at 250 and 280°C, respectively, and nitrogen (N2) was used as carrier gas with a flow rate of 6.5 mL/min.

2.5 Phytochemical screening determinations

Phytochemical screening determinations of umbu seed flours were performed on samples FP1, FP2, and FP3 in the Biofactory laboratory of the UESB.

2.6 Preparation of extracts

Initially, hydroethanolic extracts of the flours were prepared according to the methodology described by Zhao and Hall (2008), with adaptations. The samples were homogenized in hydroethanolic solution at a ratio of 80:20 v.v-1 and then transferred to test tubes, which were immersed in an ultrasonic bath for 25 min at 53°C. The solid part was submitted to two more successive extractions and then centrifuged and concentrated at a rotary evaporator. The hydroethanolic extract was stored in an amber glass flask protected from light and kept under refrigeration at -4 ± 2°C until the analysis.

2.7 Determination of phenolic compounds

The total phenolic compounds of the extracts were determined by adopting the spectrophotometric method described by Wettasinghe and Shahidi (1999) using the Folin-Ciocauteau reagent and gallic acid as the reference standard. The levels of phenolic compounds obtained were expressed as mg of GAE.100 g^{-1} of a dehydrated sample.

2.8 Determination of total flavonoids

For the determination of total flavonoids, the spectrophotometric method adapted from Santos and Blatt (1998) and Awad et al. (2001) was adopted. The results were calculated according to the rutin calibration curve and expressed in μg of rutin.100^{-1} of the sample.

2.9 Determination of total carotenoids

The total carotenoids, as well as anthocyanins and chlorophyll A and B, were determined by adopting the procedures described by Sims and Gamon (2002). For this, 0.5 g of sample was weighed and 3 mL of Tris buffer (acetone/Tris-HCl; 80:20, 0.2M v:v, pH 7.8) was added, which, after due homogenization, was centrifuged for 5 min at 2,000 rpm. The supernatant was immediately read in a spectrophotometer with wavelengths of 470, 537, 663, and 647 nm, which correspond to carotenoids, anthocyanins, chlorophyll A, and chlorophyll B, respectively. Then, the absorbance values were converted into μg.100^{-1}.

2.10 Statistical delineation

We chose the completely randomized design (CID) to carry out this research. The results obtained were reported as means followed by standard deviation. After that, the comparison test between the means of each lot was applied using the Tukey's test at a 5% probability level and statistically evaluated using the Statistica 7.0 software program.

3 RESULTS AND DISCUSSION

The yield of the umbu seed flours was calculated by the ratio between the mass of the residues, in powder form, and the mass of the residues before drying, and the results were 32.52% for FP1, 29.81% for FP2, and 28.24% for FP3.

The umbu seed flour presented physicochemical characteristics and centesimal composition are detailed in Table 1.

The protein contents found ranged from 5.35 to 6.95 g.100^{-1}. A healthy adult individual requires a daily protein intake ranging from 10 to 35% of their total energy value (TEV). This equals about 0.8 g for every kilogram of their weight of protein per day (0.8 g/kg/P). This intake should be balanced between animal (about 65%) and vegetable (about 35%) protein (IOM, 2005; Lupton et al., 2002).

Vegetable proteins have nutritional importance and have been in evidence in the diet in recent years. These proteins are being proposed as alternatives to animal protein by consumers due to several factors, especially the threat of extinction of cattle farming, beef processing, and the repercussions of climate change (Ramachandraiah, 2021).

The preparation of alternative foods rich in vegetable protein to replace animal protein has been suggested by some

Table 1. Physicochemical characteristics and centesimal composition of umbu fruit seed flours collected at Fazenda Quilombola Recruta, Vitória da Conquista, Bahia, Brazil, 2019/2020.

Analysis	FP1	FP2	FP3
(a_w)	0.56 ± 0.00^a	0.52 ± 0.00^b	0.49 ± 0.00^c
pH	3.04 ± 0.10^a	2.91 ± 0.01^c	2.93 ± 0.02^b
(°Brix)	1.33 ± 0.00^c	1.52 ± 0.05^b	1.83 ± 0.00^a
Titratable acidity (mg.$100^{-1 \text{ ac. cítrico}}$)	5.11 ± 1.42^a	2.80 ± 0.28^c	3.63 ± 0.28^b
Moisture content (%)	9.68 ± 0.00^b	9.67 ± 0.43^b	9.70 ± 0.00^a
Ash content (g.100^{-1})*	1.83 ± 0.03^a	1.62 ± 0.05^b	1.22 ± 0.37^c
Proteins (g.100^{-1})*	5.35 ± 1.19^c	6.95 ± 0.10^a	6.12 ± 0.11^b
Lipids (ether extract) (g.100^{-1})*	4.51 ± 0.23^c	5.33 ± 0.47^b	5.54 ± 1.13^a
Carbohydrates (g.100^{-1})*	0.52 ± 0.23^c	6.07 ± 0.18^b	3.18 ± 0.49^a
Total dietary fiber (g.100^{-1})*	79.15 ± 1.18^b	82.50 ± 0.45^a	80.6 ± 2.17^b
Insoluble dietary fiber (g.100^{-1})*	77.8 ± 3.16^c	80.9 ± 0.55^a	79.15 ± 0.82^b
Soluble dietary fiber (g.100^{-1})*	1.35 ± 1.03^c	1.60 ± 0.10^a	1.45 ± 0.17^b
Calcium (mg.100 g^{-1})*	-	-	104.79 ± 0.92^c
Phosphorus (mg.100 g^{-1})*	-	-	282.22 ± 0.90^c
Manganese (mg.100 g^{-1})*	-	-	14.36 ± 0.34^c
Potassium (mg.100 g^{-1})*	-	-	302.25 ± 0.92^c
Zinc (mg.100 g^{-1})*	-	-	1.87 ± 0.05^b
Selenium (mg.100 g^{-1})*			< 0.003

The mean of three triplicates of each repetition followed by ± standard deviation. Means followed by the same letter in the columns do not differ statistically from each other. Tukey's test was applied at the 5% probability level. FP1: seed flour fruit of 65 days of maturation; FP2: seed flour fruit of 90 days of maturation; FP3: seed flour fruit of 150 days of maturation. The analyses of calcium, phosphorus, manganese, potassium, zinc, and selenium were only performed in FP3 flour; *Values expressed on a wet basis.

studies, such as the use of hamburgers made with flour from byproducts of the fruit agroindustry, such as cashew nut shells (12.95 $g.100^{-1}$ of protein) (Lima, 2008), watermelon shells (21.66 $g.100^{-1}$ of protein) (Sousa et al., 2012), and passion fruit shells (17.70 $g.100^{-1}$ of protein) (Gonçalves & Magalhães, 2018).

Conversely, lipids are biomembrane components, provide energy, and allow neuronal nerve conduction, vitamin absorption, and hormone formation, among others. In plant physiology, these have several vital functions already mentioned and are found in larger quantities in fruit seeds, with the main function of carbon and energy reserve (Rai et al., 2014). The reference daily intake (RDI) is between 20 and 35% of the VET of a healthy adult individual, and of these, 20% should be from food sources of mono and polyunsaturated fats (5–10% of linoleic fatty acid) and 10% of saturated fats (IOM, 2005; Précoma et al., 2019).

The carbohydrate contents found in this research ranged from 0.52 to 6.07 $g.100^{-1}$. In addition, umbu flour showed a significant amount of total dietary fiber with values ranging from 79.15 to 82.50 $g.100^{-1}$. The Food and Nutrition Board considers that the RDI varies between 21 and 38 g/day depending on the sex and age group of the individual. Dietary fiber is considered a mandatory part of the human diet and is related to essential physiological properties for health, among which are decreased risk of hypertension (Aleixandre & Miguel, 2016), diabetes (Weickert & Pfeiffer, 2018), cardiovascular problems (Soliman, 2019), and some gastrointestinal disorders (Axelrod & Saps, 2018).

In this sense, umbu seed flour can be classified as high in dietary fiber. The total dietary fiber values are high when compared with those from other fruit seeds such as avocado seeds (47.63 $g.100^{-1}$) (Barbosa-Martín et al., 2016), orange seeds (63.60 $g.100^{-1}$) (de Moraes Crizel et al., 2013), and apple seeds (33.10 $g.100^{-1}$) (O'Shea et al., 2015).

The values of minerals found in the umbu seed flours of this study highlight potassium (302.25 $mg.100^{-1}$), phosphorus (282.22 $mg.100^{-1}$), calcium (104.79 $mg.100^{-1}$), and manganese (14.36 $mg.100^{-1}$). The recommended daily intake of these minerals for a healthy adult individual is, respectively, 4,700, 700, 1,000, and 2.3 mg, which represent about 6.5, 40.31, 10.47, and 624.35%, in that order (IOM, 2005). These minerals exert various physiological functions in the body systems, and the consumption of food sources containing them is essential for maintaining proper cellular function (Harding et al., 2017; Hodge, 2016).

For example, potassium is involved in blood pressure control and aids in better glucose control, glucose intolerance, and insulin resistance (Stone et al., 2016), while a lack of phosphorus and calcium in the body can result in several serious clinical complications, including arrhythmias, seizures, and breathing difficulties (Blaine et al., 2015). Meanwhile, the manganese observed in umbu flour is an essential element required for many biological processes, including bone and nerve health (Erikson & Aschner, 2019; Horning et al., 2015).

The amount of manganese found in the umbu seed flour is remarkable data and can be considered high compared with the amount of the mineral found in seeds of other fruits such as avocado (1.50 $mg.100^{-1}$), papaya (2.50 $mg.100^{-1}$), passion fruit (0.90 $mg.100^{-1}$), watermelon (2.60 $mg.100^{-1}$), and melon (2.50 $mg.100^{-1}$) (Morais et al., 2016).

The fatty acid profile of the umbu seed flours (Table 2) showed an emphasis on total unsaturated (71.96 $g.100^{-1} \pm 0.93$) and total saturated (28.04 $g.100^{-1} \pm 0.39$) fatty acids. Of the total unsaturated fatty acids, 33.51 $g.100^{-1} \pm 0.21$ are monounsaturated, while 38.45 $g.100^{-1} \pm 0.40$ are polyunsaturated. Among the polyunsaturated fatty acids, linoleic acid was found in the highest concentration (38.06 $g.100^{-1}$), followed by monounsaturated oleic acid (33.1 $g.100^{-1}$). The values of saturated fatty acids, palmitic acid, and stearic acid were the most concentrated with 19.1 and 8.25 $g.100^{-1}$, respectively, and a similar behavior was found in the study by Dias et al. (2019).

It is noteworthy that the intake of vegetable oils with high oleic and linoleic acid content can help reduce LDL cholesterol and, consequently, also reduce the risk of cardiovascular diseases. Moreover, they can contribute to glycemic control and reduce the risk of metabolic syndrome (Marangoni et al., 2020; Mazzola et al., 2018).

The content of lipids and the composition of fatty acids in the umbu seed have been highlighted by scientific research, highlighting the importance of the reuse of waste (umbu seeds), as a new source of fatty acids of plant origin and encouraging further studies to suggest its use as a raw material for various industries, whether pharmaceutical, cosmetic, food, or biofuel production (Dias et al., 2019; Santos et al., 2019).

This study performed the screening of bioactive phytochemicals and the investigation of the levels of pigments present in the umbu seed flours (Table 3).

Table 2. Fatty acid profile of umbu fruit seed flours collected at Fazenda Quilombola Recruta, Vitória da Conquista, Bahia, Brazil, 2019/2020.

Unsaturated fatty acids in FP3* ($g.100^{-1}$)					
Monounsaturated		**Polyunsaturated**			
Palmitoleic (C16:1)	**Oleic (C18:1-CIS)**	**Linoleic (C18:2-CIS)**	**Linolenic (C18:2-CIS)**	**EPA** C20:5)**	**DHA*** (C22:6)**
0.31 ± 0.02^{b}	33.10 ± 0.13^{a}	38.06 ± 0.06^{b}	0.14 ± 0.01^{a}	0.1 ± 0.00^{b}	0.1 ± 0.00^{a}

Saturated fatty acids in FP3 ($g.100^{-1}$)			
Myristic C14:0)	**Palmitic (C16:0)**	**Stearic (C18:0)**	**Arachidic (C20:0)**
0.46 ± 0.01^{a}	19.1 ± 0.04^{b}	8.25 ± 0.02^{a}	0.1 ± 0.00^{a}

The mean of three triplicates of one repetition followed by ± standard deviation. Means followed by the same letter in the columns do not differ statistically from each other. Tukey's test was applied at the 5% probability level; *FP3: seed flour fruit of 150 days of maturation. ($g.100^{-1}$); **EPA: eicosapentaenoic; DHA: docosahexaenoic. The results are expressed for each 100 g of lipids extracted from seed flours.

Table 3. Screening of phytochemicals present in umbu seed flours collected at Quilombola Recruta Farm, Vitória da Conquista, Bahia, Brazil, 2019/2020.

Lot	Phenolic compounds mg.g$^{-1\ GAE}$	Flavonoids mg.g$^{-1\ Rutin}$	Chlorophyll A g.100g^{-1}	Chlorophyll B μg.100g^{-1}	Anthocyanin μg.100g^{-1}	Carotenoids μg.100g^{-1}
FP1	205.61 ± 0.91^{b}	891.09 ± 9.01^{a}	0.0021 ± 0.00^{a}	0.0020 ± 0.00^{a}	0.0374 ± 0.00^{a}	1.0677 ± 0.17^{b}
FP2	194.35 ± 17.53^{b}	737.39 ± 4.87^{c}	0.0020 ± 0.00^{a}	0.0016 ± 0.00^{a}	0.0339 ± 0.00^{a}	0.9415 ± 0.05^{b}
FP3	276.21 ± 14.01^{a}	848.55 ± 1.70^{b}	0.0021 ± 0.00^{a}	0.0021 ± 0.00^{a}	0.0452 ± 0.00^{a}	1.2263 ± 0.10^{a}

The mean of three triplicates of each repetition followed by ± standard deviation. Means followed by the same letter in the columns do not differ statistically from each other. Tukey's test was applied at the 5% probability level; FP1: Seed flour fruit of 65 days of maturation; FP2: Seed flour fruit of 90 days of maturation; FP3: seed flour fruit of 150 days of maturation.

Phenolic compound contents stand out in FP3: 276.21 mg.g$^{-1\ GAE}$, while flavonoids in FP1: 891.09 mg.g$^{-1\ Rutin}$. The phytochemical screening investigation conducted with flours from umbu waste (seeds) (Louzada et al., 2015) shows higher values than in this study for total phenolics (776 mg.g^{-1}/GAE) and lower for total flavonoids (52 mg.g^{-1}/GAE).

Phenolic compounds, flavonoids, and carotenoids are substances with antioxidant potential, which promote the inactivation or removal of free radicals from the cellular environment. These compounds stand out in human food because they are known for their antioxidant activities, in which they present beneficial effects in the control of diabetes, heart diseases, neuroprotection, and, especially, the effects of controlling the main enzymes involved in the aging process and cell death (Takikawa et al., 2010; Wang et al., 2017).

Studies of the *screening* of phytochemicals from various parts of the umbuzeiro arouse researchers worldwide and go beyond its use only in food, with several indications of use, namely: chemopreventive antioxidant activity of cancer and inhibition of acetylcholinesterase (Zeraik et al., 2016), inhibition of the morphological transition of strains of the fungus of the genus *Candida*, health protective effects with gastroprotective action, and photoprotective activities in ethyl acetate extracts of umbuzeiro fruits and branches.

4 CONCLUSION

In the investigation of the physicochemical composition, centesimal, and phytochemical profile of the umbu seed flour, it was possible to observe that they showed high levels of total dietary fiber and mineral values that deserve highlighting, especially the amount of manganese when compared with other seeds. Also, the profile of fatty acids brings as expressive the amount of linoleic acid and oleic acid, revealing the potential of these flours to be used as raw material in several industries, such as food, pharmaceutical, and cosmetics.

Moreover, the *screening* of bioactive phytochemicals and the investigation of the pigment contents present in the flours showed important values for phenolic compounds and flavonoids. Thus, this study shows that umbu seed flours can be considered relevant sources of dietary fiber, minerals, and natural antioxidants. As a limitation of the study, we can observe that there is a lack of toxicological analysis on the flours because they contain an interesting composition, which opens a gap for toxicological studies to be performed and explored for different future uses.

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