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FOOD TECHNOLOGY

Nutrients and bioactive compounds of pulp, peel and seed from umbu fruit

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ABSTRACT: *The objective of this research was to evaluate the nutritional composition and bioactive compounds of whole umbu fruit, including pulp, seed and peel, and also of a commercial umbu pulp. Samples of the fractions and of commercial pulp were analyzed for determination of minerals and proximate composition, total phenolic and antioxidant activity. Pulps and peel were also analyzed for vitamin C and carotenoids contents. Commercial pulp presented better nutritional composition than fresh pulp (P<0.05) and the peel presented higher phenolic content and antioxidant activity than seed. Peel also stood out by its vitamin C (79 mg.100 g^{-1}) and total carotenoids (2,751 µg.100 g^{-1}) contents, showing that, as the main barrier of the fruit for its protection, it is a fraction rich in bioactive compounds. The highest dietary fiber and iron contents were observed in umbu seed (P<0.05). Therefore, umbu by-products may be ingredients proper for development of food richer in nutrients and bioactive compounds.*
Key words: Spondias tuberosa Arruda Câmara*, phenolic, carotenoid, ABTS assay.*

Nutrientes e compostos bioativos de polpa, casca e semente de umbu

RESUMO: *O objetivo deste trabalho foi avaliar a composição nutricional e compostos bioativos do umbu, incluindo polpa, semente, casca e uma polpa comercial do fruto. Amostras das frações e da polpa comercial foram analisadas para determinação da composição centesimal e mineral, compostos fenólicos totais e capacidade antioxidante. As polpas e casca também foram analisadas quanto aos teores de vitamina C e carotenoides. A polpa comercial apresentou melhor composição nutricional em comparação com a polpa fresca (P<0,05). A casca do fruto apresentou maior teor de compostos fenólicos e capacidade antioxidante que a semente. A casca também se destacou pelos seus teores de vitamina C (79 mg.100 g^{-1}) e carotenoides totais (2.751 µg.100 g^{-1}), mostrando que, como principal barreira do fruto para sua proteção, é uma fração rica em compostos bioativos. Os maiores teores de fibras e ferro foram observados na semente de umbu (P<0,05). Portanto, os subprodutos do despolpamento do fruto podem ser ingredientes adequados para o desenvolvimento de alimentos mais ricos em nutrientes e compostos bioativos.*
Palavras-chave: Spondias tuberosa Arruda Câmara*, fenólicos, carotenoides, ABTS.*

INTRODUCTION

Brazil is one of the world's largest producers of fruits. In addition to its traditional commodities, orange and banana, it is also known for its native fruits, which in many cases are considered sources of bioactive compounds (REETZ et al., 2015). Among native fruits from Brazil, it can be highlighted the umbu (*Spondias tuberosa* Arruda Câmara), which is a small pulpy fruit with a sweet and sour flavor and thin peel whose color goes from green to yellowish, native from Caatinga, biome characteristic of the semiarid region from Northeast Brazil (LAGO et al., 2016).

Bahia, Pernambuco, Minas Gerais, Rio Grande do Norte, Piauí and Paraíba are the main producing states of umbu, and it harvesting season is, usually, from December to March. It is estimated that in 2016 about 8,390 tons of umbu fruit were produced (IBGE, 2018).

The pulp of the fruit, which is the edible fraction, is the fraction most consumed and investigated. It presents bioactive compounds like phenolic, carotenoids and vitamin C that confer its antioxidant potential (RUFINO et al., 2010)

The antioxidant activity of these compounds can potentially contribute to reduce the damage caused by oxidative stress, which is associated with the inability of the biological system to neutralize free radicals, an instability that can lead to the development of chronic diseases such as

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cancer (WOLFE et al., 2008). Thus, consumption of foods rich in antioxidant compounds such as fruits may be a strategy to lower the risk of incidence of such diseases.

A study performed by OMENA et al. (2012) with three Brazilian native fruits showed that the umbu fractions, pulp, peel and seed, did not present cytotoxicity effects in tests using sheep corneal epithelial cells. It shows that umbu fruit can be fully exploited. The processing of umbu into pulp, its main commercial product, generates a residue composed of peel and seed that, if improperly discarded, can negatively affect the environment. Then, these fractions could be used as ingredient for new nutrient-rich food formulations.

Agroindustrial wastes have been extensively evaluated since they present great amounts of minerals and vitamins and a diversity of antioxidant compounds. INADA et al. (2015), using the fractions of jabuticaba, observed high concentrations of phenolic compounds, mainly in the peel, being this fraction rich in cyanidin-3-*O*-glucoside. SELANI et al. (2016), evaluating by-products of mango, passion fruit and pineapple, have also reported high phenolic compounds and dietary fibers concentration and suggested the use of the by-products as functional ingredients by food industry.

Therefore, the objective of this research was to evaluate the nutritional composition and bioactive compounds from the whole umbu fruit, including pulp, seed and peel, in order to evaluate their potential for application in food industry. For comparison the most commercialized product, the frozen pulp, was also evaluated.

MATERIALS AND METHODS

Fruits, from the semiarid region of Milagres, Bahia, Brazil (12º 52’ 12” S; 39º 51’ 32” W), where soils are shallow, stony and high in salt, were acquired from commercial producers in Cruz das Almas, Bahia, Brazil, in the year of 2014, where their processing was performed.

Umbu fresh pulp was extracted in a horizontal depulper, followed of manual separation of seeds and peels. The pulp was stored under freezing at -18 °C until its use in the experimental assays. Seeds were previously dried at 60 ºC for 10 h in oven with air circulation and disintegrated in a knife mill. Peels were lyophilized and disintegrated in a blender. Both flour fractions were stored under vacuum in a desiccator until further analysis.

The umbu commercial frozen pulp was supplied by a small enterprise (Itamari, Bahia, Brazil). It was a non-pasteurized pulp, without additives, packaged in 100 g polyethylene flexible bags. The pulp was transported and stored under freezing at -18 °C until its use in the experimental assays. This sample was used as a comparison reference of a commercial product as it was produced with fruits that came from the same region of fresh fruits.

Analytical methods

Proximate composition and minerals

These determinations were performed according to AOAC (2010). Moisture content was determined according to method 925.09 by gravimetric assay in oven at 70 °C under vacuum. Lipid content was determined following method 945.38 using a soxhlet device and petroleum ether as solvent. Total protein content was determined based on 2001.11 method (Kjedahl). Ash content was determined following method 923.03 by gravimetric assay at 550 °C. Dietary fiber content was determined according to the 985.29 enzymatic method. Total carbohydrate content was estimated by difference. These results were expressed in g.100 g^{-1}. The quantification of minerals sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe) and zinc (Zn) was performed following the method 990.08. These results were expressed in mg.100 g^{-1}.

Vitamin C

It was determined by the 2,6-dichlorophenol indophenol (DCPI, Vetec) titrimetric method (SILVA, 1999). Results were expressed in mg.100 g^{-1}.

TPC The total phenolic compounds was performed by spectrophotometric method using the Folin-Ciocalteu reagent (Merck®, Germany) according to the methodology described by SINGLETON & ROSSI (1965). Sample extraction was performed with acetone 70% for 30 minutes under stirring, followed by filtration. Extracts were diluted 10 times with distilled water before reaction and absorbance was measured at 760 nm. A calibration curve was made from Gallic acid standard (Sigma-Aldrich®, Brazil) and blank was prepared with distilled water. Total phenolic compounds content was expressed in mg GAE.100 g^{-1} (gallic acid equivalent).

ABTS assay Antioxidant activity was determined by the method of reduction of $ABTS^{\bullet+}$ radical (Sigma-Aldrich®, Brazil) according to RE et al. (1999). Sample extraction was conducted in two steps using methanol 50%, followed by precipitate extraction with acetone 70%. In both steps the extraction time was 1 hour. The extracts were mixed in a 25 mL volumetric flask and filled using distilled water. For the reaction, 30 µL of sample extract was added with 3 mL of $ABTS^{\cdot+}$. Absorbance was measured at 734 nm after 6 minutes of reaction. Results were expressed as micromoles Trolox equivalents per gram (µmol $TE.g^{-1}$).

TC The total carotenoids were extracted according to the methodology proposed by Rodriguez-Amaya (2001), using acetone as solvent, by means of exhaustive extraction, and quantified by spectrophotometric method at 453 nm. The carotenoid profile was analyzed by high performance liquid chromatography (HPLC) with reverse phase separation according to methodology proposed by Pacheco et al. (2014), using the same extract. For this, the chromatographic run was performed on an Waters® Alliance 2695 liquid chromatograph coupled to a photodiode array detector (Waters® 2996) set at 450 nm, using YMC Carotenoid S-3® column (4.6 × 250 mm), methanol and methyl terc-butyl ether as mobile phase in gradient elution, 0.8 $mL.min^{-1}$ flow rate, column temperature at 33 °C and 15 µL of injection volume. The identification of carotenoids was performed by comparison of retention times and absorption spectra with analytical standards. The quantification was performed by external standardization, by means of standard curves of lutein, zeaxanthin, zeinoxanthin, β-cryptoxanthin, α-carotene and β-carotene. For better resolution of the chromatogram, extracts containing the carotenoids were saponified in 10% KOH in methanol. Results were expressed in µg.100 g^{-1}.

Flavonoids

Extraction and analysis of flavonoids in samples were performed according to GODOY et al. (2013). Samples were extracted with 80% methanol for 2 h at 65 °C. After cooling, 3 mL of 2M NaOH was added and samples were subjected to stirring for 10 minutes with subsequent addition of acetic acid. The samples were centrifuged at 6000 rpm for 10 minutes and their supernatants were filled into vials. Chromatographic run was performed on an Waters® Alliance 2695 chromatograph coupled to a photodiode array detector (2996, Waters®) set at 260 nm, using Thermos BDS Hypersil C18 column (100 × 4.6 mm 2.4 µm), 1% formic acid and acetonitrile as mobile phase in gradient elution, 1.3 mL min^{-1} flow rate, column temperature at 45 °C and 25 µL of injection volume. Quantification was performed by external standardization, using standard curves of epicatechin, daidzin, naringin, rutin, myricetin, hesperidin, diosmin, quercetin, genistein, naringinin, hesperetin and kaempferol, flavonoids commonly reported in vegetables. Results were expressed in mg.100 g^{-1}.

A completely randomized design was adopted in this research and the obtained results were reported as mean ± standard deviation in dry weight (dw). Thus, four samples (fresh and commercial pulps, seed and peel) were submitted to assays in triplicate, resulting in 12 treatments. Data were subjected to one-way analysis of variance for comparison of means using Statistic 7.0 (Statsoft Inc., Tulsa, OK, USA) and significant differences were calculated according to Tukey test at 5% level.

RESULTS AND DISCUSSION

Proximate and mineral composition

Umbu fractions and commercial pulp proximate composition can be seen in table 1. The seed fraction presented the highest lipid content (8.92% dw). According to BORGES et al. (2007), the umbu seed is rich in unsaturated fatty acid (60%). It makes this fraction attractive from the health point of view, since the intake of unsaturated fatty acid has been related to the prevention of coronary diseases as well as to the improvement of cardiovascular functions (CHRYSOHOOU et al., 2016).

Protein content varied from 6.08 to 9.01 g.100 g^{-1} (dw) in the evaluated samples. Dietary fiber content varied between 12.35 and 65.00 g.100 g^{-1} (dw) in the samples. Fibers help in laxation and promote satiety, which may reduce the ingested energy amount and; therefore, the risk of obesity. They can also attenuate blood glucose levels, normalize serum cholesterol levels and reduce the risk of heart diseases. Considering the higher fiber contents observed in samples, 100 g of peel or seed provided the recommended daily intake of total fiber for an adult (OTTEN et al., 2006). Even though seed and peel are non-conventional edible fractions, they might be exploited as dietary fiber sources for food enrichment acting as ingredient in new formulations.

As expected, the carbohydrate content was higher in umbu pulps than in the peel and seed fractions. The amount of sugars reported in umbu pulps is responsible for the fruit's sweet taste, which makes it an attractive fraction for development of juice and other products. Carbohydrate content contributes to the energy value of the samples, which explains umbu pulps presenting the higher values of this parameter.

All the samples presented considerable amounts of minerals (Table 1). The minerals are essential nutrients for the good functioning of

Table 1 - Proximate and mineral composition of umbu fractions and commercial pulp.

Parameters	Fresh pulp	Peel	Seed	Commercial pulp
-Proximate composition-				
Total solids[1]	11.09 ± 0.01[c]	90.33 ± 0.01[b]	96.36 ± 0.01[a]	9.23 ± 0.04[d]
Ash[1]	2.21 ± 0.32[c]	3.38 ± 0.05[b]	2.74 ± 0.07[b,c]	4.17 ± 0.08[a]
Protein[1]	7.30 ± 0.01[b]	6.08 ± 0.05[c]	9.01 ± 0.08[a]	6.18 ± 0.00[c]
Lipid[1]	6.00 ± 1.21[b]	0.78 ± 0.05[c]	8.92 ± 0.70[a]	4.12 ± 0.61[b]
Carbohydrates[1*]	71.53 ± 0.65[a]	40.42 ± 0.05[c]	14.31 ± 0.08[d]	50.17 ± 0.19[b]
Fiber[1**]	12.35	49.34	65.00	35.32
Energy[2**]	307.21	161.49	148.03	256.34
-Mineral Composition[3]-				
Na	6 ± 0[c]	12 ± 0[b]	6 ± 0[c]	26 ± 1[a]
K	1,240 ± 12[c]	1,491 ± 9[b]	755 ± 2[d]	2,164 ± 16[a]
Mg	57 ± 1[c]	88 ± 0[b]	135 ± 10[a]	87 ± 2[b]
Ca	64 ± 0[d]	195 ± 3[b]	348 ± 1[a]	171 ± 3[c]
Fe	2 ± 0[b]	1 ± 0[b]	74 ± 4[a]	4 ± 0[b]
Zn	0.7 ± 0.0[b]	0.7 ± 0.0[b]	2 ± 0[a]	1 ± 0[b]
P	150 ± 1[b,c]	154 ± 1[b]	287 ± 20[a]	114 ± 3[c]

[1]Results expressed in g.100 g^{-1} dw. [2]Results expressed in kcal.100 g^{-1} dw. [3]Results expressed in mg.100 g^{-1} dw. [*]Determined by difference. [**]Analyses without repetition. Values are expressed in mean ± standard derivation. The values with different letters in the same line mean statistic differences (P<0.05).

human organism, acting as cofactors in enzymatic processes, for example. However, the recommended intake limits should be respected in each case (FERGUSON & FENECH, 2012; BIESALSKI & TINZ, 2016). Among the evaluated samples, it is remarkable the differences on their mineral contents (P<0.05). Commercial pulp presented the highest amounts of sodium and potassium. According to OTTEN et al. (2006) the recommended daily intake of potassium for an adult is 4.7 g. Thus, 100 g of umbu dried pulp supply 46% of individual daily requirements of this mineral.

For most of the evaluated minerals, the highest content was observed in the seed. In this fraction, Mg, Ca, Zn and P concentrations were 134.45, 347.74, 2.36, and 286.56 mg.100 g^{-1} (dw), respectively. Once the recommended daily intake for these minerals is 260, 1000, 7, and 700 mg, respectively, it represents about 52, 35, 34 and 41%, respectively, of the recommended daily intake for an adult (BRAZIL, 2005). Even though iron from vegetable matrices presents lower bioavailability than iron from animal sources, the iron content found in umbu seed (74 mg.100 g^{-1}) was high enough to be considered as a good source of this element. According to the data 100 g of seed fraction provides the individual daily requirements of an adult for this mineral (BRAZIL, 2005). INADA et al. (2015) reported that there is high prevalence of iron-deficiency in Brazil and highlighted the importance of using vegetable sources rich in iron as an auxiliary source, despite of its low bioavailability. Thus, formulated products using umbu seed might contribute to increase the intake of this mineral.

Bioactive compounds and antioxidant activity

Total phenolic compounds, total carotenoids, vitamin C and antioxidant activity of umbu samples are shown in figure 1. The phenolic contents in peel (1,775 mg GAE.100 g^{-1}) and commercial pulp (1,746 mg GAE.100 g^{-1}) were significantly higher than in the other two samples (Figure 1a). These values are higher than those reported by MELO & ANDRADE (2010) for umbu pulp and umbu peel flour, although lower than the contents in anthocyanins rich berries such as juçara and jabuticaba (INADA et al., 2015). It is important to highlight that even not as high as in berries, the umbu phenolic contents may contribute to increase antioxidant intake in human diet (SAURA-CALIXTO et al., 2007). Besides the well-known antioxidant action, phenolic compounds have also been attributed

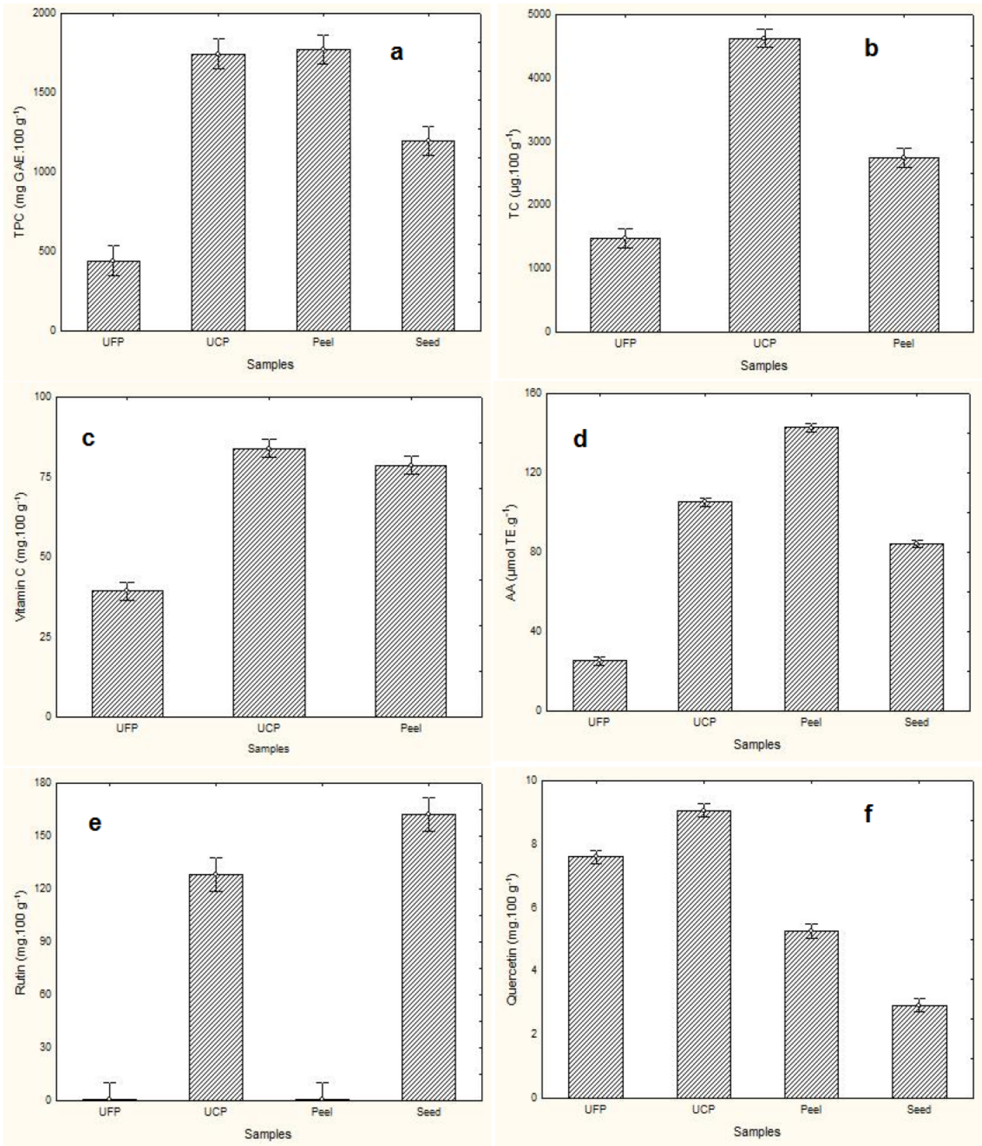

Figure 1 - Total phenolic compounds (TPC), total carotenoids (TC), vitamin C, antioxidant activity (AA), rutin and quercetin values in umbu fractions and commercial pulp. UFP (Umbu fresh pulp) and UCP (Umbu commercial pulp). The bars means average ± confidence level of 95%.

to have other biological activities such as antitumoral and cardioprotective, which reinforces the importance of the consumption of foods rich in phenolic (SAURA-CALIXTO et al., 2007; DIACOMETTI et al., 2016).

Two phenolic compounds, rutin and quercetin, were identified in the evaluated samples. The concentration of these compounds varied significantly among them (Figure 1e and 1f). Commercial pulp and seed samples presented higher rutin content ($P<0.05$). Regarding to quercetin content, it was highest in commercial sample (9.0 mg. 100 g^{-1}dw) ($P<0.05$).

The commercial pulp showed total carotenoids content (4632 µg.100 g^{-1} dw) significantly higher than the other umbu fractions, mainly fresh pulp (Figure 1b). This difference may be related to agricultural practices and ripeness stage, as well as losses during harvest, transport, storage and depulping process. However, as compared with other fruit pulps, the umbu pulp presented higher carotenoids content than those quantified in camu-camu, mangaba and cashew apple pulps (RUFINO et al., 2010).

Studies from carotenoids as lutein, zeaxanthin and β-cryptoxanthin have shown positive effects of these substances on cancers and other diseases associated to oxidative stress. β-carotene has also been attributed to anti-carcinogenic action besides of its well-known pro-vitamin A activity, which is essential for vision (RAO & RAO, 2007). This composition makes umbu fractions, pulp and peel, still more important for human nutrition, since they present rich composition in carotenoids as can be seen in table 2. It was observed the presence of lutein, zeaxanthin, zeinoxanthin, β-cryptoxanthin, α-carotene, β-carotene and its isomers in commercial pulp and peel. In fresh pulp sample zeaxanthin and zeinoxanthin were not detected.

Among the detected carotenoids, β-carotene presented the highest concentration in all the evaluated samples (P<0.05). According to the classification of carotenoid sources proposed by BRITTON & KHACHIK (2009), fresh pulp (80 μg.100 g^{-1} fw) fresh weight, commercial pulp (140 μg.100 g^{-1} fw) and peel (1,100 μg.100 g^{-1} fw) can be considered as low, moderate, and high β-carotene sources, respectively. Thus, although the umbu peel is a non-conventional edible fraction, due its bioactive composition it might be used for supplementation of human diet through new formulations development for juice, cake, cookies and other or, simply, by use as flour added to the meals. This evaluation was not performed for umbu seed.

Regarding to pro-vitamin A activity, it is known that only α-carotene, β-carotene and β-cryptoxanthin present this function (BRITTON & KHACHIK, 2009). Thus, the calculation of pro-vitamin A value was performed taking into account the activity of each carotenoid, using the relation: 1 μg β-carotene corresponding to 0.167 μg retinol equivalent (RE) and 1 μg of α-carotene and β-cryptoxanthin corresponding to 0.084 μg RE (BRAZIL, 2005). Therefore, the amount of RE, in fresh weight, was 17.14, 36.96, and 217.66 μg RE.100 g^{-1} in fresh pulp, commercial pulp and peel samples, respectively. These results show that the umbu peel can be considered a pro-vitamin A source, since a portion of 100 g provides about 36% of the recommended daily intake of retinol equivalent for an adult (BRAZIL, 2005).

The umbu pulps and the peel fraction also contains relevant values of vitamin C (Figure 1c), a bioactive compound of great importance for human health since it is associated with various biological actions in the human body, being the main one its antioxidant activity, which together with the immune system protects the organism from damage caused by oxidative stress (GUO et al., 2016).

The commercial pulp presented higher vitamin C content than the fresh pulp (P<0.05). Comparing to data reported in literature for umbu pulp, this samples presented lower vitamin C content than those evaluated by RUFINO et al. (2010). These differences among vitamin C content on umbu pulps, as well as those observed for carotenoids, may be related to soil, weather, agricultural practices and ripeness stage, as well as losses during harvest, transport, storage and depulping process.

According to Brazilian legislation (BRAZIL, 2005), the recommended daily intake of vitamin C for an adult is 45 mg. Then, the evaluated samples provide, in 100 g dry weight, at least 89% of the vitamin C daily intake.

Antioxidant activity of umbu samples is shown in figure 1d. The umbu peel showed the highest antioxidant activity value (142.78 μmol TE.g^{-1} dw) followed by commercial pulp (105.24

Table 2 - Carotenoids in umbu peel, fresh and commercial pulps.

Caratenoids[1]	Fresh pulp	Peel	Commercial pulp
Lutein	113 ± 6[b]	54 ± 2[c]	396 ± 8[a]
Zeaxanthin	ND	24 ± 2[b]	65 ± 0[a]
Zeinoxanthin	ND	441 ± 5[a]	336 ± 15[b]
β-cryptoxanthin	316 ± 13[c]	420 ± 8[b]	1,604 ± 15[a]
α-carotene	99 ± 0[a]	48 ± 2[c]	87 ± 0[b]
β-carotene	739 ± 26[c]	1,276 ± 57[b]	1,533 ± 38[a]
13-cis-β-carotene	36 ± 0[c]	60 ± 0[b]	76 ± 0[a]
9-cis-β-carotene	108 ± 0[b]	181 ± 7[a]	190 ± 8[a]

[1]Results expressed in μg.100 g^{-1} dw. ND - not detected. Values are expressed in mean ± standard derivation. The values with different letters in the same line mean statistic differences (P<0.05).

μmol TE.g^{-1} dw), seed (84.30 μmol TE.g^{-1} dw) and fresh pulp (25.24 μmol TE.g^{-1} dw). The highest antioxidant potential observed in the umbu peel is probably due to the synthesis of bioactive compounds such as phenolics synthesized by plants under stress conditions (TOMÁS-BARBERÁN & ESPÍN, 2001).

CONCLUSION

The obtained data showed that the whole umbu fruit, fresh pulp, peel and seed, as well as commercial pulp contains relevant nutrients and bioactive compounds, which makes not only the edible part but also seed and peel potential ingredients for the development of new products in food industry. This, besides of contributing to the offer of healthier processed products to consumers, is a way to reduce the discard of organic material and to add value to umbu fruit agrochain.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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