CHARACTERIZATION OF THE ANTIOXIDANT CAPACITY OF NATIVES FRUITS FROM THE BRAZILIAN AMAZON REGION[1]

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ABSTRACT - The objective of the present study was to characterize the chemistry and the antioxidant capacity in 8 species of native fruits from Amazonia. All the fruits were collected at full physiological and commercial maturity from properties located at: Boa Vista / RR, São Luiz do Anauá / RR, Manaus / AM, and Belém / PA. At the end of the experiment, the functional pattern for the camu-camu fruits showed that the total phenolic and ascorbic acid content and antioxidant assays were superior compared to the other samples. Despite the functional losses detected for the freeze-dried samples of the camu-camu fruit, all the other freeze-dried samples kept under -20ºC showed appropriate stability for long-term storage. In addition, it was also observed that fruit peel showed higher antioxidant activity than pulp or samples containing peel and pulp tissues in the same extract. When the ratio between the ORAC and total phenolic assays were observed, the uxi fruit demonstrated the highest antioxidant power compared to the other fruits studied, despite its relatively low levels of phenolic compound content and ORAC values. This means that there is a relevant contribution of these phenolic compounds to the antioxidant activity of uxi fruit.
Index terms: ORAC, DPPH, funcional compounds, nutritional.

CARACTERIZAÇÃO DA ATIVIDADE ANTIOXIDANTE DE FRUTOS NATIVOS DA REGIÃO AMAZÕNICA BRASILEIRA

RESUMO - O objetivo do presente trabalho foi realizar à caracterização química e a capacidade antioxidante em oito espécies de frutos nativos da Amazônia. Todos os frutos foram coletados em completo desenvolvimento da maturidade fisiológica e comercial em propriedades rurais localizadas em: Boa Vista / RR, São Luiz do Anauá / RR, Manaus / AM e Belém / PA. Ao final do experimento, o padrão funcional para os frutos de camu-camu mostrou que o conteúdo de fenólicos totais, de vitamina C e a atividade antioxidante foi superior em compração às demais espécies. Apesar das perdas nos componentes funcionais detectadas para as amostras liofilizadas de camu-camu, todas as amostras dos demais frutos mantidas em temperatura abaixo de -20°C mostraram estabilidade adequada para longos períodos de armazenamento. Além disso, observou-se também que a casca dos frutos apresentou maior atividade antioxidante do que a polpa dos frutos e amostras contendo tecidos da casca e da polpa no mesmo extrato. Quando a relação entre a atividade antioxidante medida pelo método ORAC e o conteúdo de fenólicos totais foram observadas, o uxi demonstrou o poder antioxidante mais elevado em comparação aos demais frutos estudados, apesar de apresentar níveis relativamente baixos no contéudo de fenólicos totais e na atividade antioxidante medida pelo método ORAC. Isso significa que existe uma contribuição considerável desses compostos fenólicos na atividade antioxidante do uxi.
Termos de indexação: ORAC, DPPH, compostos funcionais, nutricional.

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INTRODUCTION

The search for a longer, healthier life has increased concern about health and consequently the demand for high-quality nutritive foods. In this sense, the phenolic compound contents and antioxidant capacity have been the target of much scientific investigation for knowledge of the nutritional value, valorization of the commercial aspects and prevention of degenerative diseases caused, mainly, by oxidative stress.

The Brazilian Amazon region has great fruit species bioavailability with approximately 220 edible fruit producing plant species, representing 44% of native fruit diversity in Brazil. These fruits can be characterized as considerable sources of micronutrients, used in human nutrition to protect the population's health against innumerable diseases. So, knowledge of the species and characterization of the functional properties of these fruits, in function of their own biodiversity, is still an important challenge for their valorization because many of these species have not yet been studied chemically. The antioxidants can be defined as substances that have properties capable of minimizing the harmful effect of free radicals, inhibiting oxidative alterations in the molecules and are found most frequently in fruits, seeds and vegetable oils (Rahman, 2007).

Antioxidants can be of synthetic or natural origin, however, studies have shown that indiscriminate and excessive use of synthetic antioxidants may have some toxic effects (SOUSA et al., 2007). The natural antioxidants include ascorbic acid, vitamin E, B-carotene and in the phenolic compound group, flavonoids and phenolic acids (DUARTE-ALMEIDA et al., 2006; BROINIZI et al., 2007), that are frequently found in fruits. The phenolic compounds have excellent antioxidant activity according to Sousa et al. (2007), mainly because of their reducing properties and chemical structure, which play an important role in neutralizing or kidnapping free radicals and transition metal chelation, acting both at the initiation and propagation stages of the oxidative process. Thus research is being carried out to find natural products with relevant antioxidant activities accessible to the consumer to allow the possible substitution or at least association of natural and synthetic antioxidants, minimizing the risks to human health.

So, to make available information regarding the functional potential of native fruits from Amazon and consequently contribute to regional development, the objective of the present study was characterize phenolic compound and ascorbic acid content, as well the antioxidant capacity in freeze-dried samples of native species from Amazon region.

MATERIAL AND METHODS

SAMPLES

The native fruits used to carry out the present study were:

Açaí de terra firme: round fruit, 1.22 to 1.53 cm in diameter, pulp with translucent appearance and a violet-purple dark color, 1.75 to 1.92 g for fresh mass and 85.2 to 86.1% moisture content. Harvest performed in March in Manaus / AM / Brazil (winter crop – rainy season).

Araçá-boi: elliptical shape, 5.44 to 6.73 cm in longitudinal diameter and 7.40 to 7.73 cm in transverse diameter, with a white-yellow peel and a creamy-white pulp presented a mucilaginous aspect, 95.82 to 99.88 g for fresh mass and 84.5 to 85.7% moisture content. Harvest performed in November in Boa Vista / RR / Brazil.

Cajá: elliptical shape, 3.01 to 3.13 cm in longitudinal diameter and 3.50 to 3.81 cm in transverse diameter, with a yellow pulp and peel, 76.03 to 79.78 g for fresh mass and 82.8 to 83.1% moisture content. Harvest performed in March in Belém / PA / Brazil.

Cajú: pyriform shape, 4.61 to 4.88 cm in basal diameter and 3.04 to 3.33 in apical diameter pulp with fibrous aspect and of yellow-orange color and peel of reddish-orange color, 95.23 to 98.10 g (pulp and nut) for fresh mass and 89.1 to 90.8% moisture content. Harvest performed in December in Boa Vista / RR / Brazil.

Camu-camu: round fruit, 2.35 to 2.86 cm in diameter, with a red-purple peel and a translucent white-pink pulp, 9.25 to 10.44 g for fresh mass and 91.5 to 92.3% moisture content. Harvest based on color skin. Harvest performed in July (upland camu-camu) in Boa Vista / RR /Brazil.

Inajá: ovoid shape, 4.13 to 4.22 cm in longitudinal diameter and 2.55 to 2.78 cm in transverse diameter, mucilaginous pulp, interspersed with fibers, resulting in a spongy aspect with a light-yellow color, 9.34 to 9.67 g for fresh mass and 81.9 to 83.4% moisture content. Harvest performed in July in São Luis da Anauá / RR / Brazil.

Murici: round fruit, 1.7 to 2.2 cm in diameter, the pulp is fleshy and yellow translucent and the peel is yellow-orange, 2.22 to 2.37 g for fresh mass and 88.4 to 90,2% moisture content. Harvest performed in September in Boa Vista / RR / Brazil.

Uxi: drupe oblong-ellipsoid, with 5.4 to 7.1

cm in longitudinal and 4.2 to 4.6 cm in diameter, the pulp is greenish-yellow, with a smooth exocarp, and peel of yellow-orange color, 55.61 to 60.31 g for fresh mass and 91.2 to 92.2% moisture content. Harvest performed in October in Boa Vista / RR / Brazil.

METHODOLOGY

The fruits, at the complete physiological maturity stage (cajá and uxi – climacteric metabolism) and commercial maturity stage (caju, açai de terra firme, camu-camu, inajá, murici and araçá-boi – non-climacteric metabolism), following pre-tests, were collected on rural properties located in the states of Pará, Manaus and Roraima.

After harvesting, the fruits were transported to the Food Technology Laboratory at Roraima Federal University, Brazil, and selected for absence of visible damage and rot, standardized by size/caliber, epidermis coloring and washed in sodium hypochlorite (NaOCl) solution at 2.5% L^{-1} water for 30 minutes. The fruits were then washed in distilled water and dried on perforated trays and exposed during two hours to atmospheric air (25 ± 2 ºC, 70 ± 3 % RH). Next the seeds of the fruit were separated from the peel and pulp using stainless steel knives. After selected, sanitized and prepared the frozen samples were subjected to the lyophilization process in an Ilshin Freeze Drier until constant weight to obtain the samples of peel and pulp of the fruits.

The freeze-dried samples were classified into 3 extracts for the analysis of their functional compounds: peel, pulp, and both tissues in the same sample. These analyses were performed in 2 consecutive seasons. For the first season the analysis were performed at the Food Technology Laboratory of Roraima Federal University. The second season was analyzed at the Plant Bioactives & Bioprocessing Research Laboratory of Texas A&M University/USA.

ANALYSIS

The analyses were carried out over freeze-dried samples (peel and pulp) at 0 (harvest time) and 120 days of frozen storage at -20°C. The following analyses were performed:

• Ascorbic acid: was determined following the method by Carvalho et al. (1990), which is based on reduction of the indicator 2.6 – dichlorobenzene indolphenol (DCFI) by ascorbic acid. The results were expressed in mg $100mL^{-1}d$ of sample.

• Total phenols: were determined by spectrophotometric method, using the Folin–Ciocalteau reagent (Merck), following methodology described by Wettasinghe and Shahidi (1999) and standard curve of gallic acid. The results were expressed in mg of GAE $100g^{-1}$ of sample.

• Antioxidant activity by the ORAC method: was carried out according to Ou et al. (2001) adapted by Huang et al. (2002), used in microplates with fluoresceine. The analysis was carried out in 96-well microplates (Synergy HT Multi-Mode Mic. Reader, BioTek Ind., USA). A volume of 25µL of the sample was mixed with 150 µL fluoresceine (55.5nM) and incubated for 15 minutes at 37ºC in the microplate before the automatic injection of 25µL AAPH solution (155mM). The fluorescence was observed for 50 minutes by readings (λexcit. = 485nm; λemis. = 520nm). Trolox solutions were prepared for the calibration curve (8, 16, 24, 32 and 40M). All the solutions were diluted in phosphate buffer (75 mM, pH 7.4). The samples were analyzed in three dilutions taking as mean the final ORAC value as recommended by Huang et al. (2002). The antioxidant activity quantification was based on the calculation of the area under the fluorescence curve as proposed by Prior et al. (2003). The results were expressed in µmol Eq Trolox $100g^{-1}$ of sample.

• Antioxidant activity by the DPPH method: was carried out according to Blois (1958) with some modifications. The analysis was carried out in 96-well microplates (Synergy HT Multi-Mode Microplate Reader, BioTek Industries, USA), and the reduction in absorbance at 517 nm was monitored every 5 minutes until the reaction reached a plateau. The determinations were carried out by adding to each well of the microplate 250µL of the DPPH solution and 40µL methanol for the control, or the same volume for the standard solutions (BHA, BHT, ascorbic acid, chlorogenic acid and quercetin) and sample extracts. Absorbency readings were taken after 25 min reaction in a microplate spectrophotometer with incubation at 25ºC. The analyses were performed in triplicate. The DPPH remaining at the end of the reaction was determined and quantified as the DPPH radical scavenging activity using a standard curve of Trolox. The antioxidant DPPH method was expressed in µmol Eq Trolox $100g^{-1}$ of sample.

STATISTICAL ANALYSIS

The exploratory data analysis showed that the data had normal distribution; the errors were independent and presented homoscedasticity. Thus

the data was submitted to an analysis of variance by the F test and the means were compared by the Tukey test at 5% statistical probability. The experiment was carried out in a complete randomized design in a factorial arrangement consisting of two factors: different species of freeze-dry fruits/cold storage time, with 3 replications and 250 ± 15g /replication.

RESULTS AND DISCUSSION

According to the analysis presented here, high results were detected in the camu-camu fruits for the phenolic compound and ascorbic acid content and, antioxidant activity, by both methods, when compared to the other Amazon species studied (Figures 1 – 4). Reynertson et al. (2008) studied 14 Mirtaceas fruits where camu-camu fruits also presented the highest phenolic compound contents. Rufino et al. (2010) also mentioned in their studies that camu-camu fruits presented the highest levels of ascorbic acid content and antioxidant activity compared to the other native fruits of Brazil. These results were more evident in the peel than in the pulp, and even when compared to the samples containing both peel and pulp tissue in the same extract. Of all the native species analyzed, only the camu-camu extracts showed a significant decrease of the amount of phenolic compounds and ascorbic acid content during the experimental time (data not showed). Thus, it was observed in the camu-camu extracts, after 120 days of frozen storage, average losses of 31.13 % and 39.34 % of the initial amounts for the phenolic compound and ascorbic acid content, respectively, being that the same was observed by Maeda et al. (2006). These decreases were probably influenced by the exposure time of the samples to oxygen that generated oxidative reactions and consequently reduced the phenolic compound and ascorbic acid concentration, even under frozen storage.

It was further observed in the ORAC and DPPH analysis carried out on the camu-camu samples, in all the extracts analyzed (pulp, peel and peel/pulp), that the frozen storage time also influenced the antioxidant activity and significant decreases were observed after 120 days at -20ºC (data not showed). In this sense, it was observed an average decrease of 7.18 % in the antioxidant activity, by both methods, when compared to the antioxidant activity in the harvest time. This fact may be justified by the long frozen storage period to which the camu-camu samples were submitted assuming that the decreases can be greater with longer storage periods for this specie, even under controlled temperature conditions. This hypothesis was also confirmed when it was possible to state that the decreases of ascorbic acid content in the camu-camu fruits, as well as the antioxidant activity, were significant in all the extracts of this fruit during the frozen storage.

In both the antioxidant activity analyses carried out on the fruits, the ORAC method showed numerically much higher values compared to the DPPH method, where the values were numerically lower, that demonstrated the greater sensitivity of the ORAC method in detecting antioxidant activity. However, because the DPPH method is practical, fast and stable, it is considered one of the most used methods to verify antioxidant activity (ESPIN et al., 2000). Thus the joint analysis of at least two methods should be taken into consideration (HUANG et al., 2005) in the comparative analysis of the tests that quantify antioxidant activity in the species investigated, represented by the ORAC and DPPH methods in the present study.

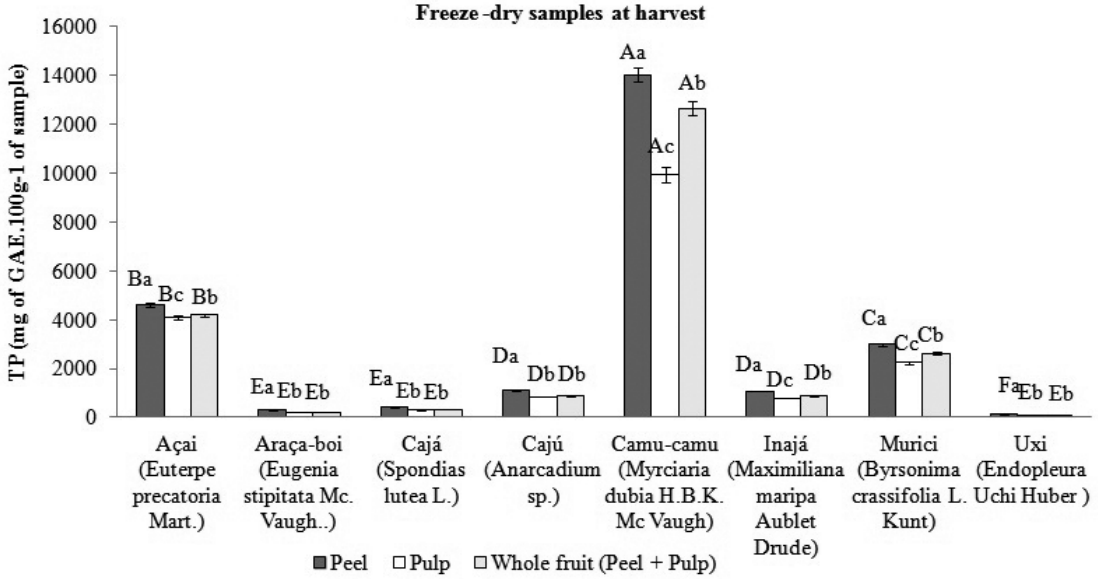
Except for the camu-camu fruits, as mentioned before, frozen storage time did not statistically influence the antioxidant activity, the phenolic compounds or the ascorbic acid content. In this sense, despite these functional losses in the camu-camu freeze-dried samples, all the others freeze-dried species kept under -20 ºC showed appropriate stability by a period up to 120 days, without any significant loss of functional compounds (data not showed). These results suggested that besides ascorbic acid, other phenolic compounds with antioxidant capacities and more stable to oxygen might be present in these fruit. It was also assumed that the ascorbic acid contents would be at high concentrations in the freeze-dried samples, in all the extracts of camu-camu fruits, influencing the antioxidant activity during frozen storage. The same was observed, but at lower concentrations, in the other samples of freeze-dried fruits that were analyzed in the same way (RUFINO et al., 2010).

In the present study the phenolic compound and ascorbic acid contents and the antioxidant activity also were numerically higher in the peel than the other extracts of all the fruits studied. Unfortunately, the peel is usually discarded before consumption, both fresh and by-product processing of these raw materials, which is representing a waste of nutrients with high functional potential, according to the results presented in this experiment.

The ratio between the ORAC and total phenolics assays (specific ORAC values) showed at Figure 5, demonstrated that uxi fruits, despite the relative low levels of phenolic compound content

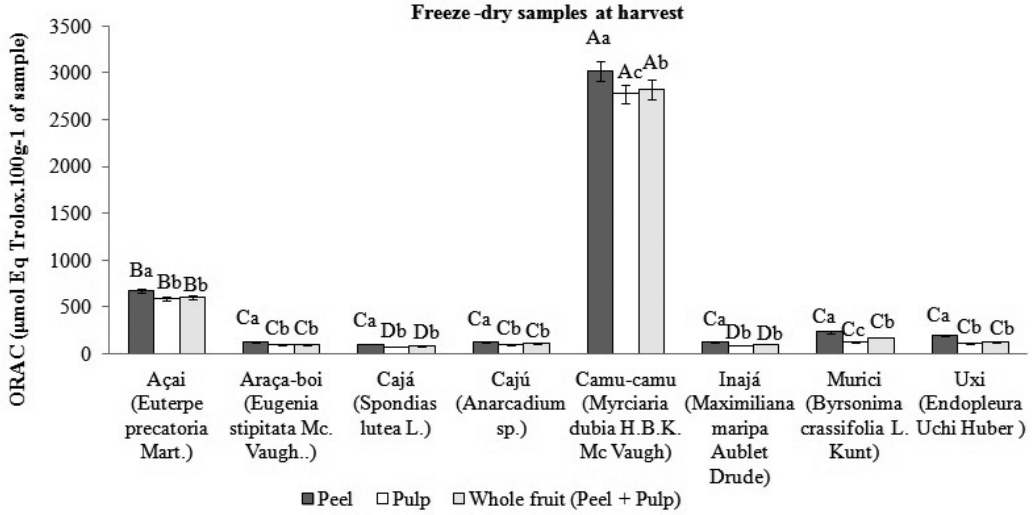
and ORAC values, showed the highest antioxidant power compared to the others fruits studied here. This means that there is a relevant contribution of these phenolic compounds to the antioxidant activity of the uxi fruits. So, the general conclusion is that other compounds have a higher importance than phenolic compound content in antioxidant activity (SCALZO et al., 2005; PATTHAMAKANOKPORN et al., 2008). However, in the study carried out by Rufino et al. (2010), and also when it was analyzed each parameter individually in the present experiment, the camu-camu fruits showed a direct relationship involving the antioxidant capacity (both methods), phenolic compound and the ascorbic acid content. As it was observed before, all species except for the freeze-dried samples of camu-camu, there were no statistical differences between all the other species and in each extract analyzed during the cold storage period. In this sense, after 120 days of -20 ºC, the mean specific ORAC values decreased 12.15, 10.87 and 13.33% for extracts made by peel, pulp and using both tissues in the same sample of camu-camu (data not showed).

This pattern reveals the need for further studies aiming to complete the characterization of phenolic compounds and supposedly, the relationship that each compound would have with the antioxidant activity. It can be asserted that there is a direct relationship between total phenolic content and antioxidant capacity of the pulp and peel of each fruit analyzed here. However, as well as mentioned, not all the pulp and peel that show the highest phenolic compound content and the higher antioxidant activity were the ones with outstanding antioxidant power when analyzing the ratio between ORAC and phenolic compound content. In this sense, the antioxidant activity of a specific phenolic compound is related with the number of available hydroxyl groups present in the chemical structure (RICE-EVANS et al., 1996). Therefore the manner that these compounds neutralize free radicals will depend on their relative concentrations in the sample matrix. So, the valuation of these and others native species of the Amazon region, in the context of functional food, requiring further studies aiming the characterization of phenolic compounds and them antioxidant activity.



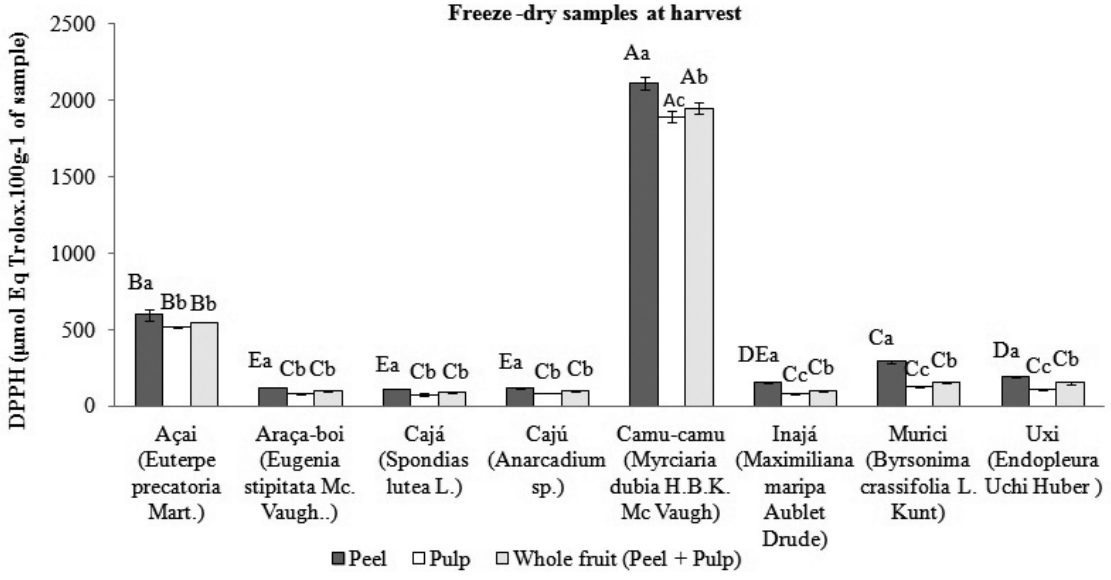
Means followed by the same capital letters (different species) and lowercase letters (inside the same specie) did not differ at the 5% level of significance by the Tukey test.

FIGURE 1 - Average between 2 seasons in the total phenolic contents (dry weight) in 3 different extracts of freeze-dried native fruit from Amazon: peel, pulp, and both tissues (peel+pulp) in the same extract. All the samples were submitted to the frozen storage at -20ºC. Boa Vista-RR, Brazil.



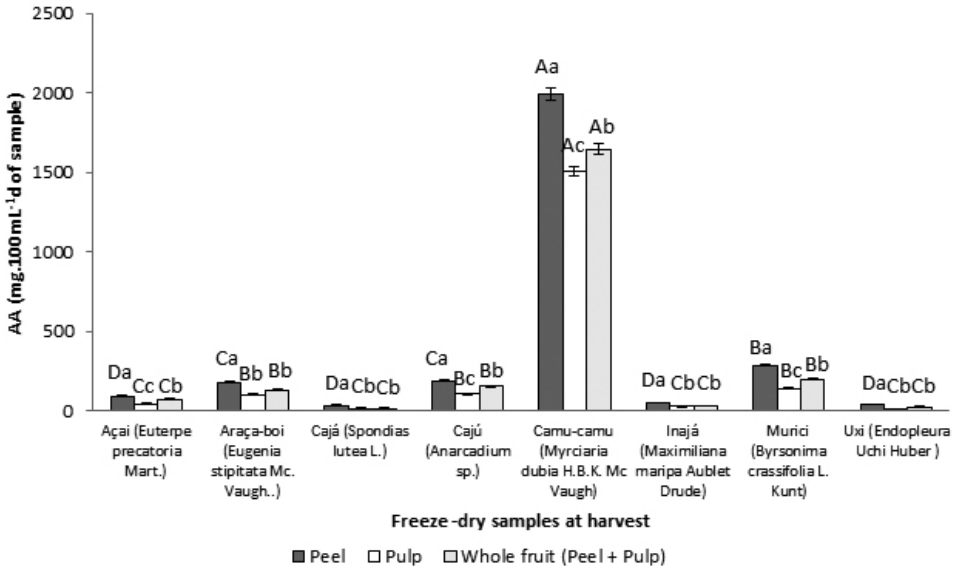
Means followed by the same capital letters (different species) and lowercase letters (inside the same specie) did not differ at the 5% level of significance by the Tukey test.

FIGURE 2 - Average between 2 seasons in the ORAC values (dry weight) in 3 different extracts of freeze-dried native fruit from Amazon: peel, pulp, and both tissues (peel+pulp) in the same extract. All the samples were submitted to the frozen storage at -20ºC. Boa Vista-RR, Brazil.



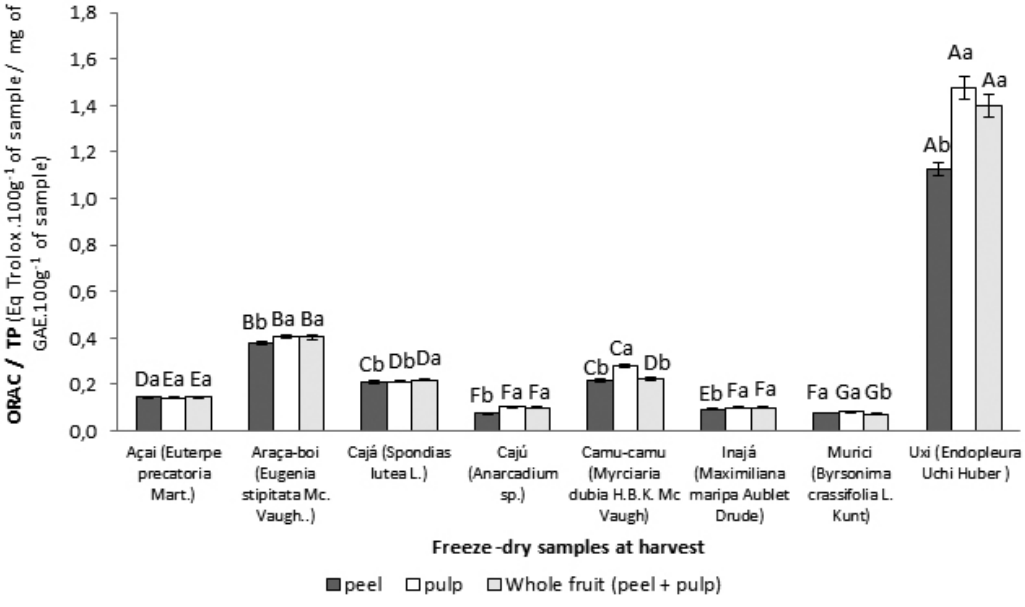
Means followed by the same capital letters (different species) and lowercase letters (inside the same specie) did not differ at the 5% level of significance by the Tukey test.

FIGURE 3 - Average between 2 seasons in the DPPH values (dry weight) in 3 different extracts of freeze-dried native fruit from Amazon: peel, pulp, and both tissues (peel+pulp) in the same extract. All the samples were submitted to the frozen storage at -20ºC. Boa Vista-RR, Brazil.



Means followed by the same capital letters (different species) and lowercase letters (inside the same specie) did not differ at the 5% level of significance by the Tukey test.

FIGURE 4 - Average between 2 seasons in the ascorbic acid content (dry weight) in 3 different extracts of freeze-dried native fruit from Amazon: peel, pulp, and both tissues (peel+pulp) in the same extract. All the samples were submitted to the frozen storage at -20ºC. Boa Vista-RR, Brazil.



Means followed by the same capital letters (different species) and lowercase letters (inside the same specie) did not differ at the 5% level of significance by the Tukey test.

FIGURE 5 - Average between 2 seasons in the specific ORAC values (dry weight) in 3 different extracts of freeze-dried native fruit from Amazon: peel, pulp, and both tissues (peel+pulp) in the same extract. All the samples were submitted to the frozen storage at -20ºC. Boa Vista-RR, Brazil.

CONCLUSIONS

1 -Thus it was concluded that the Amazon native fruit that best showed aptitude in the context of functional foods, especially regarding the phenolic compound contents and antioxidant activity, was the camu-camu;.

2 -The uxi samples, in all the 3 extracts analyzed, showed the highest specific ORAC values; and,

3 -For all the parameters analyzed the amount of functional compounds, in all the fruits, was always numerically superior in the peel than in the pulp. According to this, new scientific approaches must be performed, as the characterization of the phenolic profile of these samples, in order to detect and analyze possible new functional compounds.

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