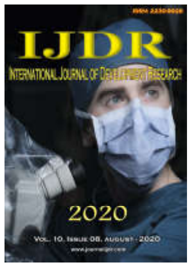
ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 08, pp. 39707-39711, August, 2020
https://doi.org/10.37118/ijdr.19722.08.2020



RESEARCH ARTICLE

OPEN ACCESS

DETERMINATION OF BOTH EXTRACTABLE AND NON-EXTRACTABLE POLYPHENOLS, AND IN VITRO ANTIOXIDANT ACTIVITY IN PULP AND CLARIFIED ASSAI JUICE

Francisco Acácio de Sousa[1], Raimundo Wilane de Figueiredo[2], Aluísio Marques da Fonseca[1], Jeferson Falcão do Amaral[1], Lívia Paulia Dias Ribeiro[1] and Maria do Socorro Moura Rufino[1*]

[1]Master's program in Sociobiodiversity and Sustainable Technologies (MASTS), University for International Integration of the Afro-Brazilian Lusophony, Redenção-CE, Brazil
[2]Department of Food Engineering, Federal University of Ceará, Fortaleza-CE, Brazil

ARTICLE INFO

Article History:
Received 29th May 2020
Received in revised form
22nd June 2020
Accepted 03rd July 2020
Published online 30th August 2020

Key Words:
Industrial juice. Functional food.
Phenolic compounds.

****Corresponding author:***
Maria do Socorro Moura Rufino,

ABSTRACT

The demand of the world population for healthier juices and foods has been growing. Assai functional juice, as well as the fruit, has functional potential, due to the high content of antioxidant dietary fiber (soluble and insoluble). In this paper, Pulp and clarified assai juice were quantified, by checking the content of extractable polyphenols (EPP), non-extractable polyphenols (MACAN) and total antioxidant activity (TAA) by the ABTS method. The following samples were analyzed: Concentrated and clarified assai juice 24° Brix (SCC1); Concentrated and clarified assai juice 24° Brix (SCC2); Concentrated and clarified assai juice 18° Brix (SCC3), and Assai pulp juice 12% solids (SP4). The statistical analysis of the data used R development core team software. In addition, we also apply principal component analysis (PCA). We found that the sample SCC1 obtained the best result with 335.68 mg EAG/g for EPP, and SCC2 obtained 769.33 mg/100g of MACAN. SCC3 sample obtained the highest TAA with 15.47 µM Trolox/g. Analysis of SP4 (PCA) showed that its value is visibly lower in relation to the total content of the analyzed parameters. Thus, clarified assai juice can be considered as a functional drink, as it has high levels of bioactive compounds and antioxidant activity.

Citation: ***Francisco Acácio de Sousa, Raimundo Wilane de Figueiredo, Aluísio Marques da Fonseca, Jeferson Falcão do Amaral, Lívia Paulia Dias Ribeiro and Maria do Socorro Moura Rufino.* 2020.** "Determination of both extractable and non-extractable polyphenols, and in vitro antioxidant activity in pulp and clarified assai juice", *International Journal of Development Research*, 10, (08), 39707-39711.

INTRODUCTION

Scientific evidence on the effects of antioxidants on human health has been established only on phenolic compounds and antioxidant activity with low molecular weight. With the advancement of new methodologies, it is possible to detect that plant foods have high concentrations of antioxidants, high molecular weight and are called macromolecular or macroantioxidant compounds (SAURA-CALIXTO, 2017). These compounds have high biological activity, as they exhibit benefits in relation to the health of the human body. They differ to low molecular weight antioxidants by having some specific physiological characteristics and some mechanisms of action that, with increased consumption, prevent chronic and degenerative diseases (ZURITA *et al*.; 2012). In Brazil, few studies seek to identify these compounds as macro-antioxidants.

The first studies were carried out by Rufino *et al*. (2010), analyzing fruits such as assai (*Euterpe oleracea*), acerola (*Malpighia punicifolia*) and cashew (*Anacardium occidentale L*.). In addition, the main researches related to assai are associated with fruit pulp. For instance, in the paper by Rufino *et al.* (2010), the assai pulp, especially the insoluble, has high levels of dietary fiber, anthocyanin and fatty acids (oleic and linoleic). Therefore, it stands out as an energy source, thus being an excellent alternative to be added to diets. Thus, it is of utmost importance to advance further research related to the macroantioxidant potential, especially of functional drinks such as clarified juice. The clarification process consists in removing suspended material (lipids and insoluble solids) and is carried out by separating the membranes and filtering to remove suspended particles. The process allows the acceptance of the product on the market, with the same characteristics as the fruit and the possibility of using juice for the preparation of new products. Rocha *et al*. (2015) already described that the pulp and clarified juice of assai have the potential to be used as functional supplements in diets, due to the high content of antioxidant dietary fiber (soluble and insoluble), which makes the consumer look for a natural product. Assai, in its various forms of commercialization, is rich in fibers and helps the human body in intestinal transit, where the low consumption of these compounds in the diet can cause serious problems, such as cancer and cardiovascular diseases (RUFINO *et al*., 2010). As mentioned above, there are two types of soluble fibers (pectins, gums, mucilages and hemicelluloses) that help to reduce the level of cholesterol in the bloodstream, in addition to insoluble ones (lignins, celluloses and hemicelluloses), which prevent the body from acquiring certain types of cancer. Both pulp and assai oil have a great antioxidant potential, attributed to the compounds present such as anthocyanins and flavonoids (RUFINO *et al*., 2011). Therefore, this work aims to analyze the pulp and clarified assai juice from the industry, by quantifying the total extractable polyphenols, the antioxidant activity and the macroantioxidant compounds (MACAN).

MATERIALS AND METHODS

Research Location: The research was carried out at the Tropical Fruit Laboratory of the Food Engineering Department (DEAL), at the Federal University of Ceará, in Fortaleza-CE (Latitude: 3°73’73’’S, Longitude: 38°'57'33"W), and at the Biochemistry Laboratory of the University of International Integration of Afro-Brazilian Lusophony in Redenção-CE (Latitude: 6°56'33"S, Longitude: 38°'58'03"W). The samples were provided in October 2018 by a juice industry, located in the State of Ceará. In total, there were four samples: three of clarified juice and one of assai pulp juice, whose processing is dated between March and July 2018. They were kept in polyethylene bottles and stored in a freezer at - 20 ºC.

Chemical reagents: The reagents Folin-Ciocalteu, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, and ABTS (2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic), were acquired from Sigma-Aldrich. Other reagents used, such as potassium persulfate, sulfuric acid, hydrochloric acid, 70% acetone, 50% methyl alcohol, 99.8% ethyl alcohol and butanol, were purchased from Neon. In addition, we also used gallic acid from Dinâmica and $FeCl_3$ from Êxodo Cientifica. All the reagents used were of analytical grade. Condensed tannin concentrate from Mediterranean carob pods was supplied by Nestlé Ltd (Vers-chez-les Blancs, Switzerland).

Preparation and analysis of polyphenol fractions: Each sample was extracted using organic solvents (methanol and acetone) through the method developed by Rufino *et al.* (2007), in which 0.5 grams were used per sample of clarified juice and juice from the assai pulp.

Total extractable polyphenols (EPP): This analysis followed the protocol developed by Montreau (1972) with adaptations by Rufino *et al.* (2007). 50 µL aliquots of assai clarified juice extracts and assai pulp juice were added in test tubes, adding up to a total of 0.5 mL. For this, distilled water was added and, in the same test tubes, 0.5mL of the Folin-Ciocalteau reagent, 1.0mL of Na_2CO_3 20% g.100mL^{-1}(Neon brand) and 1.0mL of distilled water. Then, they were homogenized, remaining at rest for a period of 30 minutes. In the same conditions, the reagents were blanched, but the extract was not added, only distilled water. After resting, readings were taken on a spectrophotometer (model UV 1800-Shimadzu) at 700nm. And the curve was made with gallic acid. The results were expressed in mg of gallic acid per 100 g^{-1} of sample.

Antioxidant activity by the free radical scavenging assai (ABTS • +): The antioxidant activity was determined by the method developed by *Miller et al.* (1993), with adaptations by Rufino *et al.* (2007a). In test tubes aliquots of the clarified juice extract and juice of the assai pulp of 30, 20 and 10 µL were used, and 3 mL of the ABTS radical were added and then homogenized. Readings were taken after 6 minutes. The results were expressed in µM Trolox/g.

Non-extractable polyphenols or MACAN: The method used was developed by Pérez-Jiménez *et al.* (2015). 0.5 grams of each sample of industrial juice was used, similarly to that performed in the EPP analysis, adding aqueous organic solvents to obtain the extracts of HPP and NEPA (SAURA-CALIXTO, 2012). For the methodology of hydrolyzable polyphenols (HPP), the samples were submitted to a temperature of 85ºC for one hour, after being cooled, they were added dropwise to the methanol and sulfuric acid of the Sigma brand. (HARTZFELD *et al.,* 2002). After that, the pH of the samples was adjusted to 5.5. Then, they were read on a spectrophotometer at 750 nm. The calibration curve was constructed from gallic acid as a sample standard. As for the procedure for the analysis of proanthocyanidins (NEPA), the fractions used were the same and the samples underwent a heat procedure in a water bath at 100ºC for one hour. After cooling, a solution composed of butanol/HCl/$FeCl_3$ was added to the samples, both solutions were made by the Sigma brand the readings were performed on a spectrophotometer, at 450 and 555 nm wavelengths. For the calibration curve, the commercial standard of proanthocyanidin (Ceratonia siliqua I.), provided by Nestlé Ltda. The sum of the HPP and NEPA values are called macro-antioxidants (MACAN) and expressed in mg/100g.

Statistical Analysis: The data were submitted to analysis of variance and comparisons of means through the Tukey test at the level of 5% with the aid of the R software (R DEVELOPMENT CORE TEAM, 2009). Principal Component Analysis (PCA) was carried out in order to identify if there were any chemical pattern between the different samples of clarified assai juice and non-clarified assai pulp, as well as to verify which chemical variable is predominant in the identification of the samples.

RESULTS

Determination of Extractable Polyphenols – EPP: The levels of total extractable polyphenols in the clarified juice and in the pulp of the assai juice varied between 102.07 to 335.68 mg EAG/g. Among the samples analyzed, the SCC1 sample stood out, with 335.68 mg EAG/g. This result may be associated with the concentration of assai pulp used in the clarified juice, which can be proven by comparing the results of the other samples. An example is the SCC2 sample, which has similar concentrations of assai with the sample mentioned above and obtained a result of 318.90 mg EAG/g. The percentage of the coefficient of variance was 3.11%. The other samples obtained inferior results compared to the samples mentioned above, as shown in Table 1.

Table 1. Quantification of total extractable polyphenols by samples of clarified juice and pulp of assai juice expressed in mg equivalent of gallic acid/100g*

Sample	Total extractable polyphenols
(SCC1)	335,68 [a]
(SCC2)	318,90 [a]
(SCC3)	210,19 [b]
(SP4)	102,07 [c]

*Mean value n = 3, Turkey test at 5% level.

Determination of Antioxidant Activity – ABTS: The results of the samples of clarified juice and pulp of assai juice varied between 8.90 to 15.47 µM trolox/g (Table 2), the samples that had the highest results were SCC2 and SCC3 with their respective values 11.36 and 15,47 µM trolox/g, both with the same brix grade. The percentage of the variance coefficient was 6.85%.

Table 2. Quantification of antioxidant activity in samples of clarified juice and pulp of assai juice by the ABTS method expressed in µM Trolox/g*

Sample	ABTS
(SCC1)	11,13 [b]
(SCC2)	11,36 [b]
(SCC3)	15,47 [a]
(SP4)	8,90 [c]

* Mean value n = 3, Turkey test at 5% level.

Determination of Macroantioxidants – MACAN: The results of the content of hydrolyzable polyphenols present in the clarified juice and pulp of the assai juice in the analyzed samples varied between 76.68 to 443.10 mg/100g, with the percentage of the variance coefficient of 2.49%. In the results of the content of proanthocyanidins, the values varied between 68.24 to 337.01mg/100g, with the percentage of the variance coefficient of 0.5%. In the results of the Macroantioxidant content, which is the sum of the results of the HPP + NEPA values, the values were 152, 14 to 780.11 mg/100g. The samples that had the most expressive results regarding the study carried out were SCC2 and SP4, with respective values 780.11 and 574.49 mg/100g (Table 3).

Table 3. Quantification of the non-extractable polyphenols (MACAN) of the elaborated extracts of clarified juice and pulp of assai juice

Sample	HPP	NEPA	MACAN
(SCC1)	76,68 [c]	75,46 [c]	152,14
(SCC2)	443,10 [a]	337,01 [a]	780,11
(SCC3)	86,47 [c]	68,24 [d]	154,71
(SP4)	393,23 [b]	181,26 [b]	574,49

*Mean value n = 3, Turkey test at 5% level.

Principal component analysis (PCA): Principal Component Analysis is a method that has a basic purpose, in which statistical data is used in a linear way that find the eigenvalues and eigenvectors of the data covariance matrix. Through the result, one can perform a dimensional reduction of the data and analyze the main patterns of variability present. It is one of the most widely used and known multivariate methods in data reduction (LYRA *et al.;* 2010). The data used already exists and the results of the analysis of this EPP, ABTS, HPP and NEPA research were used. Below in Figure 1 is the relationship between the sample scores and the weight of the variables of the main components.

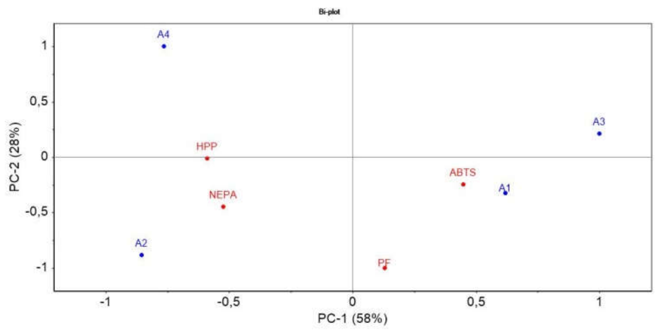


Figure 1. Bi-plot graph relates the graph of the sample scores and weights of the variables of the main component analysis, with 70% explanation in the first two components (A1 = SCC1; A2 = SSC2; A3 = SCC3 and A4 = SP4)

DISCUSSIONS

Total Extractable Polyphenols: These results corroborate the research carried out by Vissoto *et al.* (2013), in which frozen pulps of various tropical fruits were analyzed, including assai, with pulps produced in Belém-PA, whose results were 251 ± 64 mg EAG/g. In addition, in the work of Neves *et al.* (2015), whose assai pulp processed by hand, obtained values between 512.75 to 558.56 mg EAG/g. Canuto *et al.* (2010) analyzed several fruits from the Amazon, including assai, from the south-central region of Roraima, and found in the pulp of fruits harvested in the maturation stage about 408.28 mg EAG/g pulp. Studies by Rufino *et al.* (2010), evaluated the fresh pulp of assai and found results of 454 mg EAG/g. Another study, recently conducted by Camacho *et al.* (2018), in which fresh tropical and non-tropical fruits were evaluated, one of them being assai, presented a result of 454.0 mg EAG/g; these values are higher than those obtained in this research. This variation in value can be attributed to the various processes to which the fruit was subjected (CEDRIM, 2018). This research is in line with other studies carried out, where the results allow to affirm that the pulp of the juice and the clarified juice of assai are rich in phenolic compounds; these, in turn, help the human body to fight and prevent chronic diseases, anti-inflammatory, anti-hypertensive, anti-allergic among others (LOBO *et al .;* 2016).

Total Antioxidant Activity: In the research by Canuto *et al.* (2010), in which assai fruits were processed into pulps, the values found were 10.0 µM trolox/g. In the work of Coutinho *et al.* (2017), frozen assai pulps from two companies located in the state of Pará and Minas Gerais were analyzed, and their results were between 4.45 to 11.83 µM trolox/g. In another study, designed by Rufino *et al.* (2010), in which fresh assai fruits were analyzed, the results were 15.10 µM trolox/g. The inconsistency of the levels of antioxidant activity, given by the different methods used to analyze its results, depending on the polarity of the reaction medium, exert different actions with each methodology, presenting different characteristics. Because of this, it was not possible to find just one method that is efficient to demonstrate accurate and safe results and that point out the exact amount of this substance in a fruit sample (MELO, 2011).

Macroantioxidants: In a study by Pérez-Jiménez; Díaz-Rubio; Saura-Calixto (2013) the content of non-extractable polyphenols in various red fruits was evaluated, among them fresh assai, obtaining results of 1,240 mg/100g of dry matter for the content of hydrolyzed polyphenols (HPP). In the same study, the content of proanthocyanins (NEPA) in assai samples in dry matter was evaluated and values of 1210 mg/100g were obtained. Since there is not much research related to a specific fruit, we compare the research presented here with studies of other ones. Similarly, to this study, the one carried out by Rufino *et al.* (2010) also presents non-extractable polyphenols in tropical fruits, in which they analyzed fruit residues such as acerola and cashew. In this study, values of 12.1 $g.Kg^{-1}$ of dry matter for content of (HPP) for cashew. In the acerola residues, 3,9 $g.Kg^{-1}$ of dry matter were obtained in the same analysis. For the NEPA content there were no results for acerola, however the cashew obtained contents of 52,0 $g.Kg^{-1}$ of dry matter. In another study designed by Silva (2018), samples of grape marc from the wine industries were evaluated in different cycles, with results between 261.14 to 610.7mg/100g. Research on macroantioxidant compounds in foods and beverages is still limited when compared to extractable polyphenols, making further studies necessary, especially with tropical fruits and their by-products.

Principal component analysis (PCA): As shown in Figure 1, using the horizontal axis (which explains 58% of the data variability), samples SCC1 and SCC3 differ from samples SCC2 and SP4 in the following aspects: samples SCC1 and SCC3 have similarity due to the greater influence of concentration of Polyphenols and ABTS. SCC2 and SP4 samples have similarity due to the greater influence of the concentration of HPP and NEPA. Using the vertical axis (which explains 28% of the data variability), the SCC2 and SCC1 samples have similarity because they are generally richer in NEPA and Polyphenols than the SCC3 and SP4 samples. The SP4 sample is noticeably poorer in relation to the total content of the parameters, with the HPP being the only parameter with any influence. The data presented by the analysis of main components confirm the results presented in the others; in which it is identified that the samples analyzed are rich in bioactive compounds and antioxidant activity.

Conclusions

The SCC2 sample showed high levels of extractable and non-extractable polyphenols, and antioxidant activity. This result, different from the other samples, is influenced by the processing date, batch, and other factors. The data from the analysis carried out corroborate with the results presented in the principal component analysis (PCA), in which it is proven that the samples of clarified juice (SCC1, SCC2 and SCC3) showed significant results in the analysis of EPP, ABTS and HPP and NEPA. Only the juice sample from the SP4 pulp has a smaller amount of bioactive compounds than the other samples.

Acknowledgments

The support from UNILAB (Research Group: Polyphenols, antioxidants and dietary fiber in health - POLIFIBAN/CNPq), UFC (Tropical Fruit Laboratory) and National Institute of Advanced Analytical Technologies - INCTAA (CNPq, Process n° 46576/2014).

REFERENCES

CAMACHO, R. R.; RUFINO, M. S. M.; CRUZ, D. M. A.; PÉREZ, A. M. Non-extractable Polyphenols in Tropical Fruits: occurrence and health-related properties. In: SAURA-CALIXTO, F.; PÉREZ-JIMÉNEZ, J. Non-extractable polyphenols and carotenoids: importance in human nutrition and health. *The Royal Society of Chemistry*, p.88-110, 2018.

CANUTO, G. A. B.; XAVIER, A. A.O.; NEVES, L. C.; BENASSI, M. de T. Caracterização físico-química de polpas de frutos da Amazônia e sua correlação com a atividade anti-radical livre. *Rev. Bras. Frutic*., v. 32, n. 4, p. 1196-1205, 2010.

CEDRIM, P. C. A. S.; BARROS, E. M. A; NASCIMENTO, T. G. do. Propriedades antioxidantes do açaí (*Euterpe oleracea*) na síndrome metabólica. *Brazilian Journal of Food Technology.* V.21, 2018.

COUTINHO, R. M. P.; FONTES, E. A. F.; VIEIRA, L. M.; BARROS, F. A. R.; CARVALHO, A. F.; STRINGHETA, P. C. Physicochemical and microbiological characterization and antioxidant capacity of açaí pulps marketed in the states of Minas Gerais and Pará, Brazil. *Ciência Rural*, v.47, n.1, p. 1-6, 2017.

HARTZFELD, P. W.; FORKNER, R.; HUNTER, M. D.; HAGERMAN, A. E. Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *Journal of Agricultural and Food Chemistry*, v.50, n.7, p.1785-1790, 2002.

LOBO, A. C. M.; VELASQUE, L. F. L. Revisão de literatura sobre os efeitos terapêuticos do açaí e sua importância na alimentação. *Biosaúde*. v. 18, n. 2, 2016.

LYRA, W.S; SILVA, E.C; ARAÚJO, M.C.U; FRAGOSO, W.D. Classificação periódica: um exemplo didático para ensinar análise de componentes principais. *Química Nova*, Vol. 33, N. 7, 1594-1597, 2010.

MATTA, V. M. da.; CRUZ, A. P. G.; CABRAL, L.M.C.; DONÂNGELO, C. M. *Açaí clarificado por microfiltração*. Rio de Janeiro: *Embrapa Agroindústria de Alimentos*, 2010. 4p, (Embrapa Agroindústria Alimentos. Comunicado Técnico, 165).

MELO, P. S.; BERGAMASCHI, K. B.; TIVERON, A. P.; MASSARIOLI, A. P.; OLDONI, T. L C.; ZANUS, M. C.; PEREIRA, G. E.; ALENCAR, S. M. Composição fenólica e atividade antioxidante de resíduos agroindustriais. *Ciência Rural*, v.41, n.6, p.1088-1093, 2011.

MILLER, N.J.; DIPLOCK, A.T.; RICE-EVANS, C.; DAVIES, M.J.; GOPINATHAN, V.; MILNER, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Sciense*, v.84, p.407-412, 1993.

MONTREAU, F.R. Sur le dosage des composés phénoliques totaux dans les vins par la methode Folin-Ciocalteau (O teor de compostos fenólicos totais em vinhos pelo método de Folin-Ciocalteau). *Connaissance de la Vigne et du Vin*, v.24, p.397-404, 1972.

NEVES, L. T. B. C.; CAMPOS, D. C. dos S.; MENDES, J. K. S.; URNHANI, C. O.; ARAÚJO, K. G. M. qualidade de frutos processados artesanalmente de açaí (*Euterpe oleracea* mart.) e bacaba (*Oenocarpus bacaba* mart.). *Rev. Bras. Frutic.,* v. 37, n. 3, p. 729-738,2015.

PÉREZ-JIMÉNEZ, J.; DÍAZ-RUBIO, M. E.; SAURA-CALIXTO, F. Non- extractable polyphenols, a major dietary antioxidant: occurrence, metabolic fate and healt effects. *Nutrition Research Reviews*, v.26, n.2, p.118-129, 2013.

PÉREZ-JIMÉNEZ, J.; SAURA-CALIXTO, F. Macromolecular antioxidants or non-extractable polyphenols in fruit and vegetables: Intake in four European countries. *Food Research International*, v.74, p.315-323, 2015.

ROCHA, S. M. B. M.; OLIVEIRA, A. G.; COSTA, M. C. D. Benefícios funcionais do açaí na prevenção de doenças cardiovasculares. *Journal of Amazon Health Science,* v. 1, n. 1, p. 1-10, 2015.

RUFINO, M. S. M.; ALVES, R. E. *Metodologia Científica: Determinação de polifenóisextraíveis totais.* Comunicado Técnico. Embrapa, Fortaleza, 2007.

RUFINO, M. S. M.; ALVES, R. E.; BRITO, E. S.; MORAIS, S. M.; SAMPAIO, C. G.; PÉREZJIMÉNEZ, J.; SAURA-CALIXTO, F. D. *Metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre ABTS.* Comunicado Técnico n° 128.Embrapa, Fortaleza, 2007a.

RUFINO, M.S.M., PÉREZ-JIMÉNEZ, J., ARRANZ, S., ALVES, R.E., BRITO, E.S., OLIVEIRA, M.S.P., SAURA-CALIXTO, F. Açaí (Euterpe oleraceae) 'BRS-Pará': A tropical fruit source of antioxidante dietary fiber and high antioxidante capacity oil. *Food Research International*, v.44, p.2100-2106, 2011.

RUFINO, M.S.M., PÉREZ-JIMÉNEZ, J., TABERNERO, M., ALVES, R.E., BRITO, E.S., SAURA-CALIXTO, F. Acerola and cashew apple as sources of antioxidants and dietary fibre. *Food Science & Technology*, v.45, p.2227-2233, 2010.

SAURA-CALIXTO, F. Concept and Health-Related Properties of Nonextractable Polyphenols: The Missing Dietary Polyphenols. *Journal of Agricultural and Food Chemistry*, v.60, n.45, p.11195-11200, 2012.

SAURA-CALIXTO, F. Macromolecular Antioxidants: Importance in Health and Perspectives. *Arch Med Deporte*, v. 34(4), p.188-189. 2017.

VISSOTTO, L. C.; RODRIGUES, E.; CHISTÉ, R. C.; BENASSI, M. de T.; MERCADANTE, A. Z. Correlation, by multivariate statistical analysis, between the scavenging capacity against reactive oxygen species and the bioactive compounds from frozen fruit pulps. *Ciência e Tecnologia de Alimentos*, v.33, p.57-65, 2013.

****