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Reduction of antinutrients and maintenance of bioactive compounds in flour from agro-industrial residue of acerola (*Malpighia emarginata* D.C.)

Redução de antinutrientes e manutenção de compostos bioativos em farinha de resíduo agroindustrial de acerola (*Malpighia emarginata* D.C.)

Reducción de antinutrientes y conservación de compuestos bioactivos en harina de residuo agroindustrial de acerola (*Malpighia emarginata* D.C.)

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Abstract

This study was aimed at analyzing the reduction of antinutrients, preserving apparent phenolic compounds in acerola flour residue by employing drying techniques. The optimal drying conditions were then determined in the wet residue, analyzing antinutrient concentration and apparent phenolic compounds in the residue and subsequently in the acerola flour. The physicochemical characterization of the flour was carried out, determining its antioxidant activity, thereby assessing the impact of the drying process on the composition of volatile compounds. The optimal drying conditions were found for a temperature of 65ºC for 120 min, reducing water activity by 0.3 and moisture content by 13.89%, which are considered adequate conditions for flours. Carbohydrate content represented 68.72 g/100g, while proteins represented 12.55 g/100g of the centesimal composition of the flour. Significant reductions ($p<0.5$) of antinutrients were observed in saponins (35.9%), followed by phytates (32.8%) and condensed tannins (11.52%). Losses of 18.7% of apparent phenolic compounds were also observed. For volatile compounds, a significant loss of esters with a significant increase in alcohol content was noticed. Thermal processing reduced aroma complexity, but maintained important bioactive compounds, such as linalool and caryophyllene. In this regard, taking into account the results of the present study, the agro-industrial residue of acerola proved to be an alternative source of antioxidants with a reduced antinutritional impact and may be incorporated as an ingredient in the formulation of new food products. Moreover, this agro-industrial residue reduces the environmental impact caused by the fruit pulp processing industry.

Keywords: *Malpighia emarginata*; Saponin; Phytates; Bioactive Compounds.

Resumo

O objetivo de estudo foi reduzir os antinutrientes, preservando os compostos fenólicos aparentes em farinha de resíduo de acerola, para tanto, empregando a técnica de secagem. A partir do resíduo úmido foi determinada a condição ótima de secagem, nesse sentido, avaliando a concentração de antinutrientes e compostos fenólicos aparentes do resíduo e,

posteriormente, para a farinha de acerola. Acrescentou-se à caracterização da farinha a determinação da composição físico-química, de atividade antioxidante, e avaliação do impacto da secagem sobre a composição de compostos voláteis. A condição de secagem determinada como ótima foi 65ºC por 120 minutos, reduzindo a atividade de água a 0,3 e umidade a 13,89%, condição adequada para farinhas. Os carboidratos representaram 68,72 g/100 g e proteínas 12,55 g/100 g da composição centesimal da farinha. A redução significativa ($p<0,5$) de antinutrientes para saponinas foi de 35,9%, seguida pela de fitatos 32,8% e taninos condensados com 11,52%. Houve perda de 18,7% em compostos fenólicos aparentes. Em relação aos compostos voláteis foi observada uma perda de ésteres com significativo aumento de álcoois. O processamento térmico reduziu a complexidade do aroma, mas manteve importantes compostos bioativos como linalol e cariofileno. Frente a esses resultados, a farinha de resíduo agroindustrial de acerola apresentou-se como uma fonte de antioxidantes alternativa com reduzido impacto antinutricional, podendo ser incorporada como ingrediente nas formulações de novos produtos alimentares. Aliando-se a isso, agrega a redução do impacto ambiental gerado pelos resíduos da indústria processadora de polpa de frutas.

Palavras-chave: *Malpighia emarginata*; Saponinas; Fitatos; Compostos Bioativos.

Resumen

El objetivo del estudio fue reducir los antinutrientes y conservar los compuestos fenólicos presentes en la harina de residuo de acerola, para ello, mediante la técnica de secado. A partir del residuo húmedo se determinó la condición óptima de secado, evaluando la concentración de antinutrientes y compuestos fenólicos aparentes del residuo y, posteriormente, para la harina de acerola. La caracterización de la harina se añadió a la determinación de la composición fisicoquímica, de actividad antioxidante, y la evaluación del impacto del secado sobre la composición de los compuestos volátiles. La condición de secado que se determinó como óptima fue de 65ºC durante 120 minutos, reduciendo la actividad del agua a 0,3 y la humedad al 13,89%, condición adecuada para las harinas. Los carbohidratos representaron 68,72g/100g y las proteínas 12,55g/100g de la composición centesimal de la harina. La reducción significativa ($p<0,5$) de los antinutrientes para saponinas fue del 35,9%, seguida de fitatos del 32,8% y taninos condensados con 11,52%. Hubo una pérdida del 18,7% en compuestos fenólicos aparentes. En cuanto a los compuestos volátiles, se observó una pérdida de ésteres con un aumento significativo de alcoholes. El procesamiento térmico redujo la complejidad del aroma, pero mantuvo importantes compuestos bioactivos como el linalol y el

cariofileno. La harina de residuos agroindustriales de acerola se presentó como una fuente de antioxidantes con reducido impacto antinutricional, pudiendo incorporarse como ingrediente en las formulaciones de nuevos productos alimenticios. Además de esto, se suma a la reducción del impacto ambiental generado por los residuos.

Palabras clave: *Malpighia emarginat*; Saponinas; Fitatos; Compuestos Bioactivos.

1. Introduction

Acerola (*Malpighia emarginata* D.C.) is a native fruit to Central and South America, with Brazil having among the largest acerola crops in the world. Despite its industrial potential, acerola processing generates a substantial amount of residues, loss of raw material, with great environmental, social and economic impact, generating up to a total volume of 40% of discarded residue (Silva, Santana & Koblitz 2010; La Fuente, Zabalaga, & Tadini 2017, Rezende, Nogueira, & Narain 2017).

Recent studies have shown that industrial residue from acerola processing can contain higher amounts of phenolic compounds and other bioactive compounds when compared to the fruit pulp, showing that the reuse of this residue could be better exploited (Rezende et al, 2017; Rezende, Nogueira & Narain, 2018; Silva, Duarte & Barrozo, 2019; Oliveira, 2020).

The production of flours is one alternative for reusing residues, as flours can be used as ingredients in the preparation of various products (biscuits, cakes, bread, sweets, among other food products). The potential advantages of this transformation include preserving the nutritional value, flavour and characteristic aroma, besides increasing shelf life of products (Zanatta, Schlabitz & Ethur, 2010; Santos, 2006).

On the other hand, plant-based food products contain significant levels of adverse antinutritional factors, with negative nutritional and health impacts. These antinutrients include trypsin and chymotrypsin inhibitors, oxalate, saponins, tannins and phytates (Costa, Queiroz-Monici, Reis & de Oliveira, 2006; Wang, Hatcher, Tyler, Toews & Gawalko, 2010), which may be widely present in flours. These compounds interfere on digestibility, absorption or in the use of nutrients and, if consumed in high concentrations, can lead to harmful effects to health, such as gastrointestinal irritation or injury (Griffiths, Birch & Hillman, 1998; Akande, Doma, Agu, & Adamu, 2010). Most antinutrients that affect protein digestibility are heat-sensitive, with heating being an alternative for improving protein digestibility (Nergiz & Gökgöz, 2007).

Kaur, Sharma, Dar, & Singh, (2012), when submitting cereal brans to dry heating at

temperatures of 100ºC and 110ºC and to drying times of 15, 20 and 25 min, observed significant reductions in phytic acid, polyphenols, trypsin inhibitors, saponins and oxalates with the progressive increase in temperature and time (Kaur et al., 2012). However, there is no consensus regarding the ideal duration and conditions for maximum antinutrient reduction, neither for preserving bioactive phenolic compounds of interest.

Volatile compounds involved in the development of aroma mostly include thermolabile compounds which can be rearranged, cyclized or oxidized as a result of temperature increase. Losses or changes in aroma are observed when the fruit is submitted to industrial processing, corroborating the need of studies that analyze these changes, which may result in products that compromise the original aroma (Thomazini & Franco, 2000). The present work analyses antinutrient reduction, preserving the bioactive potential of phenolic compounds in acerola flour residue by drying.

2. Methodology

Following the premises of the hypothetical-deductive method, the methodology used for the research's development fits in terms of objectives as experimental, employing the collection of quantitative data and interpreted with support of statistical tests and bibliographic information (Pradanov & Freitas, 2013; Pereira, Shitsuka, Parreira & Shitsuka, 2018).

Materials

Acerola residues, consisting of pulp and skin residue, originating from two different batches, were provided by Pomar do Brasil Indústria e Comércio de Alimentos, a fruit pulp producer manufacturer in the Brazilian city of Aracaju, in the State of Sergipe. The residues were sent to the Food Chemistry and Biochemistry Laboratory, in the Department of Food Technology from the Federal University of Sergipe, stored in polyethylene packaging and maintained at a temperature of -18ºC. When defrosted, the acerola residues were maintained at a freezing temperature (4 ºC) and subsequently characterized and dehydrated in a forced air convection oven.

Processing

The acerola residue was dried in a standard forced air oven (Tecnal TE-394/2) with temperature control, according to the guidelines described in (Garcia, de Alencar, Mota, Borges & de Souza, 2017). The temperature range was of between 45ºC and 85ºC, with the samples being dehydrated until reaching constant weight and/or the moisture content determined by guidelines no. 12 established by the Brazilian National Commission on Food Norms and Standards (CNNPA), from 1978 of ANVISA (BRASIL & Agência Nacional de Vigilância Sanitária, 1978). The dry residue was subsequently crushed in a food processor (Nutri ninja TM Auto-IQTM BL480BR30) and Tamis drum sieves were used in the homogenization of flour granules.

Physicochemical Characterization

Moisture, pH, total soluble solids, titratable acidity in organic acid, ash content, lipid and protein content were determined according to the classical Kjeldahl method and following the guidelines established by (Instituto Adolfo Lutz, 2008). Carbohydrate content was determined by difference, subtracting 100 from the sum of moisture, protein, lipid and ash contents. The water activity (Aw) of each sample was determined according to the guidelines established by AOAC (1995) (Association of official analytical chemists, 1995).

Phenolic Compounds

Firstly, extracts were prepared using wet residue samples, according to the methodology proposed by (Santos, et al., 2011) with some modifications, using ethanol p.a. 1:10 ($m.v^{-1}$) (99.8% from Neon) as a solvent and stirred in a SOLAB SL222 shaker-incubator for 4 hr in the dark. The sample extracts were prepared with a final concentration of 2.5 $mg.mL^{-1}$.

Phenolic compounds were estimated using the method proposed by (Singleton & Rossi, 1965) under basic conditions.

In vitro antioxidant activity was determined by ABTS+ assays [2,2'–azinobis–(3-ethylbenzothiazoline-6- sulfonic acid)], according to the methodology described in the technical guidelines no. 128 from the Brazilian Ministry of Agriculture, Livestock and Food Supply (BRASIL, 2007), as cited in (Boroski, Visentainer, Cottica & de Morais 2015).

The methodology originally developed by (Benzie & Strain, 1996) and cited in (Boroski et al., 2015) to measure antioxidant capacity in plasma and adapted for other samples, such as food products, was used to determine antioxidant activity by the FRAP (Ferric Reducing Ability of Plasma) method, using the FeIII-TPTZ complex ([2,4,6-tris(2-pyridyl)-s-triazine] 98%, from Sigma Aldrich) to determine the reducing power.

Total monomeric anthocyanin concentration was determined by the pH differential method described by AOAC (Lee, Durst & Wrolstad, 2005) and spectrophotometrically determined.

Antinutritional factors

Condensed tannins were spectrophotometrically determined according to (Broadhurst & Jones, 1978), with modifications by (Khattab, Goldberg, Lin, & Thiyam, 2010). Saponins were determined according to the protocol established by (Monje & Raffaillac, 2006).

The analytical method employed for determining phytic acid was based on the methodology proposed by (García-Villanova, Garcia-Villanova & de Lope, 1982), with modifications by (Romero-Aguilera, Alonso-Esteban, Torija-Isasa, Cámara, & Sánchez-Mata, 2017), with quantification using 2 g for the wet residue and 0.30g for the flour sample.

Total oxalate content was determined by the method established by (Oke, 1966), with modifications by (Falade, Dara, Bello, Osuntogun & Adewusi, 2004) and expressed as sodium oxalate.

Microbiological analysis

The microbiological analyses (mould and yeast, thermotolerant coliforms, *Bacillus*, *Salmonellas sp*. and mesophilic counts) were carried out according to the protocols established by (Downes & Ito, 2001).

Extraction and identification of volatile compounds

The volatile compounds in the samples of wet residue and acerola flour residue, extracted by SPME (Solid Phase Micro Extraction) with DVB/CAR/PDMS), were determined according to the methodology adapted by (Nogueira, et al., 2018), using a gas chromatography-coupled mass spectrophotometer equipment (Agilent 7000) for a complete

desorption of volatile compounds. The compounds were identified by comparing the mass spectra with the NIST standard database (National Institute of Standards &Technology, USA) and considering the linear retention indices (LRI) of each compound, calculated considering the retention times of homologues series of n-alkanes and analyzed applying similar separations conditions. The results were expressed as normalized peak areas.

Statistical Analysis

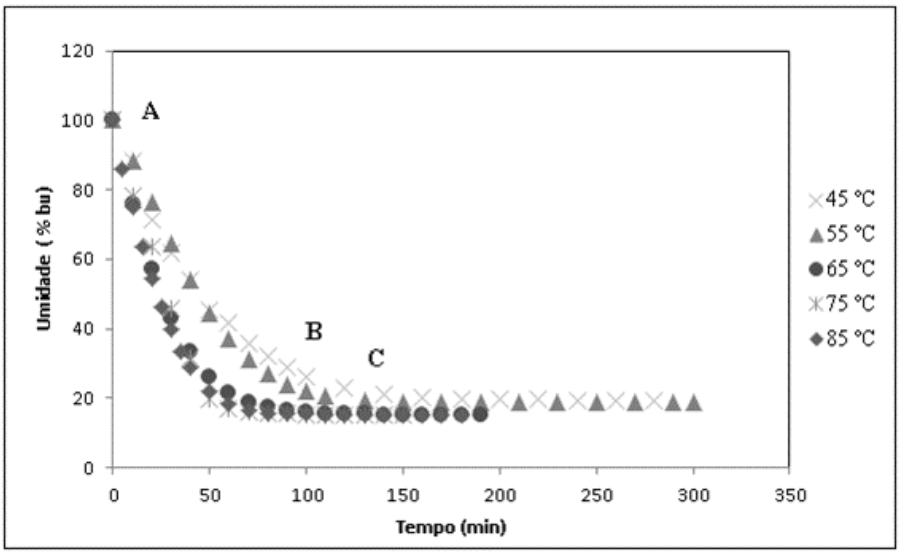
Assays were carried out in quadruplicate, with the results expressed as mean ± standard deviation. Analysis of variance (ANOVA) and Tukey's post-hoc tests were carried using the Minitab 19 software freeware, with a statistical significance of $p<0.05$.

3. Results and Discussion

Optimal drying conditions

The drying curve was obtained by monitoring moisture content throughout the drying process, estimating the decrease in moisture content of the residue over time. The final drying time was established when reaching constant moisture content, carrying out five different treatment processes, as described in Figure 1.

Figure 1. Drying curve of acerola residue representing moisture behaviour over time at different temperatures.



Note: air velocity: 2.20 m/s; Residue surface thickness: 0.50 cm. Source: Author's own compilation (2019).

In figure 1 it is possible to observe that 65ºC and 75ºC temperatures behaved similarly. Both brought the humidity to the lowest levels in the shortest time and remained stable after 100 minutes of drying, reaching equilibrium humidity, differently from what was observed 45º C and 85º C temperatures.

According to (Celestino, 2010), the interval between points A and B, as observed in Figure 1, corresponds to the period of time for which the residue is adapting to the drying conditions and when free water evaporates, corroborating the results presented in Table 2 for moisture content and water activity. After point B, evaporation rate is no longer constant, when increasingly less water is on the surface of the solid to be evaporated and the sample being increasingly drier. After point C, moisture content in the residue starts decreasing until reaching the equilibrium moisture content for the operating temperature and relative air humidity conditions, thereby terminating the drying process with the minimum moisture content, which was of 13.89% in the present work.

It is important to point out that the drying times used in the present work were lower than those found in the literature (Ferreira & Pena, 2010; Pereira, Silva, et al., 2013; Kwiatkowski, et al., 2016). These lower drying times were adopted to avoid significant losses of phenolic compounds, anthocyanins, and the reduction of antioxidant activity in acerola residue.

After residue dehydration, the first preliminary tests were carried out, in order to determine the optimal conditions for the reduction of antinutritional factors, in tannins, and the minimum loss of phenolic compounds and anthocyanins. Table 1 presents the results found for total apparent phenolic compounds, anthocyanins and condensed tannins (antinutrients) for the wet residue under the temperature conditions analysed.

Table 1. Concentration of bioactive compounds and antinutrients in the preliminary tests carried out at different temperatures of acerola flour.

Temperature (°C)	Apparent phenolic compounds (g 100 g- ¹)	Anthocyanins (mg 100 g- ¹)	Condensed tannins (mg 100 g- ¹)
25.0*	3.63 ^{a} ± 0.18	6.12 ^{a} ± 0.05	214.20 ^{a} ± 4.45
45.0	2.39 ^{d} ± 0.01	1.48 ^{c} ± 0.05	166.23 ^{b} ± 8.14
55.0	2.56 ^{cd} ± 0.02	1.39 ^{c} ± 0.06	165.25 ^{b} ± 8.23
65.0**	3.03 ^{b} ± 0.02	1.79 ^{b} ± 0.09	179.31 ^{b} ± 4.44
75.0	2.60 ^{c} ± 0.00	1.48 ^{c} ± 0.03	159.04 ^{b} ± 4.44
85.0	2.66 ^{bc} ±0.03	1.52 ^{bc} ± 0.11	169.95 ^{b} ± 0.09

Values in: *temperature of wet residue ** optimal conditions. Results regarding the mean value between both batches; mean values followed by the same letter in the same column do not differ statistically according to Tukey's test (p>0.05). Source: Author's own compilation (2019).

Table 1 shows the optimal temperature was 65ºC, significantly different (p<0.05) than

the remaining temperatures analysed, and which led to the lowest reduction of phenolic compounds and anthocyanins, one of the main objectives of this work.

No significant difference ($p>0.05$) was observed between the temperatures analysed for condensed tannins, an antinutrient. However, the resulting concentrations were significantly lower for all temperatures studied, at a 95% confidence level, when compared to the wet residue, as noted in Table 1.

Acerola contains an expressive concentration of phenolic compounds, with its strong colour being strongly contributed by polymeric anthocyanins that interfere in the determination of condensed tannins due to the formation of complexes between their molecules (Giusti & Wrolstad, 2001). This describes the behaviour of condensed tannins, like the behaviour observed for phenolic compounds and anthocyanins in optimal conditions considered in the present study.

Physicochemical characterisation of wet acerola residue and acerola flour

Table 2 presents the results of physicochemical characterisation of wet acerola residue and acerola flour produced from the former, showing the effects of thermal treatment on the flour obtained.

Table 2. Physicochemical characterisation of wet acerola residue and acerola flour.

Characteristics	Wet residue	Acerola flour
Moisture (%) (w.b.)*	$82.82^{a} \pm 0.7$	$13.89^{b} \pm 2.46$
Water activity (A_w)	$0.98^{a} \pm 0.00$	$0.3^{b} \pm 0.01$
pH	$3.51^{a} \pm 0.01$	$3.53^{a} \pm 0.01$
Acidity (% citric acid)	$4.29^{a} \pm 0.11$	$3.41^{b} \pm 0.03$
Brix^{o}	$7.75^{a} \pm 0.5$	$6.0^{b} \pm 0.4$
Ashes*		3.09 ± 0.22
Proteins*		12.55 ± 0.35
Lipids*		1.75 ± 0.03
Carbohydrates*		68.72 ± 2.36
Calorific value (Kcal)		340.5

Results in: *g 100 g^{-1}; w.b.: result expressed in wet basis; Results regarding the mean value between both batches. Mean values followed by the same letter in the same row do not differ statistically according to Tukey's test ($p>0.05$). Source: Author's own compilation (2019).

It can be seen in Table 2 that data for ashes, proteins, lipids and carbohydrates were not determined for the wet residue as these were expected to be concentrated by the drying process. Considerable amounts of protein can be seen in the residue flour, which is nutritionally relevant since it is a product obtained from fruit residue. The result shows that the determined drying condition guarantees a nutritionally interesting product, in addition to reducing costs during the process of obtaining flour.

As anticipated, the dehydration process resulted in an acerola flour with typical values of moisture content and water activity for dehydrated products.

According to the data in Table 2, after the residue's transformation into acerola flour, a significant reduction ($p<0.05$) of moisture content (69.9%) and water activity (68%) were observed, thus extending the residue's durability. Moreover, the combination of a lower drying temperature (65ºC), lower drying time (150 minutes) and air velocity (2.20 m/s) during dehydration favours the moisture content found for the flour from acerola residue, which is confirmed by the decrease in water activity. Such conditions are important to ensure microbiological stability, besides allowing to store the product at room temperature, being within the water activity range (0 and 0.6) established for dry food products, as described by (Pinho, Afonso, Carioca, Costa & Rybka, 2011).

The results of pH found for wet acerola residue (Table 2) did not significantly differ ($p>0.05$) from the results found for the acerola flour. These values characterise the acerola residue as very acid, as determined by (Nóbrega, Oliveira, Genovese & Correia, 2014).

Regarding the total acidity (% of citric acid), a significant reduction ($p <0.05$) was observed between the wet residue and the acerola flour, shown in Table 2, however on a small scale. A reference total acidity of 3.0 is established by guidelines CNNPA no. 12, of 1978 of ANVISA (Agência Nacional de Vigilância Sanitária) for flours (BRASIL & Agência Nacional de Vigilância Sanitária, 1978). The result found in the present study was higher than that established in the guidelines, which implies the need of employing a partial leaching step to the residue in order to ensure that the product is in accordance with the guidelines established by ANVISA.

Moreover, total soluble content (ºBrix) was also determinant, being an important parameter used to analyse the ripening stage of fruits. In the present study, this result was 20.5% lower ($p<0.05$) in acerola flour when compared to the wet residue (Table 2). Sugars are the main soluble solids found in fruits. A higher content of soluble solids is indicative of fruits harvested at a more advanced ripening stage, taking into account all compounds responsible for their aroma, flavour and organoleptic characteristics (Companhia de Entrepostos e

Armazéns Gerais de São Paulo, 2016).

Regarding ash content, the acerola flour presented a result of 3.09 ± 0.22 g 100 g-¹. This parameter reveals the total content of minerals (Na, K, Ca, etc.) present in the sample. Moreover, flours with a high ash content exhibit greater buffer capacity (Monteiro, Mársico, Soares Junior, Caliari & Conte-Junior, 2019).

The lipid content found was of 1.75 ± 0.03 g 100 g-¹ for the flour from acerola residue. (Sancho, et al., 2015) and (Abud & Narain, 2018) observed distinct results of lipids for dehydrated acerola residue, with values of 2.92 g 100 g-¹ and 2.28 g 100 g-¹, respectively. (Narain, Almeida, Galvão, Madruga & Brito, 2004) stressed the importance of lipids, which are enzymatically converted for the formation of volatile compounds.

The carbohydrate content was determined by difference, with the result found for acerola flour being of 68.72 ± 2.36 g 100 g-¹. The acerola flour analysed in the present study was in accordance with the values established by guidelines CNNPA no. 12, of 1978 from ANVISA for sugars in flours (72.0 g 100 g^{-1}) (BRASIL & Agência Nacional de Vigilância Sanitária, 1978).

Phenolic compounds, anthocyanins, antioxidant activity and antinutritional factors of the wet acerola residue and flour from acerola residue

The results regarding phenolic compounds, anthocyanins, antioxidant activity and antinutritional factors of wet acerola residue and acerola residue dehydrated at 65ºC are presented in Table 3.

Table 3. Bioactive compounds, antioxidant activity and antinutritional factors of the wet acerola residue and acerola flour.

Characteristics	Wet Residue	Acerola Flour
Apparent Phenolic Compounds (g 100 g¹)	3.63 ^{a} ± 0.18	2.95 ^{b} ± 0.07
Anthocyanins (mg 100 g- ¹)	6.12 ^{a} ± 0.05	2.88 ^{b} ± 0.04
FRAP (µmol 100 g- ¹)	41605 ^{a} ± 1078	43055 ^{a} ± 4422
ABTS (µmol 100 g- ¹)	10721 ^{a} ± 400	6729.2 ^{b} ± 97.5
Condensed Tannins (mg 100 g- ¹)	214.20 ^{a} ± 4.45	189.51 ^{b} ± 5.80
Phytates (g 100 g- ¹)	0.61 ^{a} ± 0.06	0.41 ^{b} ± 0.03
Saponins (g 100 g- ¹)	0.78 ^{a} ± 0.01	0.50 ^{b} ± 0.01
Oxalate (g 100 g- ¹)	1.90 ^{a} ± 0.05	1.35 ^{b} ± 0.06

Results regarding the mean value between both batches; mean values followed by the same letter in the same column differ statistically according to Tukey's test ($p<0.05$). Source: Author's own compilation (2019).

Table 3 presents results of wet residue characterization in relation to the phenolic compounds, antinutrients and antioxidant potential, as well as the impact caused by thermal

processing in the optimum condition established to these parameters when presenting the results obtained in flour characterization. Based on these data, the impact percentage suffered by them with processing was determined.

Regarding apparent phenolic compounds in the wet acerola residue and in the acerola flour, a significant reduction of 18.7% in content was observed after the dehydration process. Within the classes of phenolic compounds, anthocyanins are pigments responsible for the colour in acerola fruits, being strongly impacted by thermal processing (Ribeiro, et al., 2018). The result found for monomeric anthocyanins (Table 3) showed a significant decrease ($p<0.05$) of 52.9% after the drying process, which demonstrates the impact of dehydration. However, despite the negative impact of dehydration, dry acerola residue is an important source of anthocyanins when compared to other dry fruit residues (guava, cashew, mango and papaya) (Sancho, et al., 2015).

The results found for antioxidant activity of the wet residue are presented in Table 3. Antioxidant activity in the residue extract was determined by ABTS and FRAP assays. The advantage of these techniques includes their brevity and simplicity, though the results are influenced by several factors, such as the interaction of various bioactive compounds and nutrients, with different action mechanisms.

Regarding the antioxidant activity of wet acerola residue and acerola flour using the ABTS assay, a significant reduction ($p<0.05$) of 37.2% was observed after the drying process. When applying the FRAP method, a slight increase of 3.48% in antioxidant activity was observed, though not significant when compared to the wet residue ($p>0.05$).

The drying process had a greater effect on the antioxidant potential determined by ABTS assays, showing that the antioxidant compounds which were most affected by the increase in temperature and exposure time were those with an antioxidant mechanism of free radicals. These compounds are represented by the reduction in apparent phenolic compounds. On the other hand, compounds acting as antioxidants through redox reactions were little or not at all affected by the drying conditions employed, with a slight change in concentration due to the removal of free water.

To determine condensed tannin content, the acidified vanillin method, in which vanillin reacts with condensed tannins to yield coloured compounds. After dehydration, a significant reduction ($p<0.05$) of 11.52% in condensed tannin content was observed. This substantial reduction is important as tannins have been associated with the reduction of protein digestibility and poor absorption of iron (Udomkun, et al., 2019). No reference has been found in the literature regarding the toxic effects of tannins nor regarding their

Reference Daily Intake (RDI).

The results found for phytates in the wet acerola residue and acerola flour are presented in Table 3, with a significant reduction ($p<0.05$) of 32.8% being observed after thermal treatment. Phytates can form very stable and insoluble complexes with minerals such as calcium, iron and zinc, as well as the chelation of amino acids, thereby decreasing the bioavailability of these nutrients (Udomkun, et al., 2019). This indicates that the reduction in phytate content in the acerola flour obtained can improve the absorption of these metals, ensuring a safer intake of these nutrients.

In the present study, a significant reduction (35.9%, $p<0.05$) of saponins was also observed. The adverse effects of saponins include changes in reproduction, growth, interaction with proteins, carbohydrates and lipids due to changes in the permeability of the cell membrane, thus reducing the absorption of these nutrients (Marques, Corrêa, Lino, Abreu, & Simão, 2013). However, no reference was found in the literature to the acceptable daily intake of saponins.

Oxalate content was significantly reduced (28.9%, $p<0.05$) after drying the acerola residue. It is worth noting that oxalates are present in the composition of most plant-based food products and are strongly attracted to Ca^{2+} ions, thereby forming stable complexes and reducing Ca^{2+} content available for absorption (Gordiano, Tondin, Miranda, Baptista, & Carvalho, 2014).

On the other hand, a research carried out by the Canadian Urological Association, on the development of a guideline for the evaluation and medical management of patients with renal stones, reported the importance of oxalate in the development of calcium nephrolithiasis (which accounts for 85% of all types of nephrolithiasis) and recommended calcium supplementation taken with meals, ensuring greater oxalate sequestration and excretion, maintaining significant amounts of calcium ion available for absorption (Dion, et al., 2016).

Therefore, the reduction in oxalate levels in plant-based processed food products, such as in the case of acerola flour, contributes to greater bioavailability of calcium. Due to the greater presence of oxalate in composite flours, oxalates must be carefully considered for patients with renal stones, limited to a total oxalate intake of (50-60 mg/day) (Massey, Palmer, & Horner, 2001; Udomkun, et al., 2019). In this regard, the flour from acerola residue obtained in this work, with a reduced oxalate content (1.35 ± 0.06 g.100 g^{-1}), can be considered a safe alternative for human consumption, especially for patients with kidney disease.

Another relevant aspect to be considered, which brings greater added value to the results of the reduction of antinutrients presented in this study, is related to the need of

carefully controlling children's diet, as childhood is the stage when greater intakes of calcium and iron minerals are required (Leal, et al., 2010). Consequently, a certain percentage of acerola residue, processed in the conditions presented herein, could be used in the formulation of children's meals, ensuring the intake of functional compounds present in flours with low antinutrient content.

Another consumer group which could take advantage from the consumption of flours produced from acerola residue could be vegetarian and vegan consumers, who would benefit from the properties of flours with lower antinutrient content.

Microbiological quality of flour from acerola residue

The results in Table 4 show that the flour produced from acerola residue complied with Guidelines CNNPA no. 12, of 1978 from ANVISA (BRASIL & Agência Nacional de Vigilância Sanitária, 1978), presenting adequate microbiological quality and are suitable for human consumption.

Table 4 - Results of the microbiological analyses of flour from acerola residue.

Microorganism	Count	Compliance
Standard mesophillic plate count	$3.5x10^5$ UFC/g	Compliant
Thermotolerant coliforms	<3LPN/g	Compliant
Salmonelas sp.	Absent in 25g	Compliant
Mould and yeast	$1.9x10^2$ UFC/g	Compliant
Bacillus cereus	1x10 UFC/g	Compliant

*LPN: least probable number. Results regarding two batches. Note: due to technical infeasibility, it was not possible to carry out the analyses of sulphite-reducing bacteria *Staphylococcus aureus* and *Clostridia* (at 44ºC). Source: Author's own compilation (2019).

One important factor for microbiological quality is pH, with microorganism growth being observed in the range from 5 to 8 (Storck, Basso, Favarin, & Rodrigues, 2015). The present study found a pH (3.3±0.01), which is adequate for microorganism growth inhibition.

Volatile compounds

The volatile compounds detected and identified in the samples of wet acerola residue and acerola flour are presented in Table 5, demonstrating drying impact on the volatile

compounds profile.

The compounds predominantly present in the wet residue are esters, namely 4-Pentenyl butyrate, Ethyl caproate and Ethyl octanoate, accounting for 39% of all compounds present, followed by alcohols such as 1-Octen-3-ol and 1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol, representing 18% of the total compounds present. 1-Octen-3-ol is one of the key compounds contributing to the aroma of acerola (Nogueira, et al., 2018). Compounds such as Nonanal, (E)-β-ocimene, (E)-2-hexenal were only detected in the wet residue and are part of the group of compounds key for the aroma of the fruit (Nogueira, et al., 2018). These results indicate that the wet residue preserves the aromatic characteristics of the fruit *in natura*. It is worth pointing out that the sensory feedback of aroma is not associated to high concentrations of this compound in the sample, and compounds in lower concentrations may have a greater impact (Parker, 2015).

Table 5. Volatile compounds found in wet acerola residue and acerola flour.

Compounds	LRI(Exp.)	LRI(lit.)	WR Area(%)	F Area(%)
Pentanal	984	974	0,195	-
Ethyl butanoate	1069	1052	0,638	-
Hexanal	1150	1111	1,457	6,322
3-Hexenal	1180	1176	0,454	0,759
D-Limonene	1194	1204	0,099	1,414
2-Hexenal, (E)-	1219	1222	2,772	5,418
Methyl caproate	1227	1219	2,718	-
Furan, 2-pentyl-	1232	1231	-	0,813
β-cis-Ocimene	1255	1237	0,192	-
Heptanal	1264	1284	0,064	-
ND	1280	*	0,193	-
Octanal	1281	1280	-	2,957
Ethyl caproate	1287	1267	13,452	-
1-Octen-3-one	1288	1283	-	0,958

Ethyl hexanoate	1288	1248	-	1,728
4-Pentenyl butyrate	1303	1305	22,128	2,706
6-Methyl-5-heptene-2-one	1337	1339	-	1,852
1-Hexanol	1344	1339	2,589	-
3-Hexenoic acid, ethyl ester	1355	1345	0,146	-
4-Hexen-1-ol, acetate	1363	1346	0,203	-
3-Hexen-1-ol, (Z)-	1373	1371	1,226	-
2-Hexen-1-ol, (E)-	1384	1388	1,807	-
Ethyl heptanoate	1393	1373	0,651	-
Nonanal	1397	1400	0,92	11,871
Ethyl caprylate	1401	1404	4,214	3,167
3-Methyl-3-butenyl isovalerate	1409	1379	0,522	-
3-Octanol	1414	1427	2,491	-
3-Methyl-3-buten-1-yl 3-methylbutanoate	1423	1434	0,254	-
cis-3-Hexenyl butyrate	1436	1429	1,074	-
5-Hepten-2-one, 6-methyl-	1436	1441	0,318	-
trans-2-Hexenyl Butyrate	1440	1461	0,355	-
Ethyl cis-4-octenoate	1449	*	0,287	-
1-Octen-3-ol	1450	1456	13,789	-
2-Octenal, (E)-	1467	1468	0,149	0,915
Hexyl butanoate	1494	1473	1,012	-
3-Methylbut-3-enyl (E)-2-methylbut-2-enoate	1501	1519	0,441	-
Benzaldehyde	1509	1508	0,297	2,365
4-Pentenyl hexanoate	1517	1510	9,806	9,569
3-Methyl-2-butenyl hexanoate	1562	1571	0,264	-
Linalol	1571	1566	0,589	2,179

Caryophyllene	1576	1577	0,59	2,309
1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol	1644	1631	4,081	24,535
β-Cyclocitral	1649	1638	1,039	5,244
Benzeneacetaldehyde	1693	1659	-	0,849
Sesquicineole	1730	1733	1,013	-
Octanoic acid, 3-methylbut-2-enyl ester	1738	1759	-	0,525
β-Damascenone	1780	1792	-	0,977
2-Buten-1-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	1799	1815	-	0,665
5,9-Undecadien-2-one, 6,10-dimethyl-	1816	1864	-	1,351
Ethyl (2E,4Z)-2,4-nonadienoate	1828	*	0,457	-
Isomethyl-α-ionol	1844	1848	0,761	-
α-Ionone	1884	1879	0,674	0,859
2-Butanol, 4-(2,2-dimethyl-6-methylenecyclohexylidene)-	1898	*	0,298	-
trans-Geranylacetone	1916	1877	0,294	-
3-Methyl-3-butenyl benzoate	1938	1933	0,147	-
2-Butanol, 4-(2,2-dimethyl-6-methylenecyclohexylidene)-	1944	*	-	0,811
trans-β-Ionone	1970	1953	2,88	6,882
Total	-	-	100,00	100,00

*: Not found in the literature; LRIexp. - obtained from experimental data; LRILiter. – obtained from online databases, such as NIST, Flavor net, Pherobase, Pubchem and ChemSpider; WR=wet residue; F=flour. Source: Author's own compilation (2019).

In the composition of volatile compounds from acerola flour (Table 5), 4-Pentenyl butyrate, which was the major constituent of the wet residue, was drastically reduced, only representing 9% of the acerola flour. In turn, higher concentrations of Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol (24.5 %) were observed, being considered the major component. Therefore, drying at 65ºC reduced ester content but increased the presence

of alcohols in the sample. Such behaviour was already anticipated, as esters are significantly more volatile.

Aldehydes were also a considerable part of acerola flour, with Hexanal, (E)- 2-Hexenal and Nonanal, combined, representing 23% of all compounds present. Such effect can be attributed to the partial oxidation of the lipid fraction combined with the occurrence of the Maillard reaction, as aldehydes are formed in the final stages of the reaction (Belitz, 2004). Nevertheless, the increase in the concentration of aldehydes key for acerola aroma in the profile of volatile compounds present in the flour are not necessarily positive, as they can lead to olfactory fatigue and can be responsible for off-flavours, taking into account the maximum olfactory perception in humans (Parker, 2015).

Another important aspect to be considered was the reduction of 40% in the total number of volatile compounds identified in the acerola flour (Table 5), being indicative of a reduction in the aroma complexity as the matrix was exposed to a temperature of 65ºC for 2 hours. Nevertheless, the acerola flour maintained important bioactive compounds in its aromatic profile, namely Linalol, which adds floral and citric notes and exhibits antimicrobial activity (Buettner, 2017), as well as Caryophyllene, which exhibits anti-cancer and analgesic properties (Fidyt, Fiedorowicz, Strządała, & Szumny, 2016), contributing to the woody notes in the aroma (El-Shemy, 2017).

4. Final Considerations

The ideal drying conditions to produce some flour from agro-industrial acerola residue was of 65ºC for 2 hours. Under these conditions, a significant reduction in antinutrients was observed, namely of saponins, phytates and tannins. The presence of phenolic bioactive compounds was maintained, and the remainder content of volatile compounds showed the maintenance of important aroma and bioactivity properties. Considering these results, the flour produced from agro-industrial residue of acerola proved to be an alternative source of antioxidants with a reduced antinutritional impact, which may be incorporated as an ingredient in the formulation of new food products. This application is of extreme importance, especially for reducing the environmental impact of residue from the fruit processing industry.

New research investigating reduction of flour total acidity and proteases inhibitors are necessary to increase nutritional quality and expand the potential for its application in new products.

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