Caracterização de rutina, compostos fenólicos e capacidade antioxidante de polpas e subprodutos de frutas tropicais

Characterization of rutin, phenolic compounds and antioxidant capacity of pulps and by-products of tropical fruits

Caracterización de rutina, compuestos fenólicos y capacidad antioxidante de pulpas y subproductos de frutas tropicales

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Raimundo Wilane de Figueiredo
Resumo
Nos últimos anos, o consumo de frutas tropicais aumentou, principalmente devido aos seus benefícios à saúde. Este estudo teve como objetivo identificar e quantificar o conteúdo de rutina por cromatografia líquida de alta eficiência (HPLC) e outros compostos bioativos na polpa e nos subprodutos de frutas tropicais do Brasil (acerola, goiaba, manga e caja-umbu). Também foram analisados os níveis de polifenóis totais, flavonóides amarelos, antocianinas e atividade antioxidante total. Os resultados mostram a grande quantidade de compostos bioativos encontrados em subprodutos, que possuem propriedades benéficas para a saúde e que são desperdiçados principalmente. A presença de rutina foi observada em todas as polpas e subprodutos, mas foi maior no subproduto da polpa de caja-umbu e acerola. Este estudo fornece novas informações sobre o conteúdo de rutina na polpa e subprodutos de frutas tropicais, essencial para futuras aplicações na indústria de alimentos. Além disso, contribui para a utilização de subprodutos do processo agroindustrial.

Palavras-chave: Antioxidantes; Fitoquímicos bioativos; Subproduto; Cromatografia; Frutas.

Abstract
In recent years the consumption of tropical fruits has increased, mainly due to their health benefits. This study aimed to identify and quantify the content of rutin using high performance liquid chromatography (HPLC) and other bioactive compounds in the pulp and in the by-products of tropical fruits from Brazil (acerola, guava, mango and caja-umbu). The levels of total polyphenols (Folin-Ciocalteau), yellow flavonoids, anthocyanins and total antioxidant activity (ABTS method) were also analyzed. The results shows the large amount of bioactive compounds found in by-products, that have beneficial properties for health, and which are mostly wasted. The presence of rutin was observed in all pulps and by-products, but it was highest in the by-product of caja-umbu and acerola pulp. This study provides new information about the content of rutin in the pulp and by-products of tropical fruits, which is essential for future applications in the food industry. Moreover, it contributes to the recovery of by-products from the agro-industrial process.

Keywords: Antioxidants; Bioactive phytochemicals; By-product; Chromatography; Fruits.
Resumen
En los últimos años, el consumo de frutas tropicales ha aumentado, principalmente debido a sus beneficios para la salud. Este estudio tuvo como objetivo identificar y cuantificar el contenido de rutina por cromatografía líquida de alto rendimiento (HPLC) y otros compuestos bioactivos en la pulpa y en los subproductos de frutas tropicales de Brasil (acerola, guayaba, mango y caja-umbu). También se analizaron los niveles de polifenoles totales (Folin-Ciocalteau), flavonoides amarillos, antocianinas y actividad antioxidante total (ABTS method). Los resultados muestran la gran cantidad de compuestos bioactivos que se encuentran en los subproductos, que tienen propiedades beneficiosas para la salud y que en su mayoría se desperdician. La presencia de rutina se observó en todas las pulpas y subproductos, pero fue más alta en el subproducto de pulpa de caja-umbu y acerola. Este estudio proporciona nueva información sobre el contenido de rutina en la pulpa y los subproductos de las frutas tropicales, que es esencial para futuras aplicaciones en la industria alimentaria. Además, contribuye a la recuperación de subproductos del proceso agroindustrial.
Palabras clave: Antioxidantes; Fitoquímicos bioactivos; Subproductos; Cromatografía; Frutas.

1. Introduction

Concerning the use of healthy diets and well-being, South America has a wealth of underexploited native and exotic fruits of great interest for food technology, science and industrial applications, with many nutritional and functional properties yet to be uncovered (Garcia-Ruiz et al., 2017). Rutin (3-O-rutinoside, quercetin) is used in the prevention or treatment of different health problems. Also known as vitamin P, rutin has antiviral, antiallergic, anti-inflammatory, antitumor and antibacterial properties due to its polyphenolic nature, and also has a radical scavenging activity and metal chelating properties (Nasirizadeh et al., 2012).

The major problem in processing fruits is a lot of agro-industrial by-products generated. These by-products have excellent nutritional quality, as recent studies have shown that the bioactive compounds present in fruits are concentrated mostly in the peels and seeds (Leao et al., 2017). Adding value to these by-products is of economic and environmental interest.

There is a potential which has not yet been discussed with regard to the use of pulps and by-products of tropical fruits, and that is to identify and isolate specific phytochemical
compounds for application in nutraceutical supplements, as food additives, and in the production of new foods and pharmaceutical products. This would enable these by-products of the agro-industrial process to be used (Ayala-Zavala et al., 2011), reducing manufacturing costs and offering new consumer products.

Thus, the purpose of this study was identify and quantify rutin in the pulp and by-products of tropical fruits (acerola - *Malpighia glabra*, mango - *Mangifera indica*, guava- *Psidium guajava* and cajá-umbu - *Spondias tuberosa*), from Brazil.

2. Methodology

The present research was characterized as a quantitative study, where the results were interpreted in numbers, opinions and information, classified and analyzed, in a descriptive character. It consisted of research investigations, whose purpose was to describe the characteristics that determine population or phenomenon, or to establish relations between variables. It was a cross-sectional study, that is, the data were collected in a single moment (Kauark et al., 2010; Pereira et al., 2018).

2.1 Raw material

The fruits used in this study were acerola (*Malpighia glabra* - 60566), mango (*Mangifera indica* - 60565), guava (*Psidium guajava* - 60567) and cajá-umbu (*Spondias tuberosa* - 60564), all of which were acquired in the local market in the state of Ceará, Brazil. Initially the fruits were washed with food detergent and sanitized with sodium hypochlorite solution at 200 ppm of free chlorine/15 minutes.

The acerola and guava fruits were pulped using a domestic multiprocessor (Philips), obtaining the pulp and the by-product. The mangos and the cajás-umbu were peeled manually with a knife. For the mangos, only the peel was considered to be a by-product.

The cajás-umbu seeds were dried in an oven with air circulation at 37 °C for 48 hours and ground in a mill saws steel (Star Model FT-100 - Fortinox) and the peels were dried at the same temperature for 24 hours and ground in a domestic blender. Then, they were mixed and used for analysis. For the other samples, after processing they were subjected to freezing (-18 °C) until the time of analysis.

2.2 Determination of bioactive compounds
The analysis of total anthocyanins and yellow flavonoids were determined according to the methodology described by Francis (1982). The total polyphenols were determined using the methodology described by Larrauri, Rupérez & Saura-Calixto (1997). The total antioxidant activity was determined by the method which captures the radical 2,2-azinobis (3-ethylbenzthiazoline-sulfonic acid 6) (ABTS) as methodology retrofitted by Rufino et al. (2010).

2.3 Identification and quantification rutin by HPLC

The extraction of rutin was performed according to method described by Wach, Pyrnska & Biesaga (2007). Before being injected into the HPLC, extracts were filtered through a 0.45 µm syringe filter.

The analyses were conducted in high-efficiency liquid chromatograph Shimadzu (HPLC), controlled by LC Solution Software, a manual injector with a fixed volume of 20µL, pump model LC-20DA, CTO-20A column oven set at 40 °C and UV-VIS detector model SPD-20A. The detection of rutin was performed at 350 nm.

One column Luna C18 (150mm x 4.6mm Phenomenex, 25 cm) was used. The mobile phase was prepared using MilliQ grade water acidified with 0.1% phosphoric acid (H3PO4) to pH 2.8 (Solution A) and acetonitrile (solution B) in the ratio 80A: 20B. The flow rate was 1.0 ml min\(^{-1}\) with a run time of 15 minutes and injected volume of 20µl. The injections were performed in triplicate.

The identification was made by comparing the retention times and also from the co-injection of the sample and standard, for confirmation. To obtain the standard, rutin obtained from Sigma-Aldrich was used. The quantification was performed by external standardization (Ribani et al., 2004). The calibration curve was constructed by injecting triplicate standards rutin solutions in ten different concentrations covering the range of expected concentration of the samples.

The detection limit was defined as the lowest peak height detected three times greater than the baseline. The limit of quantification was defined as the concentration of compound which could be determined with the lowest calibration curve.

2.4 Statistical analysis

Results were expressed as mean, and standard deviations between samples were assessed by comparison of means by analysis of variance (ANOVA) and Tukey's test at 5% significance level using the 7.7 beta software Assistat.
3. Results and Discussion

3.1 Bioactive compounds determination

Among the pulps studied, the acerola had the highest content of total extractable polyphenols (1337.34 mg GAE 100g⁻¹) (Table 1).

Table 1. Total extractable polyphenols levels and antioxidant activity of pulps and by-products from acerola, mango, guava and caja-umbu.

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Total extractable polyphenols (mg GAE* 100g⁻¹)</th>
<th>Total antioxidant activity (ABTS) (μM de Trolox g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulps</td>
<td>By-products</td>
<td>Pulps By-products</td>
</tr>
<tr>
<td>Acerola</td>
<td>1337.34 ± 34.20⁵⁶</td>
<td>717.78 ± 30.13⁵⁶</td>
</tr>
<tr>
<td>Mango</td>
<td>69.93 ± 10.73⁵⁶</td>
<td>892.07 ± 97.42⁴⁶</td>
</tr>
<tr>
<td>Guava</td>
<td>261.22 ± 4.46⁵⁶</td>
<td>429.52 ± 23.87⁴⁶</td>
</tr>
<tr>
<td>Caja-umbu</td>
<td>116.05 ± 0.91⁵⁶</td>
<td>380.28 ± 31.0⁴⁶</td>
</tr>
</tbody>
</table>

Source: Authors
The results are expressed as mean ± standard deviation (n = 3). *GAE = Gallic acid equivalent. Equal capital letters in the same column and lowercase letters in the same row do not differ significantly (p> 0.05) between them.

The guava pulp showed total polyphenol content (261.22 mg GAE 100g⁻¹) statistically superior (p<0.05) to the mango pulp (69.93 mg GAE 100g⁻¹) and caja-umbu (116.05 mg GAE 100g⁻¹), and these did not differ from one another.

With respect to by-products, the mango by-product had the highest total phenolic content (p<0.05), followed by that of acerola. The by-products of guava and caja-umbu were not statistically different (Table 1).

The by-products of mango, guava and caja-umbu showed higher levels of total polyphenols compared with their respective pulps. This result shows the great amount of bioactive compounds present in these products, which have beneficial properties for health, that most of the time were wasted. Silva et al. (2014) found similar results for pineapple, guava and cashew apple. Dorta et al. (2015) found high levels of bioactive compounds for mango peel and seed extracts.

In the analysis of total anthocyanins (Table 2), the acerola pulp had statistically higher levels (7.15 mg 100g⁻¹) than the other fruit pulps studied.

Table 2. Total anthocyanins and yellow flavonoids levels in pulps and by-products from acerola, mango, guava and caja-umbu.

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Total anthocyanins</th>
<th>Yellow flavonoids</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6
The results are expressed as mean ± standard deviation (n = 3). Equal capital letters in the same column and lowercase letters in the same row do not differ significantly (p > 0.05) between them.

The same behavior was observed for the by-products analyzed, except for the mango and guava by-products that were statistically different, with total anthocyanin contents of 4.10 and 0.35 mg 100g⁻¹, respectively.

For the analysis of yellow flavonoids (Table 2), acerola, mango and caja-umbu pulps showed levels that do not differ statistically (p < 0.05). The by-products of mango and caja-umbu showed the highest levels, but did not differ from each other. It was found that the by-products analyzed had higher levels than their respective pulps, especially the mango and caja-umbu by-products.

The acerola pulp presented total antioxidant activity statistically higher than the other pulps analyzed (Table 1).

The acerola by-product showed total antioxidant activity statistically higher than the other samples, with 51.62 µM Trolox g⁻¹ (Table 1), and the same result was observed for the acerola pulp. The mango, guava and caja-umbu by-products obtained higher values than their respective pulps.

Batista et al. (2017) found high level of phenolic compounds and antioxidant activity for peel and seeds of Red Jambo (S. malaccense) as Raudone et al. (2017), analyzing peel of six Lithuanian grown apple cultivars. These results demonstrate the wealth of existing bioactive compounds in byproducts of the fruit pulp processing industry.

### 3.2 Identification and quantification rutin by HPLC

Rutin was detected at 350 nm with a retention time of about 3.93 minutes (Figure 1). The detection limit was 0.0205 g l⁻¹ and the quantification limit was 0.041 g l⁻¹.

![Rutin chromatogram](Figure 1)
The rutin concentration varied from 0.94 mg 100g
\(^{-1}\) to 185.24 mg 100g
\(^{-1}\) (Table 3). Regarding the by-products, caja-umbu had the highest content (185.24 mg 100g
\(^{-1}\)).

Table 3. Rutin concentration in the pulp and by-products from acerola, mango, guava and caja-umbu.

| Fruits         | Rutin concentration (mg 100g
\(^{-1}\)*) |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pulps</td>
</tr>
<tr>
<td>Acerola</td>
<td>18.46 ± 12.40(^{Aa})</td>
</tr>
<tr>
<td>Mango</td>
<td>0.94 ± 0.74(^{Ca})</td>
</tr>
<tr>
<td>Guava</td>
<td>2.82 ± 2.16(^{Ca})</td>
</tr>
<tr>
<td>Cajá-umbu</td>
<td>12.04 ± 1.86(^{Bb})</td>
</tr>
</tbody>
</table>

Source: Authors

The results are expressed as mean ± standard deviation (n = 3). *dry matter. Equal capital letters in the same column and lowercase letters in the same row do not differ significantly (p> 0.05) between them.

The results of this study have not yet been addressed in the literature. Thus, the identification and quantification of rutin in the samples can be considered a primary strategy for its application in food.

Measuring the availability of rutin in commonly consumed foods can lead to new studies, aiming at the extraction and use of this compound as an alternative supplementation.

The pulps showing the highest levels of rutin were acerola and caja-umbu (Figure 2).
The mango and caja-umbu by-products had higher rutin levels than those found in their respective pulps, showing again the richness of these by-products in terms of bioactive compounds. Khallouki et al. (2017) found rutin in mature Argan fruits from Morocco.

Fruit by-products have excellent nutritional quality, as recent studies have shown that the bioactive compounds present in fruits are concentrated mostly in the peels and seeds (Abrahão et al., 2010; Melo et al., 2008; Leao et al., 2017).

4. Final considerations

These results demonstrate the wealth of existing bioactive compounds in byproducts of the fruit pulp processing industry.

In all fruit pulps and by-products the presence of rutin was verified. The caja-umbu by-product, which has high levels of rutin, could be used as a raw material for manufacturing various functional food products, with the possibility of isolation and pharmaceutical use.

The assessment of toxicity, bioaccessibility, stability of these extracts and in vivo studies are suggestions for further studies, aiming the use of these extracts industrially.
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References


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