

Determination of flavonoids in fruit and vegetable residues with their application as a potential functional food ingredient

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Summary

This study aimed to determine the content of quercetin, rutin and isoquercitrin in by-products of certain fruits and vegetables sold in northeastern Brazil and propose their use as functional ingredients in food production. Skins from avocado (*Persea americana* Mill.), acerola (*Malpighia emarginata* DC.), beet (*Beta vulgaris* L.), cajarana (*Spondias dulcis* Parkinson), yellow and red onion (*Allium cepa* L.), guava (*Psidium guajava* L.), kiwi (*Actinidia deliciosa* (A. Chev.) E. F. Liang and A. R. Ferguson), seriguela (*Spondias purpurea* L.) and umbu (*Spondias tuberosa* Arr. Cam.) were freeze-dried and subjected to extraction with organic solvents. The extracts obtained were analysed for selected flavonoids using high-performance liquid chromatography. The highest levels of quercetin were found in red onion (19836.11 mg·kg⁻¹), yellow onion (4917.11 mg·kg⁻¹), acerola (60.28 mg·kg⁻¹) and umbu (55.98 mg·kg⁻¹). Rutin had high contents in cajarana (4198.46 mg·kg⁻¹), umbu (3265.37 mg·kg⁻¹), acerola (1538.29 mg·kg⁻¹) and yellow onion (1362.91 mg·kg⁻¹). Isoquercitrin was found only in red onion (1475.76 mg·kg⁻¹), acerola (388.46 mg·kg⁻¹), guava (295.37 mg·kg⁻¹) and umbu (288.91 mg·kg⁻¹). The levels of flavonoids found may be sufficient to use them as functional ingredients and fortify certain foods with health-promoting compounds, thereby adding value to food processing waste materials.

Keywords

rutin; quercetin; isoquercitrin; fruit skin; vegetable skin; functional food

The food system is a complex network involving agricultural producers, processors, distributors, wholesalers, retailers and, ultimately, consumers. A part of food is wasted at all these stages. At the same time, this system requires large amounts of inputs, such as irrigation water, pesticides, fertilizers and energy, which generate environmental impacts, in particular soil erosion, loss of biodiversity, water pollution and greenhouse gas emissions. The lost food contributes to greater waste of resources and negatively impacts the environment. However, this could be avoided [1]. Brazil is among the world's top fruit producers. In 2022, the total production of the primary fruit species was estimated at 41 million tons. Regarding vegetables, onions are produced in the most significant quantity (1.5 million tons) [2].

According to the Food and Agriculture Or-

ganization [3], a third of the food generated for human consumption is discarded, resulting in 1.30 billion tons of wasted edible parts annually, while 821 million people worldwide suffer from some form of malnutrition [3]. This considerable amount of wasted food could be used to increase food and nutritional security for vulnerable populations. Nevertheless, the agro-industrial by-products are generally discarded and to the harm of the environment. Ideally, these residues should be managed such that there is a simultaneous reduction in food waste and repurposing of residues as alternative sources of food products for human consumption [4].

Various studies investigated the nutritional content of fruit and vegetable residues to encourage the full use of food and sustainable food patterns [5–7]. By-products and residues can be repurposed

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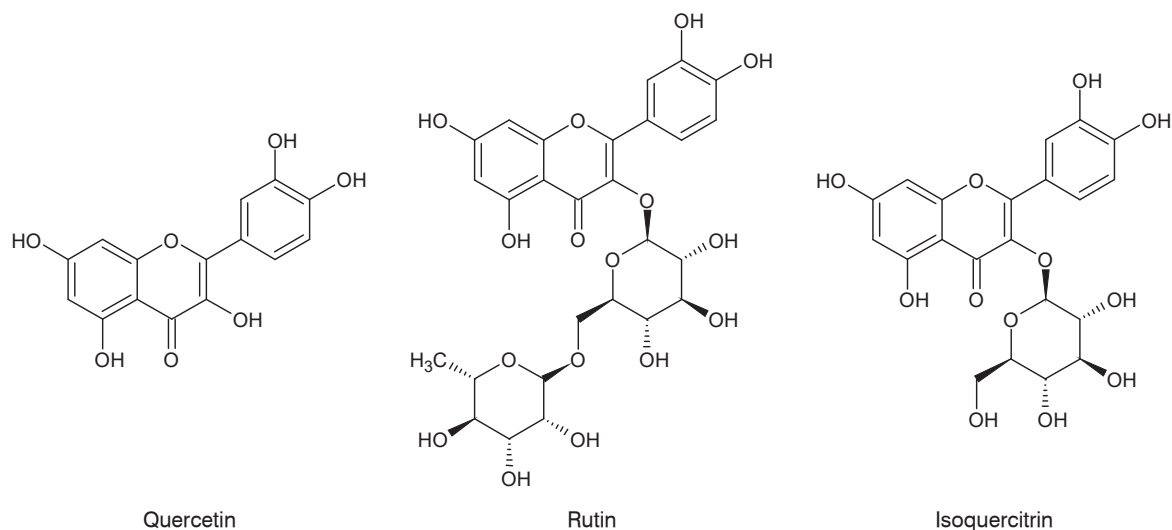


Fig. 1. Structures of quercetin, rutin and isoquercitrin.

for several applications that exploit their functional characteristics associated with health benefits [8–10]. Several bioactive compounds are present in fruits and vegetables, especially in skins. Among them are flavonoids that belong to the group of polyphenols, many of which have antioxidant and anti-inflammatory characteristics that can have positive impact on cardiovascular and cerebrovascular diseases, diabetes, and respiratory tract infections of consumers [11].

There has been substantial interest in these substances in foods and their properties in recent years. Quercetin (Fig. 1) is the flavonoid discussed in the most significant number of scientific publications [12]. This compound has been shown to exert protective effects against Alzheimer's disease, diabetes and cardiovascular disease [13]. The effects of quercetin against COVID-19 have also been studied. Previous research involving coronaviruses such as SARS and MERS suggested that quercetin can be used as a potential treatment for COVID-19. A study showed that quercetin could reduce the entry of the virus into the cell by blocking the angiotensin converting enzyme 2 (ACE2) receptor and reducing levels of interleukin-6 (IL-6) in patients with SARS and MERS [14].

Rutin and isoquercitrin (Fig. 1) are quercetin glycosides that have relevant biological activities. Rutin has cytoprotective, vasoprotective, neuroprotective, cardioprotective and anticarcinogenic properties [15], while isoquercitrin can act against oxidative stress, cancer, diabetes, cardiovascular diseases, and allergic reactions [16]. These substances are already widely used in the pharmaceutical industry in nutraceuticals [12].

There are no daily intake recommendations for flavonoids. However, the average estimated population intake is approximately 900.00 mg·d⁻¹ [17]. The consumption of these compounds in the usual Brazilian diet ranges from 50.00 mg·d⁻¹ to 374.00 mg·d⁻¹. Among European adults, values can reach 246.00 mg·d⁻¹ [18], while the adult population in the United States ingests around 200.00 mg·d⁻¹ to 250.00 mg·d⁻¹ [19]. In Australia, the consumption of these bioactive compounds is higher, reaching 626.00 mg·d⁻¹ [20]. In Japan, the consumption of flavonoids can reach up to 2000 mg because the oriental diet is rich in vegetables, soya and tea [18].

This study was based on taking into account the potential of compounds present in by-products of fruits and vegetables and their effects on human health. We attempted to encourage the consumption of alternative sources of these compounds and full use of food. Therefore, this study aimed to quantify and compare the major flavonoids found in by-products from avocado, acerola, beet, cajariana, yellow onion, red onion, guava, kiwi, seriguela, and umbu commercialized in Brazil. After extraction and analysis, the fresh by-products were dried in order to use them as a potential functional ingredient.

MATERIALS AND METHODS

Chemical reagents and standards

Quercetin dihydrate (purity ≥ 95.0 %), rutin (purity ≥ 95.0 %) and isoquercitrin (purity ≥ 95.0 %) standards were obtained from Sigma-

Aldrich (St. Louis, Missouri, USA). Methanol and ethanol solvents of analytical-grade used in the extraction were obtained from Sigma-Aldrich. The high-performance liquid chromatography (HPLC) solvents methanol and acetonitrile were from J. T. Baker (Phillipsburg, New Jersey, USA). The buffer solution was composed using the Nuclear phosphoric acid brand analytical-grade (São Paulo, Brazil) and ultra-pure water (18.2 MΩ·cm, 25 °C) obtained from the Simplicity model (Millipore, Billerica, Massachusetts, USA).

Obtaining and preparing samples

The skin samples were obtained from ripe fruits and vegetables purchased in Fortaleza, Ceará, Brazil. The following species were selected: avocado (*Persea americana* Mill), acerola (*Malpighia emarginata* DC.), sugar beet (*Beta vulgaris* L.), cajarana (*Spondias dulcis* Parkinson), yellow onion (*Allium cepa* L.), red onion (*Allium cepa* L.), guava (*Psidium guajava* L.), kiwi (*Actinidia deliciosa* (A. Chev.) E. F. Liang and A. R. Ferguson), seriguela (*Spondias purpurea* L.) and umbu (*Spondias tuberosa* Arr. Cam.). The ripeness of the fruits was between RS3 and RS4 according to classification [21].

The samples were rinsed with a sodium hypochlorite solution (2.5 g·l⁻¹) during 15 min and manually peeled. The skins were frozen and lyophilized at -50 °C, under a vacuum of 0.67 Pa, for 24 h in a bench lyophilizer (Edwards; Irvine, California, USA). The skins were then macerated and the material obtained was stored in a hermetically closed glass container.

Flavonoids extraction

Extraction of flavonoids were carried out as described by CHUA [15]. The extracts of the ten samples were prepared using approximately 2.50 g of lyophilized sample and 100 ml of either ethanol or methanol was added. The samples were heated for 30 min with constant agitation and controlled temperature (approximately 70 °C).

After heating, the samples were filtered using a filter paper Qualy (J. Prolab, São Paulo, Brazil). Adding solvent to the sample followed by heating was repeated two to three times for each sample with ethanol and about two to four more times with methanol to ensure extraction efficiency. The alcohol extracts were concentrated until dryness in a rotary vacuum evaporator (Quimis, São Paulo, Brazil) and lyophilized. The concentrates were transferred to a 10 ml volumetric flask, dissolved in methanol and analysed using HPLC. The calculation of the yield of plant extracts was made concerning the weight of 2.50 g of freeze-dried skins.

High performance liquid chromatography

The analyses were carried out at the Chemical Laboratory of the Technological Development Park, located at the Federal University of Ceará (PADETEC/UFC, Ceará, Brazil). A Shimadzu LC 10Avp chromatograph (Shimadzu, Kyoto, Japan) was used with LC-10ADvp pump, 20 µl volume manual injector, CTO-10Avp oven at 40 °C, SPD-M10Avp detector and SCL-10Avp controller. The column was Kromasil C18 (4.6 mm × 150 mm, particle size 5 µm; Kromasil, Bohus, Sweden). The method used to analyse flavonoids was that described by DA SILVA et al. [22]. For identification and quantification of rutin and quercetin, a mobile phase consisting of ultra-pure water (18.2 MΩ·cm, 25 °C) acidified to pH 2.80 using phosphoric acid (H₃PO₄) (solution A) and acetonitrile (solution B) was used, in gradient mode using the following conditions: up to 12 min, A:B of 20:80; from 12 min to 23 min, A:B of 40:60 and from 23 min to 25 min, A:B of 20:80. The flow rate was 1.2 ml·min⁻¹, the injection volume was 20 µl. Detection was at 350 nm and the total run time per sample was 25 min at 40 °C. The parameters obtained in the quantification are shown in Tab. 1.

The determination of linearity was performed by constructing the calibration curve with a standard solution of quercetin and rutin at four

Tab. 1. Method validation for chromatographic analysis of rutin, quercetin and isoquercitrin.

Parameter	Quercetin	Rutin	Isoquercitrin
Detection limit [mg·l ⁻¹]	3.0 × 10 ⁻⁴	2.0 × 10 ⁻⁴	1.6 × 10 ⁻⁴
Quantification limit [mg·l ⁻¹]	1.0 × 10 ⁻³	1.5 × 10 ⁻³	1.2 × 10 ⁻³
Linearity	4 × 10 ⁷ x + 34 992	2 × 10 ⁷ x + 17 387	2 × 10 ⁷ x + 17 387
Correlation coefficient (R ²)	0.9990	0.9996	0.9996
Retention time [min]	10.89	3.20	3.87

concentrations (0.001; 0.01; 0.05; 0.1 mg·ml⁻¹ in methanol), obtaining Eq. 1 for quercetin, with $R^2 = 0.9990$ and Eq. 2 for rutin, with $R^2 = 0.9996$.

$$y = 4 \times 10^7 x + 34992 \quad (1)$$

$$y = 2 \times 10^7 x + 17387 \quad (2)$$

Initially, injections were made with the standards of flavonoids and then samples of extracts from the fruit and vegetable skins were injected for comparison, thereby identifying the presence of the compounds. For isoquercitrin, a 0.1 mg·ml⁻¹ solution (in methanol) was prepared with standard and injected to determine its retention time (3.87 min). For each sample, analyses were performed in triplicate ($n = 3$).

The calculation of isoquercitrin content was performed as described in the European Pharmacopoeia by multiplying the average of the peaks of the areas of this substance by the corresponding correction factor ($FC = 0.8$), and the result obtained was used in the same equation of the rutin [23]. Area means were used to calculate concentration and area values to calculate the standard deviation (SD).

Development of functional ingredient

After analysis by HPLC of the samples, the vegetable with the highest content of flavonoids was chosen to be further processed as described below. The skin samples of red onion were obtained from ripe fruits and vegetables purchased in Fortaleza, Ceará, Brazil. The samples were rinsed with a sodium hypochlorite solution (2.5 g·l⁻¹) during 15 min and manually peeled. The red onion skins were dried in a microwave oven (Panasonic, Osaka, Japan) at a power of 800 W for 5 min (maximum power). After drying, the skins were crushed in a blender with stainless steel blades, cup capacity 2.5 l and polypropylene cup material (Mondial, São Paulo, Brazil) at 900 W power for 3 min, by which a homogeneous powder was obtained. To confirm that there was no reduction in flavonoid levels, a sample was taken and analysed by HPLC.

Statistical analysis

For all analyses, determinations were made in triplicate as independent experiments. Data analysis was performed using Graph Pad Prism v8.01 program (Dotmatics, Boston, Massachusetts, USA). Differences between variables (rutin, quercetin and isoquercitrin content) were tested for significance by one-way analysis of variance (ANOVA). Significantly different means

($p < 0.05$) were separated by the Tukey's test. Data are presented as mean \pm SD .

RESULTS AND DISCUSSION

The yield data from the extraction of fruit and vegetable skins are shown in Tab. 2. The extracts of ten samples analysed by HPLC showed peaks of quercetin and rutin with retention time close to 10.89 min and 3.20 min, respectively, confirming the presence of these flavonoids in all the skins of the analysed fruits and vegetables. The highest levels of quercetin were found in red onion, yellow onion, acerola and umbu residues. Rutin showed the highest concentration in cajarana, umbu, acerola and yellow onion extracts. Isoquercitrin was found only in four of the ten samples analysed and red onion was the residue that presented the highest content of this substance in its skin. The results of the analyses are displayed in Tab. 2. The chromatogram obtained by analysis of flavonoid standards and the guava skin extract sample was used as an example to demonstrate that the three identified substances were present, as represented in Fig. 2A and Fig. 3A, respectively. The UV absorption spectra of the standards (Fig. 2B, 2C, 2D) were used to confirm the analysed substances (Fig. 3B, 3C, 3D).

Among the species studied, red onion had the highest quercetin content in its skins, which was higher than that of yellow onion with the quercetin content four-fold lower. These results corroborate previous findings by GORINSTEIN et al. [24], who found that red onion skins contained a higher content of total quercetin than white onions. Another study reported lower levels of total quercetin in white onion skins (89.30 ± 38.50 mg·kg⁻¹ and 101.00 ± 18.90 mg·kg⁻¹) and higher in red varieties (280.20 ± 41.50 mg·kg⁻¹ and 304.30 ± 81.20 mg·kg⁻¹) [25]. Thus, the red onion that possesses a higher content of flavonoids presents more significant antioxidant activity [25].

Recent studies evaluated the effect of quercetin from onion extracts, highlighting the importance of this vegetable as a functional food. The effect of quercetin glycosides extracted from varieties of onions and garlic on cancer cells in vitro were studied and showed that quercetin-4'-O-glycoside is a potentially potent anticancer agent [26]. Another study demonstrated that the addition of a powder obtained from onion skins to wheat flour samples increased the food's antioxidant potential due to the presence of quercetin, suggesting the use of these by-products to add nutritional value [27].

Tab. 2. Flavonoid content in freeze-dried fruit and vegetable skins and heat-treated vegetable skins.

Popular name	Concentrated extract		Content [mg·kg ⁻¹]		
	Weight [mg]	Yield [%]	Quercetin	Rutin	Isoquercitrin
Avocado	311.30	12.5	48.26 ± 0.45 ^c	89.24 ± 0.61 ^g	ND
Acerola	1 570.40	62.8	60.28 ± 0.07 ^c	1 538.29 ± 0.42 ^d	388.46 ± 0.25 ^c
Beet	831.20	33.2	14.48 ± 0.10 ^d	22.09 ± 0.07 ⁱ	ND
Cajarana	600.20	24.0	12.05 ± 0.17 ^d	4 198.46 ± 1.58 ^d	ND
Yellow onion	214.00	8.6	4 917.11 ± 0.09 ^b	1 362.91 ± 0.20 ^b	ND
Red onion	482.40	19.3	19 836.11 ± 1.38 ^a	1 049.55 ± 0.26 ^f	1 475.96 ± 1.14 ^b
Guava	708.80	28.3	44.68 ± 0.29 ^c	156.98 ± 0.70 ^d	295.37 ± 0.55 ^a
Kiwi	1 264.50	50.6	9.75 ± 0.00 ^d	72.87 ± 0.29 ^h	ND
Seriguela	988.20	39.5	18.29 ± 0.18 ^d	472.76 ± 0.90 ^a	ND
Umbu	1 073.40	42.9	55.98 ± 0.16 ^c	3 265.37 ± 1.47 ^c	288.91 ± 0.02 ^d
Red onion*	506.60	20.2	20 184.23 ± 0.32 ^a	998.95 ± 0.26 ^e	1 135.80 ± 0.13 ^b

All tests were performed in triplicate ($n = 3$) and the results were expressed as mean ± standard deviation. Values with different superscript letters are significantly different by ANOVA and Tukey's test ($p < 0.05$).

* – flavonoid content in heat-treated vegetable skins. ND – not detected.

Other foods and their residues were also previously studied as sources of quercetin. A study from northeastern Brazil identified the presence of quercetin in acerola and its by-products [28], detecting values lower than we found because those authors included the pulp in their analysis (2 960.00 mg·kg⁻¹). However, the values found in the umbu-cajá skin (33.80 mg·kg⁻¹) were similar to the umbu residue extract analysed in this study. Another study found 24.27 mg·kg⁻¹ of quercetin in guava pulp residues (including skin and seeds) [29], which is less than we reported here. KOSIŃSKA et al. [30] quantified quercetin and its derivatives in avocado skin with values of 23.00–80.00 mg·kg⁻¹, which are similar to those found in the present study. By-products represent approximately 30 % of the total weight of Hass avocados, with 14 % referring to its skin and 16 % to its pit. In another study from Portugal, quercetin was analysed in avocado skin and it was found that flavonoids were the second-largest class of biologically active compounds found in this material, the first being those from the catechin family [31].

Quercetin has anti-inflammatory and antioxidant properties, which are mainly due to its effect on the oxidative balance through various mechanisms, such as induction of the production of glutathione (GSH). That compound acts by removing reactive oxygen species (ROS), interference with enzymatic activity and signal transduction pathways, fighting the ROS that are caused by environmental and toxicological factors [32]. In relation to obesity, quercetin may attenuate

its effects, as it directly interferes with adipose tissue signalling pathways. Due to the reduction of pro-inflammatory cytokines and enzymes such as tumour necrosis factor alpha (TNF- α), monocyte chemoattractant protein-1 (MCP-1), IL-6 and cyclooxygenase (COX), quercetin promotes reduction in insulin resistance and has anti-adipogenic activity through the regulation of AMP-activated protein kinase (AMPK) and acetyl coenzyme A (acetyl-CoA) carboxylase phosphorylation in pre-adipocytes, then regulates the metabolism of fatty acids. In addition, quercetin is able to increase the level of uncoupling protein 1 (UCP1) in adipose tissue that acts on thermogenesis and activate AMPK, which suppress proinflammatory mediator expression and play an important role in increasing caloric expenditure, thus preventing obesity. However, more clinical studies are needed to clarify the correct dosage of quercetin supplementation and its effects in humans [33].

Regarding rutin, a study found an average of 162.40 mg·kg⁻¹ in the umbu, lower values were reported here as pulp was subjected to analysis [34]. A recent study from Portugal analysed the total phenolic compounds of industrial waste obtained from kiwi processing and identified ten types of phenolics, including rutin (59.00 mg·kg⁻¹), although the values were lower than those in this study [35]. Application of different extraction and analytical methods could also contribute to differences between the levels of flavonoids found in the studies.

Experimental studies suggest that rutin may

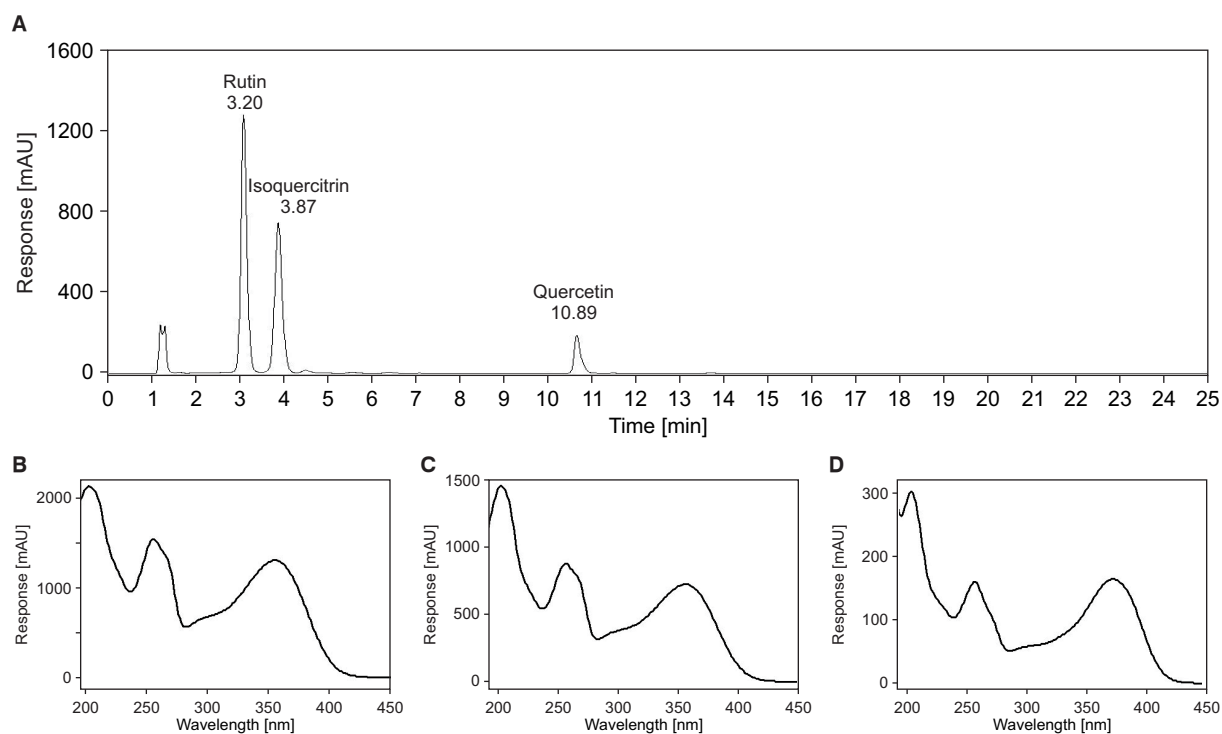


Fig. 2. Chromatogram and absorption spectra of rutin, isoquercitrin and quercetin standards.

A – chromatogram, B – absorption spectra of rutin, C – absorption spectra of isoquercitrin, D – absorption spectra of quercetin.

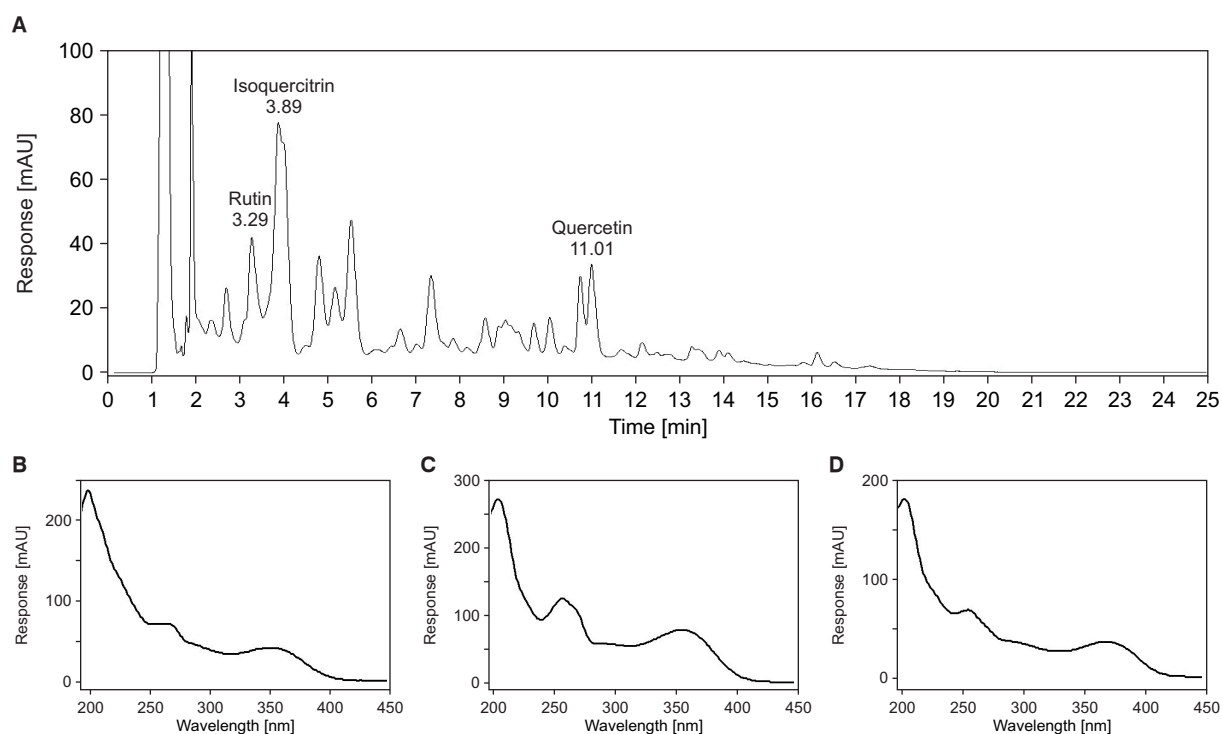


Fig. 3. Chromatogram and absorption spectra of rutin, isoquercitrin and quercetin in guava skin extract.

A – chromatogram, B – absorption spectra of rutin, C – absorption spectra of isoquercitrin, D – absorption spectra of quercetin.

prevent and treat diabetes and other diabetes-related illnesses. Proposed mechanisms for the hypoglycemic effect of rutin include reduced carbohydrate absorption in the intestine, inhibition of tissue gluconeogenesis, increased tissue glucose uptake, stimulation of pancreatic beta-cell insulin secretion and protection of the islets of Langerhans against degeneration. Rutin also decreases the formation of sorbitol, ROS, advanced glycation products and inflammatory cytokines. All these effects result in the protective activity of rutin against diabetic nephropathy, dyslipidemia, neuropathy, liver damage and cardiovascular diseases [36].

Isoquercitrin may also improve type 2 diabetes by inactivating glucagon-like peptide-1 (GLP-1), an intestinal hormone that increases insulin secretion. A study in rats demonstrated that this compound promotes glucose consumption by liver cells and is also responsible for improving clinical symptoms of diabetes by controlling blood glucose concentration. The possible protective effects of isoquercitrin in cancer are not very well elucidated, but there are hypotheses that it is attributed to its action on lipid peroxidation and its interference with cell signalling pathways that can modulate the activity of oncogenic proteins, inducing apoptosis of preneoplastic cells. In addition, this flavonoid is able to reduce pro-inflammatory proteins and other cytokines, such as TNF- α and tumor necrosis factor beta (TGF- β). However, more studies are necessary to better understand the benefits of isoquercitrin in this disease [37]. The skins of fruits that are generally discarded have a higher content of phytochemicals than the pulp. Several studies demonstrated the application of these residues in the improvement of food and the development of new culinary preparations, for example, in the preparation of cookies [38].

In this study, it was observed that the red onion residues had the highest levels of the 3 flavonoids under study. Thus, the red onion residues were selected for development of a product with potential application as a functional ingredient. The microwave pre-treatment of fruit and vegetable residues was used as a simple drying method. Quantification of flavonoids was performed by HPLC with diode-array detection after the application of heat treatment in red onion residues and the flavonoids content was again analysed. The results obtained by drying by lyophilization and by microwave presented only a small difference (Tab. 2), indicating that this way of drying does not decompose these flavonoids to a large extent. The results obtained by the HPLC analysis of dried onion residues treated by freeze-drying and microwaves were in

agreement with the work published by ROHN et al. [39]. According to that work, the thermal treatment (at 180 °C) led to degradation of quercetin glycosides and the main product was quercetin aglycone, which remained stable during further roasting (180 °C). In our work, the drying conditions (regarding temperature and time) used were milder and the glycosylated flavonoids rutin and isoquercitrin had their content reduced by a small amount while the content of quercetin aglycone also increased by a small amount.

Although official recommendations for daily intake of flavonoids are not quantified, doses from oral quercetin supplements evaluated in studies ranged from 3.00 mg·d⁻¹ to 1000.00 mg·d⁻¹ [19]. If the preparation from red onion skins prepared this work would be considered to reach the minimum dose of supplementation, only 24.00 g of the powder obtained from its grinding would be needed. This could be added to culinary preparations such as bread dough, pizza or cookies.

According to the Phenol-Explorer, the first database on the content of polyphenols in foods, the content of isoquercitrin in 36 items listed ranged from 0.07 mg·kg⁻¹ to 419.50 mg·kg⁻¹ [16]. Based on these data and the recommendation to consume 500.00 g of fruits and vegetables per day, the average daily dose of this compound can be estimated at 3.00–12.00 mg [16]. Thus, using 8.00 g of dried red onions skins, as described in this work, it would be possible to guarantee the consumption of the minimum recommended dose of isoquercitrin per day.

For rutin, the daily intake providing some beneficial health effects has been reported to range from 40.00 mg to 100.00 mg [40]. In the study reported here, 45.00–50.00 g of red onion skin residue would be enough to provide this amount of rutin. The prepared dry powder can be used to enrich bakery products such as cakes or cookies. Thus, based on the values obtained in the analysis after the heat treatment, we can reach the recommended daily consumption of rutin, quercetin and isoquercitrin using approximately 50.00 g of dry red onion powder.

The method proposed in this work allows processing of low-cost vegetable waste in an accessible, inexpensive and reproducible way. The products can be used in the food and pharmaceutical industry as nutraceuticals or food supplements to nutritionally enrich food products. In addition, the use of the waste generated by the food industry could reduce environmental pollution and, consequently, ensure greater food and nutritional security.

CONCLUSIONS

This study showed that the by-products of several fruits and vegetables are sources of flavonoids and can be used to produce a functional ingredient. This means exploitation of low-cost resources, providing a positive economic and environmental impact. The use of already small amounts of these ingredients can provide the recommended daily intake of these substances. Red onion stood out for the presence of large amounts of quercetin in its skin, highlighting the importance of detailed individual characterization of bioactive compounds, as research in this format is scarce, especially in Brazil. More studies are needed to verify the content of these substances in fruits and vegetables by-products, encourage their use and analyse their effects on human health. Further, cell or animal tests may be needed to verify its functional properties. The data obtained in this study add valuable information to current knowledge of flavonoids in Brazilian fruit and vegetable residues.

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