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Recovery of ascorbic acid, phenolic compounds and carotenoids from acerola by-products: An opportunity for their valorization

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ABSTRACT

Ascorbic acid, phenolic compounds and carotenoids were extracted from acerola (*Malpighia emarginata* DC.) by-products using gas-expanded liquids (GXLs) based on carbon dioxide expanded ethanol at 40 °C and 7 MPa. The by-products generated in the processing of acerola: bagasse (seed and peel) and non-pomace (from the juice clarification step), were studied separately. The overall extraction yields obtained were 3.8 and 12.9 g/100 g for bagasse and non-pomace, respectively. Ascorbic acid was not detected in the bagasse extract, while 158.2 mg/$g_{extract}$ was found in non-pomace extract, and 193.7 mg/g was found in the acerola juice powder. The higher the concentration of ascorbic acid, the higher the antioxidant activity of the extracts. The phytochemical profile obtained by LC-Q-TOF-MS/MS showed a higher number of phenolic compounds (hydroxycinnamic acids and flavonoids) in the bagasse and non-pomace extracts when compared to acerola juice. In addition, GXL promoted the extraction of a pool of carotenoids (lutein and β-carotene), pheophytin and chlorophyll derivatives detected by LC-APCI-MS/MS. The obtained extracts, rich in bioactive compounds, open an opportunity for the valorization of acerola by-products, meeting the current demand for natural nutraceuticals rich in vitamin C and polyphenols.

1. Introduction

Acerola (*Malpighia emarginata* DC.) is a tropical fruit known for its high vitamin C content. In addition, many other compounds contribute to acerola being recognized as a "super fruit". Currently, natural acerola extract has been presented as an alternative to synthetic ascorbic acid by many acerola extract manufacturers (Belwal et al., 2018). Multinational companies such as Dupont, Döhler, Duas Rodas, Amway and Diana Foods offer acerola products rich in ascorbic acid for various industrial applications, including food and nutraceutical supplements, such as tablets for daily intake. These companies claim the use of acerola from South America, meanwhile Amway and Diana Foods have their own acerola plantations in northeastern Brazil. The most popular benefits of vitamin C are strongly associated with colds prevention caused by respiratory viruses and relieving their duration and symptoms (Hemilä, 2017). On the other hand, it is important to find alternative sources of vitamin C in order to surpass the activity of "vitamin cartels" like those of 1990's (European Commision, 2003). In fact, nowadays the vitamin C market is dominated by China producers, who faced price hikes as high as three times over a year in 2017 (Gelski, 2017). Currently, with the spread of the new coronavirus (Covid-19), the vitamin C market is in ascending demand due to consumers interest in boosting their immunity (NutritionInsight, 2020).

Recently, a metabolomic analysis of immature and mature acerolas identified differences in the level of several classes of compounds, including phenolics and carotenoids. Although immature acerola has a lower level of accumulated compounds, it shows higher antioxidant activity than mature acerola, mainly due to the high content of vitamin C (Xu et al., 2020). For this reason, immature acerolas are industrially processed when the focus is on the production of vitamin C concentrates. Among the phenolic acids, ferulic, *p*-coumaric and caffeic were identified in the pulp of acerola, in addition to chlorogenic acids, flavonoids (rutin, kaempferol and quercetrin), isoflavonoids, polyphenols and carotenoids (lutein, β-carotene and phytofluene) (Xu et al., 2020); no

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chlorophyll or chlorophyll-derivatives were cited in that study.

An important fraction of compounds generally remains in the by-products generated in the processing of fruits, including phenolics, dietary fibers and proteins, among others (Beres et al., 2017). Approximately 60% of ascorbic acid is reported in the acerola waste, when compared to the fruit pulp (Rezende, Nogueira, & Narain, 2017). According to the Brazilian company located at latitude 3°51′12″ S and longitude 41°5′10″ W (Ceará, Brazil), the processing of acerola generates about 40% of by-products; 30% of seeds and peels (bagasse) from the pressing step, and 10% of sludge from the juice clarification step (non-pomace). Considering the increasing demand for vitamin C, these by-products can be an alternative source of ascorbic acid, phenolic compounds and carotenoids, promoting their valorization.

Ascorbic acid is an unstable and thermolabile compound, requiring low temperatures for its extraction. Gas-expanded liquid (GXL) is a green extraction process, which uses a liquid solvent (e.g., ethanol) acting as a primary solvent, and a compressible gas (e.g., CO_2) to assist in the extraction (Herrero, Mendiola, & Ibáñez, 2017). The extraction conditions of GXL result in a solvent with greater extraction power, even at low temperatures. Furthermore, ethanol is considered as a safe solvent, and ethanolic extracts can be further used for food applications, for example.

Considering that ascorbic acid is the main antioxidant compound found in immature acerolas, and an important amount of other valuable antioxidant compounds still remains in the residues after acerola processing, the valorization potential of acerola by-products was evaluated in this work using a GXL procedure, according to the following steps: 1) evaluation of the overall extraction yield obtained from bagasse and non-pomace by-products; 2) determination of the ascorbic acid content recovered from each by-product; 3) phytochemical profiling of phenolics and pigments (carotenoids, chlorophylls and pheophytin) extracted along with ascorbic acid. By tackling these issues, it was possible to characterize the extracted fractions from acerola by-products with the highest concentration and the broadest variety of health-promoting compounds. The composition of acerola by-products and acerola juice was also compared.

2. Material and methods

2.1. Raw material

Acerola (*Malpighia emarginata* DC.) by-products and acerola juice were supplied by the company located at latitude 3°51′12″ S and longitude 41°5′10″ W (Ceará, Brazil). Bagasse (seeds and peels) from the pressing step, and non-pomace from the juice clarification step (centrifugation), were collected and stored at freezing temperature (−18 °C). Samples were dried in a vacuum oven at 40 °C until constant weight, milled and sieved (40-mesh). The juice was freeze-dried for further characterization.

2.2. Gas-expanded liquid (GXL) extraction apparatus and procedure

The conditions of GXL process to extract bioactive compounds from acerola by-products were based on previous studies performed by Foodomics group for other biomasses (Reyes, Mendiola, Ibañez, & Del Valle, 2014; Rodríguez-Pérez, Mendiola, Quirantes-Piné, Ibáñez, & Segura-Carretero, 2016). High pressure extractions were carried out in a PrepMaster supercritical fluid extractor under continuous flow mode, as described by (Reyes et al., 2014). PrepMaster unit (Suprex-Pittsburgh, PA, USA) maintained the CO_2 pressure set to 7 MPa and a flow rate of 1 mL/min. Ethanol was pumped by a PU2080 HPLC pump (Jasco Corp., Tokyo, Japan) set to achieve 50% CO_2 flow – 0.5 mL/min. The extraction cell and the feeding tube (where the solvents are mixed) were placed in a hot air circulation oven set to 40 °C. The overall yield curve was constructed to define the extraction time for both acerola by-products. The ethanol present in the extracts was removed under nitrogen flow, and the mass obtained was used to calculate the extraction yield ($g_{extract}$/100 g by-product).

2.3. Chemical composition of extracts

Total phenolic content (TPC) was determined by Folin-Ciocalteu method (Koşar, Dorman, & Hiltunen, 2005). First, 10 μL of sample (10 mg/mL) was mixed with 600 μL of ultrapure water and reacted with the reagent Folin-Ciocalteu (50 μL). After 1 min, 150 μL of 20% (w/v) sodium carbonate (Na_2CO_3) was added, and the final volume was adjusted to 1 mL with water. The mixture was stirred and incubated for 2 h at room temperature in the dark. Subsequently, 300 μL of the reaction mixture was transferred to a 96-well microplate spectrophotometer reader (Synergy HT, BioTek Instruments, Winooski, VT, USA) and the absorbance was measured at 760 nm. The values were converted to mg GAE (gallic acid equivalent) per g of extract by a calibration curve (0–2 mg/mL) prepared with standard gallic acid (Sigma-Aldrich, Madrid, Spain).

Total carbohydrates were analyzed according to the phenol-sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), reacting 278 μL of sample, 167 μL of phenol (5% w/v) and 1 mL of H_2SO_4. After 30 min at room temperature, absorbance was measured at 490 nm and concentrations were calculated according to the standard glucose (Sigma-Aldrich, Madrid, Spain) curve (0.2–1 mg/mL). Protein content was determined according to the Bradford method (Bradford, 1976) by reacting 10 μL of sample and 500 μL of Bradford reagent at room temperature for 10 min. Absorbance was measured at 562 nm and the concentration calculated using standard bovine serum albumin (BSA, Sigma-Aldrich, Madrid, Spain) curve (50–750 μg/mL).

2.4. Antioxidant activities

2,2-Diphenyi-*l*-picrylhydrazyl (DPPH, Sigma-Aldrich, Madrid, Spain) radical scavenging activity was performed according to Brand--Williams, Cuvelier, and Berset (1995) reacting 25 μL of sample with 975 μL of a methanolic solution of DPPH (6×10^{-5} M) at room temperature for 1 h in the dark. Then, 300 μL were taken out and placed in a 96-well microplate and the absorbance was measured at 516 nm. Oxygen radical absorbance capacity (ORAC) assay was performed using fluorescein as a fluorescent probe (Prior et al., 2003). The reaction was performed in a 96-well microplate containing 100 μL of extract at different concentrations (5–100 μg/mL) in ethanol/H_2O (1:9, v/v), 100 μL of peroxyl radical generator (2,2-azobis(2-amidinopropane) dihydrochloride – AAPH, 590 mM) in 30 mM phosphate-buffered saline (PBS) at pH = 7.5, 25 μL of fluorescein (10 μM) in PBS buffer and 100 μL of PBS buffer. Fluorescence was measured (λ excitation = 485 nm; λ emission = 530 nm) every 5 min at 37 °C for 60 min. The results were expressed as EC50 in μg/mL. EC50 represents the concentration required to reduce to 50% the maximum absorbance value of the radicals.

2.5. Phytochemical profiling of acerola by-product extracts

2.5.1. Phenolic composition by UHPLC-q-TOF-MS/MS

Phenolic compounds extracted from acerola by-products were characterized using an ultra-high performance liquid chromatography (UHPLC) system 1290 from Agilent (Agilent Technologies, Santa Clara, CA, USA) coupled to a quadrupole-time-of-flight mass spectrometer (q-TOF-MS) Agilent 6540, equipped with an orthogonal ESI source (Agilent Jet Stream, AJS, Santa Clara, CA, USA), and controlled by the Mass Hunter Workstation software 4.0 (MH) from Agilent. The chromatographic method employed a Zorbax Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm particle diameter, Agilent Technologies, Santa Clara, CA) at 30 °C. The flow rate was 0.5 mL/min, the injection volume was 20 μL, the mobile phases were composed of water (0.01% formic acid, solvent A) and acetonitrile (0.01% formic acid, solvent B) for acquisition in negative ionization mode (ESI-), and the elution gradient was as follows:

0 min, 0% B; 12 min, 80% B; 14 min, 100% B; 16 min, 100% B; 17 min, 0% B.

The mass spectrometer was operated in MS and MS/MS modes using the following parameters: capillary voltage, 3000 V; nebulizer pressure, 40 psi; drying gas flow rate, 11 L/min; gas temperature, 300 °C; skimmer voltage, 45 V; fragmentor voltage, 110 V. The MS and Auto MS/MS modes were set to acquire m/z values ranging between 50–1100 and 50–800, respectively, at a scan rate of 5 spectra per second. Auto MS/MS mode was operated selecting 4 precursor ions per cycle at a threshold of 200 counts, as reported by Ballesteros-Vivas, Álvarez-Rivera, Ibáñez, Parada-Alfonso, and Cifuentes (2019). The reference compound solution for internal mass calibration of the Q/TOF at mass spectrometer contained 5 μM of purine ([C5H5N4]+ 121.050873m/z) and 2.5 μM HP-0921, hex-S-5akis(1H,1H, 3H-tetra- fluoropropoxy) phosphazine ([C18H19O6N3P3F24]+ at 922.009798m/z) in acetonitrile-water (95:5, v/v) from Agilent. A solution containing a mixture of standard polyphenols (catequin, caffeic acid, hydroxycinnamic acid, rutin, quercetin and kampferol) was used for confirmation purposes.

2.5.2. Carotenoid and chlorophyll composition by HPLC-APCI-MS/MS

Carotenoids and chlorophylls were characterized by HPLC-DAD (diode-array detector) using an Agilent 1200 series liquid chromatograph equipped with autosampler injector, binary pump, and online degasser (Santa Clara, CA, USA) coupled to an ion trap mass spectrometer (Agilent Ion Trap 6320) with an atmospheric pressure chemical ionization (APCI) source. The reverse phase-HPLC separation was performed at room temperature with 10 μL injection volume on a YMC-C30 column (150 × 4.6 mm i.d., 3 μm particle size; YMC Europe, Schermbeck, Germany) protected by a YMC-C30 (10 × 4 mm, 5 μm particle size) pre-column. The mobile phases were A (methanol:methyl *tert*-butyl ether:water; 80:18:2 v/v/v) and B (8:90:2 v/v/v), both containing 400 mg/L of ammonium acetate, according to Schex et al. (2018). The elution gradient at constant flow rate of 0.3 mL/min was as follows: from 10% to 55% B (25 min), from 55% to 100% B (2 min) and an isocratic hold at 100% B (3 min). Final reconditioning from 100% to 10% solution B (2 min) and then maintained isocratically for 5 min.

The DAD recorded visible spectra in the range of 380 and 700 nm (peak width 0.1 min (2 s), slit 4 nm). Data were acquired with LC ChemStation 3D Software Rev. B.04.03 (Agilent Technologies, Santa Clara, CA, USA). MS analysis was operated using the following parameters: capillary voltage, −3.6 kV; drying temperature, 350 °C; vaporizer temperature, 400 °C; drying gas flow rate, nebulizer gas pressure, 45 psi; 7 L/min; corona current, 4000 nA.

Operating in positive ionization mode (APCI+), full scan spectra were obtained in the range from m/z 150 to 1100 at a scan speed of 13000 m/z per second. Automatic MS/MS data dependent-scans were carried out, fragmenting the two highest precursor ions (10000 counts threshold; 1 V fragment amplitude) with an isolation width of 2.0 Th for precursor ions.

2.5.3. Identification of compounds

Phenolic compounds and carotenoids were identified according to data from the high-resolution MS analysis, MS/MS fragmentation patterns and information reported in literature and on-line databases (Metlin; HMDB). When available, commercial standards were used for unambiguous identification.

2.5.4. Quantification of ascorbic acid, lutein and β-carotene

L-ascorbic acid was quantified by UHPLC-q-TOF-MS, using the operating conditions described in Section 2.5.1. A calibration curve ranging from 5 to 100 μg/mL was prepared. Carotenoids (all-E)-lutein and (all-E)-β-carotene were quantified using a calibration curve ranging from 25 to 400 μg/mL and 5–200 μg/mL respectively, using the operating conditions described in Section 2.5.2. Quantitation of (Z)-isomers was performed using the corresponding (all-E)-carotenoids. The linear regression curve of the target standards is shown in Table 1.

Table 1
Linear regression curves for ascorbic acid, lutein and β-carotene quantification.

No	Standard	Linear regression curve	R^2
1	L-ascorbic acid	Y = 42346 X - 216176	0.9997
2	(all-E)-lutein	Y = 23878 X – 339	0.9947
3	(all-E)-β-carotene	Y = 25219 X + 107	0.9948

2.6. Statistical analysis

The experimental determinations were carried out in triplicate and the results were expressed as mean ± standard deviation using Microsoft Excel.

3. Results and discussion

The characterization of the extracts obtained from the different by-products of acerola (bagasse and non-pomace) was carried out in order to identify differences in their composition and guide future applications.

3.1. Extraction yield

The kinetic study of GXL extraction of bioactive compounds from acerola by-products is shown in Fig. 1. The non-pomace and bagasse yields show a similar trend with well-defined extraction periods, as described by Rodríguez-Pérez et al. (2016). A period of constant extraction rate (CER) was observed in the first 20 min, characterized by the extraction of solutes from the particle surface dominated by the convection mechanisms. After 20 min, the yields obtained were 3.4 $g_{extract}$/100 g bagasse and 10.5 $g_{extract}$/100 g non-pomace, representing 72% and 63% of the maximum extraction capacity, respectively. After this period, the curves were characterized by a falling extraction rate (FER) between 60 and 80 min. Finally, a diffusion-controlled rate (DCR) period takes place, which controls the extraction of compounds from inside of the matrix particles at an almost null mass transfer rate. The maximum yields obtained after 160 min was 4.7 $g_{extract}$/100 g bagasse and 16.5 $g_{extract}$/100 g non-pomace. The optimal extraction time was defined as the one that provides 90% of the maximum yield. In this case, extraction times of 90 and 60 min for non-pomace and bagasse, respectively, were selected.

The different yields obtained from the two by-products can be attributed to their different composition. The non-pomace is made up of fragments of fruit pulp, polysaccharides, gums, proteins and polyphenols (Haas et al., 2016; Kilara & Van Buren, 1989). In the

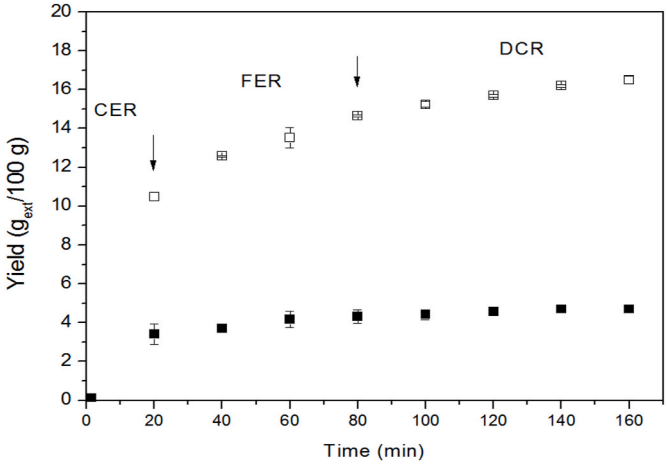

Fig. 1. Kinetic study of extraction yield under GXL conditions (40 °C, 7 MPa and 50% ethanol) for (□) non-pomace and (■) bagasse. CER, FER and DCR are the periods of extraction (see text for explanation).

clarification step, the enzymes help to precipitate the polysaccharides that cloud the juice. In this step, the polysaccharides are aggregated, carrying with them still linked compounds such as polyphenols and other soluble compounds. However, in bagasse, seeds are the main constituents and show a high content of insoluble fibers (Marques, Corrêa, Lino, Abreu, & Simão, 2013) and, as indicated by the extraction yield, this fraction had a lower content of extractable compounds under GXL conditions (7 MPa, 40 °C, CO_2:EtOH 2:1).

3.2. TPC and antioxidant activity

Differences can be observed in total phenolic content (TPC) and antioxidant activities (DPPH and ORAC) of non-pomace and bagasse extracts (Table 2). Regarding TPC, while non-pomace extract showed 203.3 mg GAE/$g_{extract}$, the bagasse exhibited half of that content (106.8 mg GAE/$g_{extract}$). TPC was shown to affect antioxidant activity values, as suggested by the higher antioxidant activity of non-pomace extract compared to bagasse extract. However, taking the immature acerola juice powder as reference in terms of phenolic content and antioxidant potential, the non-pomace extract showed comparable values, mainly for ORAC activity, despite the higher TPC in the juice. These results can be explained by the presence of other compounds such as carotenoids and flavonoids in the non-pomace extract (discussed in Sections 3.4 and 3.5), which were not detected in acerola juice.

3.3. Ascorbic acid recovery

The presence of ascorbic acid in the acerola extracts was confirmed by HPLC-HRMS/MS analysis. Ascorbic acid exhibited experimental $[M-H]^-$ ion at m/z 175.0250, and MS/MS fragmentation at m/z 87.0098, 59.0146, 71.0146 and 115.0036 in good agreement with the reference MS/MS databases. The retention time was 0.7 min according to the standard (L-ascorbic acid) used for quantification.

The ascorbic acid content in non-pomace extracts was 158.2 mg/$g_{extract}$, representing approximately 81% of the content found in powdered acerola juice (193.7 mg/g as shown in Table 2). However, ascorbic acid was not detected in the extract obtained from the bagasse (seed and peel). Differences in the vitamin C content were reported in extracts obtained from acerola by-products (Carmo, Nazareno, & Rufino, 2018), where higher values (three-fold) of vitamin C in the juice clarification residue (non-pomace) were found. The acid extraction of peel and pulp of camu-camu also showed concentrations of vitamin C up to three-fold higher in the pulp (Cunha-Santos, Viganó, Neves, Martínez, & Godoy, 2019), indicating that this compound is in the edible fraction of the fruit. The bagasse fraction could be explored to obtain other compounds, such as oligosaccharides, due to its rich composition in dietary fibers. The proximal composition of acerola by-products has been reported by Carmo et al. (2018) and Marques et al. (2013).

Few studies report the recovery of compounds from acerola by-products. Most of these studies make use conventional methods (Carmo et al., 2018; Marques, Cesar, Braga, Marcussi, & Corrêa, 2018), although ultrasound-assisted extraction (Rezende et al., 2017) was also used. The use of GXL extraction to recover ascorbic acid is a powerful alternative, since 81% of ascorbic acid was recovered (based on the content found in the juice). The conditions of low pressure (7.0 MPa) compared to supercritical fluids (CO_2), and low temperature (40 °C) compared to conventional or subcritical water extraction, reduce energy consumption and processing costs. In addition, the tunability of the solvent allows the extraction of compounds that would not be soluble under atmospheric conditions. Thus, despite the low solubility of ascorbic acid in ethanol under atmospheric conditions, the changes in the physicochemical properties of ethanol under GXL conditions allowed the extraction of this compound. Detailed discussion of the applicability and advantages of GXL to obtain bioactive compounds can be found in the work of Herrero et al. (2017).

The recovery of ascorbic acid from acerola by-products makes a positive economic and environmental impact in the industry of acerola processing. In Fig. 2, the mass balance of acerola processing shows that 0.25 kg of extract can be recovered from the non-pomace generated in the processing of 100 kg of acerola. This value corresponds to approximately 15% of the powder juice yield (1.65 kg), which can be considered remarkable when the yields are compared. In addition, the ascorbic acid content in the non-pomace extract represents 15.8% (w/w), indicating that a large amount of this compound remains in the fruit waste when compared to the amount of ascorbic acid in the powdered juice.

It is important to notice the higher ascorbic acid recovery values obtained using GXL compared to other methods. For example, Rezende et al. (2017) tested three extraction methods: agitation in shaker, maintenance in refrigerator and ultrasound-assisted extraction (UAE). They got the best results using UAE obtaining 489 ± 19 mg ascorbic/100 g acerola by-product. As can be seen in Fig. 2, using GXL in the present study we obtained 39.5 g ascorbic/1.93 kg acerola residue, which means 2046.6 mg ascorbic acid/100 g acerola by-product, more than 4 fold the recovery of UAE reported in literature.

Table 2
Comparative results of the extracts obtained from acerola by-products and acerola juice powder: extraction yield, chemical composition and antioxidant activity.

	Non-pomace	Bagasse	Juice powder
Extraction yield ($g_{extract}$/100 g)	[a]12.9 ± 1.1	[a]3.8 ± 0.1	[b]2.7 ± 0.1
Chemical composition (mg/$g_{extract}$)			
Total phenolic content (TPC)	203.3 ± 7.2	106.8 ± 5.5	314.5 ± 5.8
L-ascorbic acid	158.2 ± 0.4	nd	193.7 ± 3.7
Total carbohydrates	141.0 ± 1.0	169.0 ± 5.8	277.7 ± 4.6
Protein	0.027 ± 0.002	0.037 ± 0.004	Nd
(all-E)-lutein	11.1	3.4	Nd
(9Z)-lutein	3.0	0.8	Nd
(9′Z)-lutein	4.1	1.0	Nd
(all-E)-β-carotene	5.8	<0.05	Nd
β-carotene isomer	0.6	<0.05	Nd
Antioxidant activity (EC50 μg/mL)			
DPPH	6.87 ± 0.54	38.17 ± 1.01	4.24 ± 0.13
ORAC	2.13 ± 0.01	7.86 ± 0.06	2.44 ± 0.38

The values correspond to the mean of three extraction assays for non-pomace and bagasse, and three analytical determinations for the juice sample. Standard deviation was <0.05 in carotenoid analysis; nd: not detected.

[a] Yield obtained at 90 min for non-pomace and 60 min for bagasse in the conditions of GXL extraction.

[b] Corresponds to the yield of immature acerola juice after freeze-drier.

3.4. Carotenoid and chlorophyll composition

Carotenoids and chlorophylls composition was determined by HPLC-DAD-APCI-MS/MS analysis. Table 3 summarizes the retention time, UV–vis maxima, molecular ions and main MS/MS fragments of 15 pigments detected in acerola by-products. All compounds were detected in non-pomace and bagasse extracts, although higher abundance was observed in the non-pomace sample. However, no carotenoid or chlorophyll-derivatives was identified in the juice. The chromatographic profile of the identified compounds is shown in Fig. 3. Two characteristic absorption bands in the visible region were observed in the compounds characterized as chlorophylls and derivatives. The first band was observed around 400–450 nm and the second between 600 and 700 nm. The gap between the two bands is known as the green window of chlorophylls. Thus, several chlorophyll-derivatives (peaks 1–4, 11) were tentatively identified based on absorption spectra (λ_{max}) and the fragmentation patterns (Viera, Roca, & Perez-Galvez, 2018). (Z)-isomers of lutein (5, 7, 8) and β-carotene (14, 15) were identified according to the commercial standards, exhibiting experimental [M-H]+ ion at m/z 551.6 and 537.6, respectively. Pheophytin a and b showed a base peak at m/z 872.3 (13) and 886.0 (12), respectively, yielding abundant product ions at 593 and 533 for pheophytin a and 857, 607, 547 for pheophytin b. Less abundant peaks (6, 9, 10), identified as carotenoid precursors,

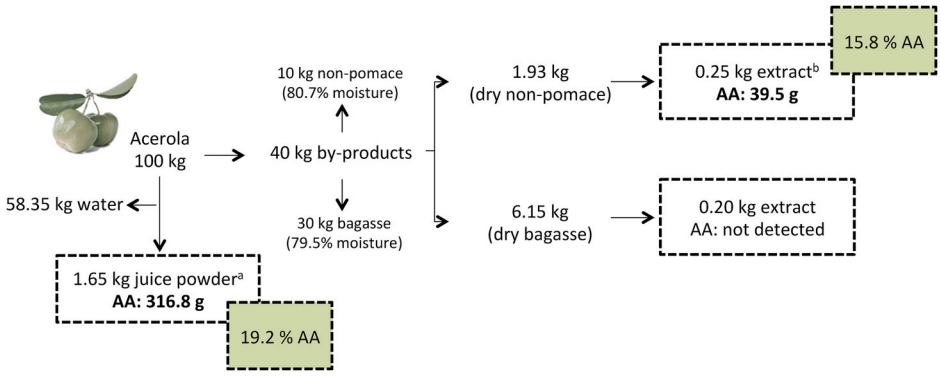

Fig. 2. Mass of ascorbic acid (AA) recovered from acerola juice and acerola by-product (non-pomace). [a] Powdered juice after freeze-drying; [b] GXL extract.

Table 3
HPLC-DAD-APCI-MS/MS data of pigments (carotenoids and chlorophylls) identified in acerola by-products.

Peak No	RT (min)	Tentative identification	λ_{max} (nm)	$[M-H]^+$ (m/z)	MS/MS product ions (+) (mz)
1	4.75	Chlorophyll-derivative	435, 450	614.6	595, 521
2	5.31	Chlorophyll-derivative	410, 664	726.8	545, 262
3	6.08	Chlorophyll-derivative	405, 662	617.6	602, 635
4	6.32	Chlorophyll-derivative	405, 662	753.1	573, 262
5	6.67	(all-E)-lutein[b]	423, 445, 473	551.6[a]	533, 495, 429, 397
6	7.60	Carotenoid	420, 445, 472	575.7	558, 500, 418
7	8.34	(9Z)-lutein	420, 443, 470	551.6[a]	533, 495, 451, 429
8	9.52	(9′Z)-lutein	420, 442, 470	551.6[a]	533, 495, 457, 429
9	10.17	Carotenoid	418, 442, 470	656.8	638.8
10	12.00	Carotenoid	418, 442, 470	664.9	647, 629, 551
11	14.63	Chlorophyll-derivative	418, 625	697.2	679, 661
12	15.64	Pheophytin b	418, 450	886.0	857, 607, 547
13	17.09	Pheophytin a	405, 460	872.3	593, 533
14	17.82	(all-E)-β-carotene[b]	424, 450, 475	537.6	481, 457, 445, 413, 399
15	19.39	β-carotene isomer	422, 446, 472	537.6	ND

RT: Retention Time; λ_{max}: UV/vis absorption maxima.
[a] In-source elimination of water [M + H–H_2O].
[b] Identified with commercial standard co-injection.

were also detected. Chlorophylls and pheophytin are naturally occurring pigments in plants, especially in immature fruits, and associated to the high antioxidant activity found in plant extracts (Sarker, Oba, & Daramy, 2020).

The contents of (all-E)-lutein, (all-E)-β-carotene and their isomers are shown in Table 2. The (all-E)-lutein content (11.1 mg/g) was higher in the non-pomace extract compared to that found in bagasse (3.4 mg/g). Higher (all-E)-β-carotene content (5.8 mg/g) was also found in the non-pomace extract. Carotenoids were not detected in the juice, probably due to the low solubility in polar medium, remaining attached to polymeric structures in cell walls. The presence of these compounds in the GXL extracts is due to the conditions used in the extraction process, which allows the extraction of non-water-soluble compounds. Therefore, the carotenoids profile found in the by-product extract makes it more nutritionally and biologically attractive, due to the potential benefits to human health attributed to lutein (related to eye protection), and β-carotene acting as a precursor to vitamin A (Amengual, 2019).

Although different carotenoids have been reported in acerola fresh fruits, as well as in derived products (Mezadri, Pérez-Gálvez, & Hornero-Méndez, 2005; Xu et al., 2020), this is the first report of carotenoids and chlorophylls in acerola by-products.

3.5. Phenolic composition

Phenolic compounds tentatively identified in the extracts of acerola by-products and juice are shown in Table 4. It is important to highlight that the presence of phenolic compounds provides the extract with an additional antioxidant potential to that assigned to ascorbic acid, enhancing the bioactivity of the extract. In addition, the consumption of vitamin C along with other compounds from fruits, such as phenolics and nutrients, is considered preferable, as the presence of these compounds can increase the bioavailability of vitamin (Carr and Vissers, 2013). The phenolic compounds identified were classified into hydroxycinnamic acids, hydroxybenzoic acids, and flavonoids. Catequin (3), caffeic acid (5), hydroxycinnamic acid (7), rutin (10), quercetin (14) and kampferol (18) were confirmed by retention time, MS and the corresponding MS/MS fragment ions of commercial standards. The compounds listed in Table 4 are in agreement with those already identified in immature acerola pulp (Xu et al., 2020).

The phenolic composition of the obtained extracts was compared with acerola immature juice (Fig. 4). The extraction conditions promoted the recovery of some compounds in the extracts, which were not detected in the acerola juice, such as quercetin, trihydroxyflavanones and kampferol, probably because these compounds are insoluble or sparingly soluble in aqueous medium. The main difference between both by-products relies on the absence of catequin, epicatequin and flavones in the bagasse sample, indicating that non-pomace extract has a greater variety of compounds.

Many health benefits are associated with the antioxidant and anti-inflammatory activities of phenolic compounds, although few studies presented biological activities of compounds extracted from acerola. Gallic acid, catequin, epigallocatechin-gallate, epicatechin, syringic acid, *p*-coumaric acid, and quercetin were identified in methanolic extracts of acerola by-product (without seeds) and assayed as *in vitro* digestive enzymes inhibitor (α-amylase and β-glucosidase) (Marques et al., 2016), and thrombin-like enzymes (phospholipases and proteases) (Marques et al., 2018). The authors concluded that the compounds

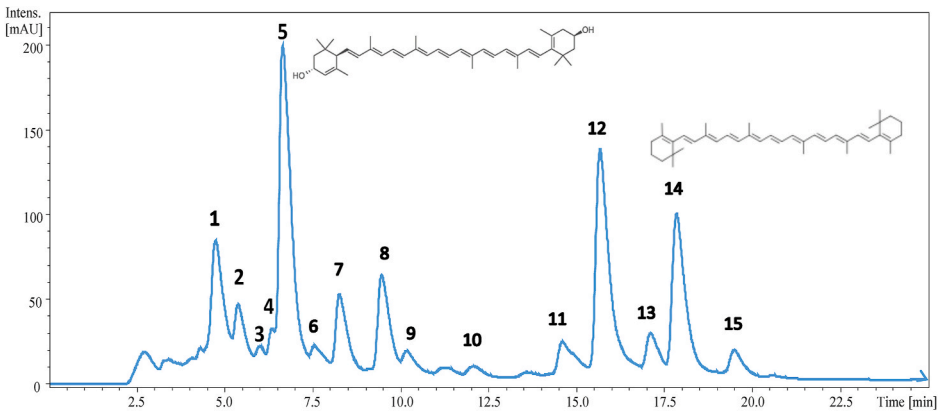

Fig. 3. HPLC-DAD chromatogram of carotenoids identified in non-pomace extract (detection wavelengths: 450 nm). See Table 3 for peak annotation.

Table 4
HPLC-MS/MS information on phenolic compounds tentatively identification in extracts of acerola by-products and juice.

Peak No	RT (min)	Tentative identification	Formula	[M-H]$^{-}$[a]	Error (ppm)	MS/MS product ions (−) (mz)	% Heights[b]		
							Non-pomace	Bagasse	Juice
1	2.867	3.4-Dihydroxyhydrocinnamic acid[1]	$C_9H_{10}O_4$	181.0506	−0.9	135.0448, 119.0507, 134.0367, 163.0381	2.2	0.7	12.0
2	3.221	4-Hydroxybenzoic acid[2]	$C_7H_6O_3$	137.0244	0.2	137.0252, 92.0269, 136.1084, 108.0218	0.7	2.0	nd
3	3.647	Catechin[3]	$C_{15}H_{14}O_6$	289.0718	−0.1	123.0455, 109.0284, 203.0711, 125.0268	2.0	nd	5.0
4	3.761	Trihidroxy(iso)flavone[3]	$C_{15}H_{10}O_5$	269.0455	−2.4	269.0461, 185.0223, 96.96, 197.0602	0.4	nd	1.7
5	3.922	Caffeic acid[1]	$C_9H_8O_4$	179.0350	0.1	135.045, 134.0371, 136.0482, 107.0498	6.7	5.8	11.3
6	4.277	Epicatechin[3]	$C_{15}H_{14}O_6$	289.0717	−0.2	109.0309, 125.0258, 205.0503, 289.0741	4.8	nd	11.1
7	5.010	2-Hydroxycinnamic acid[1]	$C_9H_8O_3$	163.0400	−4.1	93.0356, 119.0507, 118.039, 65.0412	23.0	18.7	32.9
8	5.291	Salicilic Acid[2]	$C_7H_6O_3$	137.0244	−4.2	119.0508, 120.0537, 93.0357, 44.9986	1.0	3.1	nd
9	5.291	Ferulic acid[1]	$C_{10}H_{10}O_4$	193.0506	0.2	134.0375, 116.9298, 135.0406, 96.9616	0.7	1.6	nd
10	5.331	Rutin[3]	$C_{27}H_{30}O_{16}$	609.1461	−0.1	609.1467, 610.1487, 300.0269, 611.147	2.6	2.4	6.5
11	6.108	Quercetrin[3]	$C_{21}H_{20}O_{11}$	447.0933	2.0	300.0277, 301.0342, 447.0934, 448.0966	28.5	22.8	19.5
12	6.670	Coumaroylquinic acid[1]	$C_{16}H_{18}O_8$	337.0929	2.3	161.0441, 193.0492, 143.0334, 163.0505	0.5	0.6	nd
13	6.718	Kaempferol-3-rhamnoside[3]	$C_{21}H_{20}O_{10}$	431.0983	2.6	284.0332, 285.0399, 431.0972, 432.1027	1.7	1.6	nd
14	7.656	Quercetin[3]	$C_{15}H_{10}O_7$	301.0354	2.2	151.0037, 178.9992, 301.0351, 121.03	13.6	13.5	nd
15	7.893	Coumaric acid derivative[1]	$C_{10}H_{10}O_3$	177.0557	−4.4	117.0347, 118.0420, 159.8930, 70.9385	0.3	1.1	nd
16	8.017	Trihydroxyflavanone-I[3]	$C_{15}H_{12}O_5$	271.0612	−1.8	253.0514, 227.0697, 271.0269, 274.0333	0.3	0.7	nd
17	8.220	Trihydroxyflavanone-II[3]	$C_{15}H_{12}O_5$	271.0612	−1.8	151.0037, 178.9992, 301.0351, 121.03	1.4	3.1	nd
18	8.330	Kaempferol[2]	$C_{15}H_{10}O_6$	285.0405	−0.1	285.0411, 186.0443, 151.0022, 185.0607	7.0	7.8	nd
19	8.647	(Iso)Formononetin[3]	$C_{16}H_{12}O_4$	267.0663	−1.5	252.0436, 267.0647, 253.0454, 268.0695	2.3	14.1	nd

RT: Retention Time; nd: not detected.
[1]Hydroxycinnamic acid family.
[2]Hydroxybenzoic acid family.
[3]Flavonoid family.
[a] Theoretical mass.
[b] Calculated from the sum of peak heights (total peak area: 5.4×10^5 for non-pomace; 4.9×10^5 for bagasse; 2.0×10^5 for juice).

found in the acerola by-product had enough effect to be used as an aid in the treatments of obesity and thrombosis. Glycosylated forms of quercetin and kaempferol were also found in methanolic extracts from edible parts of acerola fruits. The compounds were associated with protective effects against oxidative damage of macromolecules such as lipids and proteins (Alvarez-Suarez et al., 2017). In addition, some studies showed that the antioxidant activity found in acerola juice, attributed to phenolic compounds and vitamin C, helps to reduce oxidative stress in

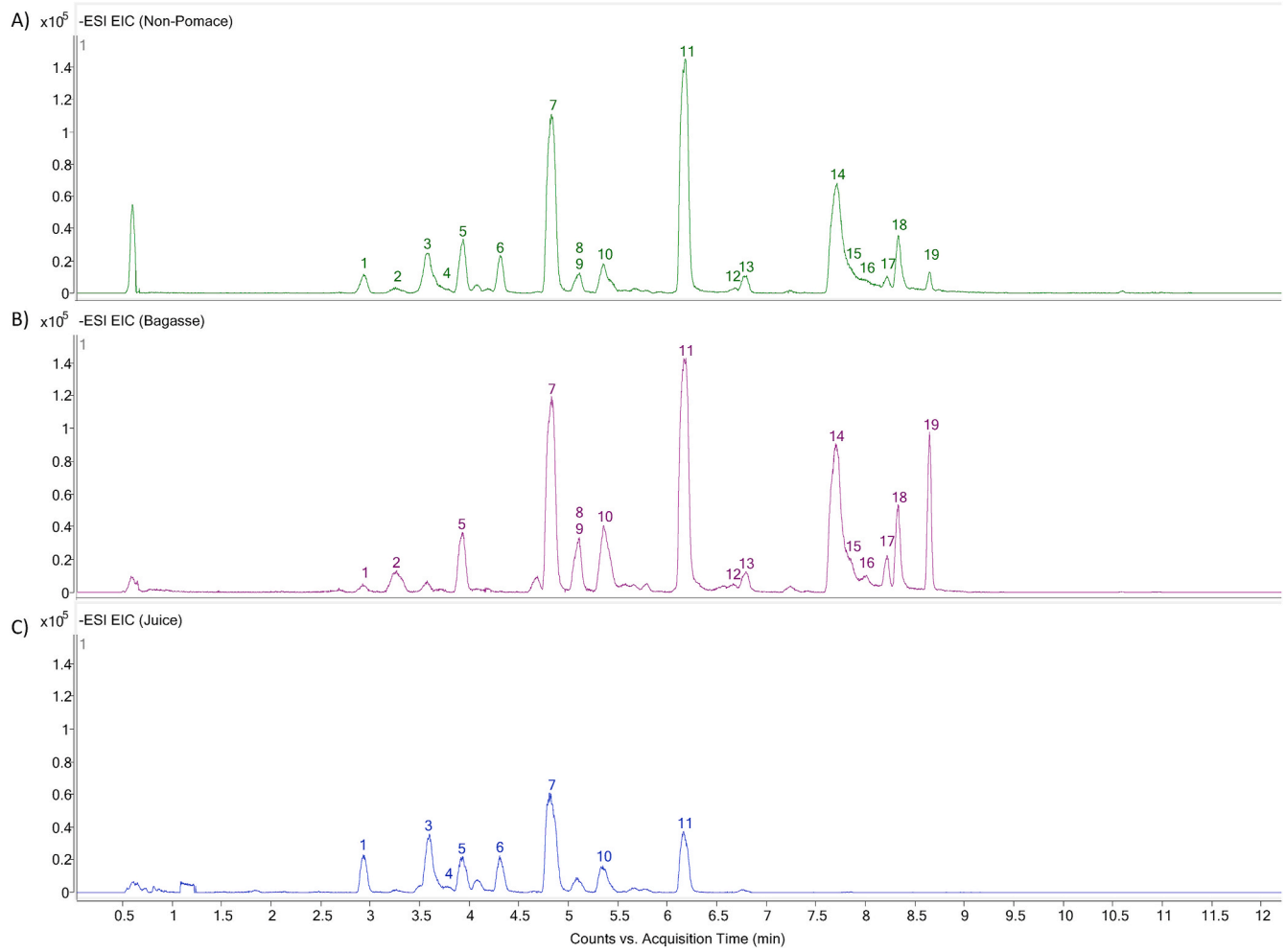

Fig. 4. HPLC-ESI(−)-QTOF-MS extracted ion chromatogram of phenolic compounds detected in extracts of acerola by-products and juice. A) non-pomace, B) bagasse, C) juice.

obese individuals, preventing weight gain and restoring the metabolic and inflammatory pathways to normal levels (Dias et al., 2014; Leffa et al., 2017).

Concomitant extraction of ascorbic acid and phenolic compounds by the GXL technique can be an advantageous alternative for the production of nutraceuticals. Commercial products, rich in vitamin C from acerola, usually add phenolic extracts from other fruits in their formulations, such as pomegranate and grapefruit extracts, claiming antioxidant protection. The extracts of acerola by-products showed a higher number of phenolic compounds compared with acerola juice (peaks 12 to 19 - Table 4 and Fig. 4), and the presence of carotenoids (Table 3). This pool of compounds could be used in the development of new formulations using the acerola's own bioactive compounds.

4. Conclusions

Acerola by-products were proved to be a valuable natural source of ascorbic acid in addition to a pool of molecules with high antioxidant activity, including phenolics and carotenoids. Ascorbic acid was only identified in the non-pomace extracts, and higher concentrations of carotenoids were also found in this by-product, compared to bagasse. Non-pomace extracts was also obtained at a higher extraction yield, which represented 15% in relation to the powder juice yield, while vitamin C content reached 81% of that found in the acerola juice powder, and with comparable antioxidant capacity. GXL extraction has demonstrated to be a more efficient extraction process to increase ascorbic acid recovery, compared to previously published methods (more than four-fold). The non-pomace extract obtained by GXL technique can be further used in the development of new nutraceutical products or food ingredients, with a rich composition of polyphenols and carotenoids that are not found in acerola juice.

CRediT roles

Patrícia Poletto: Conceptualization, Methodology, Formal analysis, Data curation, Writing, Review and Editing; Gerardo Alvarez-Rivera: Supervision, Writing, Investigation, Review; Gerson-Dirceu López: Formal analysis and Writing; Otília M. A. Borges: Formal analysis; Jose A. Mendiola: Writing, Review and Editing; Elena Ibañez: Conceptualization, Writing and Review; Alejandro Cifuentes: Conceptualization, Writing, Review and Supervision, Project Resources.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Patrícia Poletto: Conceptualization, Methodology, Formal analysis, Data curation, Writing, Writing – review & editing. **Gerardo Álvarez-**

Rivera: Supervision, Writing – original draftWriting – original draft, Investigation, Writing – review & editing, Review. **Gerson-Dirceu López:** Formal analysis, and, Writing – original draft. **Otília M.A. Borges:** Formal analysis. **Jose A. Mendiola:** Writing – review & editing. **Elena Ibáñez:** Conceptualization, Writing – review & editing. **Alejandro Cifuentes:** Conceptualization, Writing – review & editing, and, Supervision, Project administration, Resources.

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