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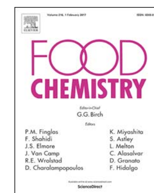

Evaluation of nutritional and chemical composition of yacon syrup using ^{1}H NMR and UPLC-ESI-Q-TOF-MS^{E}

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A R T I C L E I N F O

Chemical compounds studied in this article:
Chlorogenic acid (PubChem CID: 1794427)
1-Kestose (PubChem CID: 440080)
Nystose (PubChem CID: 166775)
1F-β-fructofuranosylnystose (PubChem CID: 3085157)

Keywords:
Prebiotic
Chlorogenic acid
Fructooligosaccharides
Smallanthus sonchifolius

A B S T R A C T

A complete characterization of yacon syrup was performed by analytical techniques, including NMR and UPLC-QTOF-MS^{E}. The effect of the different stages of yacon syrup production on fructooligosaccharides (FOS) and chlorogenic acid (CGA) contents were also evaluated. As a result, in addition to higher levels of FOS and CGA, some mineral elements, such as K, Ca and P, and essential amino acids, such as tryptophan, valine, and threonine, were determined in yacon syrup. Twenty-five compounds were putatively identified, and the main compounds were phenolics derived from quinic and *trans*-cinnamic acids. Considering the different stages of yacon syrup production, the results indicate that the contents of FOS and CGA were maintained in the pulping, enzymatic maceration and microfiltration, leading to a concentration of these components in the last stage of processing (vacuum concentration). These results will be used to fortify this innovative and promising product in the area of functional foods.

1. Introduction

Yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] is a perennial herbaceous plant of the family *Asteraceae* native to the Andean regions of South America. Nowadays, this tuberous root is currently cultivated in other countries, such as Brazil, Japan and United States (Caetano et al., 2016). It is known as an abundant source of β-$(2 \rightarrow 1)$ fructooligosaccharides (FOS) and phenolic compounds, especially chlorogenic acid (CGA).

The high content of bioactive compounds in yacon roots is recognized to offer health benefits (e.g., reduction of glycemic index, body weight and the risk of colon cancer), arising from its antioxidant and prebiotic properties (Campos et al., 2012; Caetano et al., 2016; Silva et al., 2017), exhibiting great public interest as a dietary supplement (Caetano et al., 2016). However, fresh yacon roots are perishable due to its high water content (> 80%) (Campos et al., 2012), phenolic compounds types and concentration, and the activity of the polyphenol oxidase (Dionísio et al., 2013). Therefore, in order to increase the yacon shelf-life, several processes have been reported as its transformation into juice, dehydrated products, or concentrated products, such as syrup (Dionísio et al., 2015; Silva et al., 2017).

Yacon syrup could be classified as a functional product due to its naturally high FOS content and presents similar physical and sensorial characteristics to those of honey or sugar cane syrup (Genta et al., 2009). However, although the characterization and quantification of FOS and phenolic compounds were reported for yacon roots and leaves (Biazon et al., 2016; Campos et al., 2012; Russo, Valentão, Andrade, Fernandez, & Milella, 2015), the assessment of nutritional and functional compounds present in different syrups are focused mainly in the FOS contents (Genta et al., 2009; Geyer, Manrique, Degen, & Beglinger, 2008; Manrique, Párraga, & Hermann, 2005).

Recently, a process to obtain a yacon syrup with high levels of FOS and CGA, which include enzymatic maceration, followed by microfiltration and vacuum concentration was developed (Silva et al., 2017). This process uses mild conditions of temperature aiming to preserve its functional compounds. However, the effect of each step processing on FOS and phenolic compounds, especially CGA, is still unknown.

In the current study, we investigate the complete characterization of yacon syrup using several methods, including UPLC-ESI-Q-TOF-MS^{E} and NMR. The effects of the processing steps to produce yacon syrup on

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the phenolic profile, FOS and CGA contents were also evaluated. These results will be used to elucidate the nutritional and functional properties of this innovative and promising product in the area of functional foods.

2. Material and methods

2.1. Chemicals

Pectinex® Ultra SP-L (mainly polygalacturonase) from *Aspergillus aculeatus* and Celluclast® 1.5 L (cellulase) from *Trichoderma reesei* were obtained from Novozymes Investment Co. Ltd. (Denmark). The enzyme activities were 9500 polygalacturonase units (PGU) mL^{-1} for Pectinex® Ultra SP-L and 700 β-glucanase units (EGU) g^{-1} for Celluclast® 1.5 L. All chemicals were of analytical or higher grade from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

2.2. Yacon syrup preparation

The raw yacon was obtained in a local market in Fortaleza, Ceará State, Brazil. Then, yacon pulp was processed as previously reported (Dionísio et al., 2013). After washing and sanitizing, the yacon peel was removed manually and the edible portion was cut (1 cm^3) and immersed in a citric acid solution (2.4% w/v) for 8 min to inactivate polyphenol oxidase enzymes. These small pieces were homogenized in an industrial blender to obtain the yacon pulp, and stored at −18 ± 1 °C. The yacon pulp was treated with Celluclast® 1.5 L and Pectinex® Ultra SP-L (500 mg L^{-1} of each enzyme at 45 °C, 175 rpm for 2 h). After enzymatic inactivation (85 °C for 5 min), the pulps was refrigerated to 30 °C, and filtered through a microfiltration system (Pall industrie, model Membralox, Saint-Germain-en-Laye, France). Then, yacon juice was concentrated until 71 °Brix. The evaporation temperature was 60 ± 5 °C and a vacuum pressure of 560 mmHg. The yacon syrup was stored at 5 °C, and subjected to specific analysis. All the analyses were carried out in three independent replicates.

2.3. Methods of analyses

2.3.1. Proximate composition

The proteins were determined using the Kjeldahl method (920.87 AOAC, 2005); total lipid contents were determined by Soxhlet extraction method (925.38 AOAC, 2005); ash was determined by incinerating at 550 °C in a muffle furnace for 6 h (923.03 AOAC, 2005), the moisture was determined by AOAC 925.09 method, and carbohydrate by difference (AOAC, 2005).

2.3.2. Mineral analyses

An aliquot of 1 mL was placed into a digestion tube and reacted overnight with nitric acid and perchloric acid (3:1 v/v). Digestion was carried out using a digester dry block at 200 °C for 4 h and after cooling the volume was brought to 50 mL with deionized water. Reagent blanks were prepared similarly to the samples. The digests were analyzed by atomic absorption spectrometry for calcium, magnesium, copper, zinc and manganese determination. Potassium and sodium were determined by flame photometry while phosphorus and sulfur were determined by colorimetric and turbidimetric methods, respectively.

2.3.3. Water activity, pH, soluble solids, turbidity and color

Water activity (a_w) was measured at 25 °C using a water activity meter (Aqualab Decagon Devices Inc. Pullman, model CX-2T, Washington, EUA). The pH of the sample was measured using a digital pH meter (Hanna Instruments, model HI2211, Romania), following AOAC's methods (2005) (AOAC, 942.15), and the soluble solids content (°Brix) was determined using a refractometer (Atago, model Pocket PAL-3, Tokyo, Japan) at 20 °C, as recommended by AOAC (2005). Turbidity was measured using a turbidimeter (Tecnopon, model TB-1000, Piracicaba, SP, Brazil) and the results were expressed in NTU (Nephelometric Turbidity Units). The color was performed in a colorimeter (Konica Minolta Sensing, Inc., model CR-400, Osaka, Japan), with results based on three color coordinates: L^* (whiteness or brightness/darkness), a^* (redness/greenness), and b^* (yellowness/ blueness).

2.3.4. Fructooligosaccharides content (FOS) and simple sugars

The FOS were determined as described by AOAC 999.03 method (2005), and the results were expressed as % FOS. Glucose, fructose, maltose and sucrose were quantified by HPLC, after extraction of sugars (Burgner & Feinberg, 1992). A sample of 10 mL was homogenized in 100 mL solution containing deionized water, zinc acetate 1.0 M and potassium ferrocyanide 0.25 M and filtered on qualitative filter paper. The sugar solution was then filtered through a PVDF membrane of 0.22 µm pore diameter and injected into a liquid chromatograph (Varian, model Pro Star 210, Mulgrave, Australia), equipped with a refractive index detector (Varian, model ProStar 350) and a normal phase column (Zorbax Carbohydrate, 250 × 4.6 mm, 5 µm, Agilent), and oven at 35 °C. The mobile phase consisted of acetonitrile and water (80:20 v/v), the flow rate was 1.5 mL min^{-1} and the injection volume was 20 µL. Quantification was performed by external calibration curves of each sugar from 0.02 g mL^{-1} to 1.0 g mL^{-1}.

2.3.5. Oligosaccharides degree of polymerization

The quantification of the oligosaccharides [GF_6, GF_5, GF_4 (1F-β-fructofuranosylnystose), GF_3 (nystose) and GF_2 (1-kestose)] was performed by thin layer chromatography (TLC), using a silica gel on Sigma-Aldrich TLC plates (20 × 20 cm, 60 Å medium pore diameter). Samples of 3 µL were applied on the plate at 1 cm from the bottom and 1.0 cm separation distance from each other. The solvent system used to separate the carbohydrate mixture was an *n*-butanol/2-propanol/H_2O (10:5:4 [vol/vol/vol]) mixture (Shiomi, Onodera, & Sakai, 1997). The TLC plate was irrigated by the solvent system two times. After each ascent, the solvent was dried using a hairdryer. To visualize the separated carbohydrates on the plates, a fine spray containing *n*-butanol (80% w/w), phosphoric acid (6.78 mL), urea (3 g) and ethanol (8 ml) in 100 mL was used. The plates were oven heated at 120 °C for 10 min to make the spots visible. The readings were performed at a wavelength of 450 nm on a densitometer (Camag, TLC scanner 4, Muttenz, Switzerland). The oligosaccharides were quantified using the Planar winCATS Chromatography Manager software.

2.3.6. Total antioxidant activity (ABTS and FRAP) and total polyphenols

The total antioxidant activity was measured by the ABTS and FRAP methods. For extraction, the procedure developed by Larrauri, Rupérez, and Saura-Calixto (1997) was used. The samples were extracted sequentially with 4 mL of methanol/water (50:50, v/v) at 25 °C for 1 h, centrifuged at 25,400*g* for 15 min. After, the supernatant was recovered. Then, 4 mL of acetone/water (70:30, v/v) was added to the residue at 25 °C, which was extracted for 1 h, and then centrifuged with the same conditions. Methanolic and acetonic extracts were combined in a 10 mL flask, adding water to complete the flask volume. The $ABTS^{\cdot +}$ assay was based on a method developed by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993). For FRAP assay, the procedure described by Benzie and Strain (1996) was used. Both methods were used with the modifications suggested by Rufino et al. (2010). The results of the ABTS and FRAP assays were expressed as µM Trolox and µM Fe_2SO_4 per g of yacon syrup, respectively.

The total polyphenols were determined by the Folin–Ciocalteu method (Obanda, Owuor, & Taylor, 1997) and the results were expressed as mg GAE (gallic acid equivalent) per g of yacon syrup.

2.3.7. Chlorogenic acid (CGA)

The quantification of CGA was performed as described by Jaiswal, Deshpande, and Kuhnert (2011) with modifications. The analysis was performed in a HPLC-PDA instrument (Varian, Model 920-LC, Walnut

Creek, CA, USA) coupled with a photodiode array detector (PDA). A Shim-pack CLC-ODS (M) column (Shimadzu C_{18} 4.6 × 150 mm, 5 µm) was used at a flow rate of 0.6 mL min^{-1}, kept at 30 °C. The samples were filtered (PVDF 0.45 µm, Tedia, Brazil) and the injection volume was 40 µL. The mobile phase consisted of a combination of solvent A (0.1% formic acid in water) and solvent B (methanol). The gradient varied linearly from 10% to 40% B (v/v) in 22 min, to 70% B at 24 min, and held there for 6 min. The PDA detector was set to 324 nm. Quantification was performed using an external calibration curve of CGA from 10 µg g^{-1} to 200 µg g^{-1} and the results were expressed as µg of CGA per g of yacon syrup.

2.3.8. UPLC-ESI-Q-TOF-MS^E measurements

UPLC-ESI-Q-TOF-MS^E analysis was performed using an Acquity UPLC-QTOF-MS (Xevo™, Waters®, Milford, MA, USA) system with an electrospray ionization (ESI) source. The mobile phases were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient used consisted: (0–15) min, 2–95% B; (15.1–17) min, 100% B; (17.1–19.1) min, 2% B. A Waters Acquity UPLC BEH column (150 × 2.1 mm, 1.7 µm) with flow rate 0.4 mL min^{-1} kept at 40 °C was used. The injection volume was 5 µL. The MS conditions were as follow: negative ionization mode; acquisition range: 110–1180 Da; source temperature: 120 °C; desolvation gas temperature: 350 °C; desolvation gas flow: 500 L h^{-1}; extract cone voltage: 0.5 V; capillary voltage was 2.6 kV; acquisition mode by MS^E. Leucine enkephalin was used as lock mass. The equipment was controlled by Masslynx 4.1 (Waters® Corporation) software. The compounds were putatively identified based on their exact mass and comparison with published data. In this study, the term putative was employed for compound annotation since the MS and/or MS/MS data are not enough for an unequivocal identification (Sumner et al., 2007).

2.3.9. NMR spectroscopy analysis

The samples were prepared by dissolving 10 mg of the yacon syrup into 550 µL of D_2O (99.9%) with 1% of the trimethylsilylpropanoic acid, (TSP, v/m). The final solution was transferred to 5 mm NMR tubes. The NMR experiments were acquired in a spectrometer (Agilent, model DD2 600 MHz, Santa Clara, CA, USA) equipped with a 5 mm (H-F/^{15}N-^{31}P) inverse detection One Probe™, operating at 298 K. The spectra were recorded with 32 free induction decays (FID) into 21 K of data points for 13,157.9 Hz of spectral width, and relaxation delay of 2 s. For the molecular identification of the compounds, homo and heteronuclear 2D experiments were acquired. The spectra were referenced to the TMSP-d4 resonance at 0.0 ppm. The identification of the constituents within the yacon syrup was performed through ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC experiments. The results were compared to the existing data in open access databases and literature reports.

2.3.10. Effects of the different processing steps on phenolic profile, FOS and CGA contents

Samples of the following processing steps to produce yacon syrup were evaluated: (a) pulping, (b) enzymatic maceration, (c) microfiltration and (d) vacuum concentration. The analysis performed were: total FOS analysis (according to item 2.3.4.) and their different degrees of polymerization [GF_6, GF_5, GF_4 (1F-β-fructofuranosylnystose), GF3 (nystose) and GF2 (1-kestose)] (according to item 2.3.5.), chlorogenic acid – CGA (according to item 2.3.7.) and phenolic profile by UPLC-ESI-Q-TOF-MS^E (according to item 2.3.8.). The results of total FOS and their different degrees of polymerization and CGA were subjected to ANOVA analysis at 5% probability by the F test and Tukey's multiple-comparison test ($P < .05$), using XLSTAT software (version 5.01, New York, NY, USA).

Table 1
Yacon syrup characterization.

Characteristics	Contents
FOS and simple sugars (%)	
FOS	21.84 ± 1.31
Glucose	9.71 ± 1.00
Fructose	16.32 ± 0.21
Maltose	1.06 ± 0.00
Sucrose	11.99 ± 0.39
Total polyphenols, chlorogenic acid and antioxidant activity	
Total polyphenols (µg GAE g^{-1})	1,202.25 ± 30.02
Chlorogenic acid (µg g^{-1})	175.13 ± 5.38
ABTS (µM trolox g-^{1})	6.99 ± 0.09
FRAP (µM F_2SO_4 g^{-1})	16.19 ± 0.66
Proximate composition (%)	
Moisture	31.46 ± 0.13
Ash	2.11 ± 0.10
Protein	1.61 ± 0.05
Lipids	0.07 ± 0.01
Carbohydrates	64.90 ± 0.25
Mineral composition (mg 100 g^{-1})	
Phosphorus	162.00 ± 2.65
Potassium	691.00 ± 33.96
Calcium	40.67 ± 3.79
Magnesium	45.67 ± 10.12
Sulfur	42.00 ± 2.65
Sodium	17.00 ± 1.73
Copper	0.80 ± 0.00
Iron	1.43 ± 0.12
Zinc	0.20 ± 0.00
Manganese	0.10 ± 0.00
General characteristics	
Water activity	0.78 ± 0.00
pH	3.71 ± 0.02
Soluble solids (°Brix)	71.03 ± 0.06
Turbidity (NTU)	75.37 ± 0.04
L^*	47.50 ± 0.38
a^*	1.43 ± 0.03
b^*	21.81 ± 0.23

All values are expressed as mean ± standard deviation.

3. Results and discussion

3.1. Yacon syrup characterization

The characterization of the yacon syrup is presented in Table 1. The syrup contains a low concentration of protein (1.61%), lipids (0.07%) and ash (2.11%), and high values of carbohydrates (64.90%). Considering the carbohydrate composition, yacon syrup presented 21.84% of FOS, 16.32 and 11.99% were fructose and sucrose, respectively, whereas glucose and maltose contents were less than 10%.

The yacon syrup obtained by Geyer et al. (2008) was composed of 2.3%, 0.4% and 67.0% for protein, lipids, and carbohydrates, respectively. Moreover, Genta et al. (2009) obtained a yacon syrup with 2.16% proteins, 0.14% lipids and 67.04% carbohydrates, and Manrique, Párraga, and Hermann (2005), using two cultivars of yacon, produced syrups with the following characteristics: 0.1 and 0.0% of lipids, 1.3 and 1.0% of protein, 64.0 and 78.1%. All these authors produced yacon syrup using concentration temperatures up to 120 °C.

Although the high content of carbohydrates and low concentration of protein and lipids were general characteristics of all yacon syrups reported in the literature, the results of simple sugars and FOS varies greatly. Genta et al. (2009) obtained a yacon syrup with 25.65% of simple sugars and 41.39% of FOS, almost twice the content we obtained. The syrups produced by Manrique, Párraga, and Hermann (2005), using different cultivars, presented FOS content from 10.9 to 47.6%. The variation of FOS occurred due to the cultivars used, physiological factors (e.g., plant age), harvest and post-harvest conditions

(e.g., storage duration use of the traditional sun exposition treatment), and others.

The concentrations of the different minerals analyzed in the yacon syrup are presented in Table 1. The yacon syrup showed 1.43 mg 100 g^{-1} of iron, 0.8 mg 100 g^{-1} of copper, 691.00 mg 100 g^{-1} of potassium, 0.20 mg 100 g^{-1} of zinc and 162.00 mg 100 g^{-1} for phosphorous. All the other minerals were found at a concentration below 1.5 mg 100 g^{-1}. The syrup also presents 0.78 of water activity, pH of 3.71 and 71 °Brix. Those conditions are desirable for preservation by avoiding the development of pathogens and others microorganisms, which could impact the product shelf-life.

Genta et al. (2009) produced a yacon with 73 °Brix and pH of 5.4. The difference in the acidity of the product is due to the acidification that occurs in the first step of the yacon syrup preparation (Dionísio et al., 2013). This process is important to inactivate the polyphenol oxidases and, as a consequence, to preserve the phenolic compounds, bioactive molecules that contribute to the functional properties of yacon syrup. Through UPLC-ESI-Q-TOF-MS^E it was observed that the pulping process without acid inactivation, resulted in a partial degradation of the phenolic compounds. Specifically for CGA, the degradation occurred totally (see Supplementary Material).

It is well known that the phenolic content plays an important role in the antioxidant capacity of foods. To perform this evaluation, two methods were employed: ABTS and FRAP (Table 1). For yacon in natura, Campos et al. (2012) evaluated 35 accessions, and the values for ABTS ranged from 3.2 to 20.1 µM TE g^{-1} fresh weight (FW). The authors concluded that yacon exhibit an important antioxidant activity due its phenolic compounds, such as CGA. Recently, Pereira et al. (2016) measured the total antioxidant activity of yacon in natura by the DPPH and ABTS assays. The authors correlated its antioxidant activity with the total phenolic content and tannins, and suggest that yacon can be used as an alternative food source of phenolic compounds that help prevent degenerative processes caused by oxidative stress.

3.2. UPLC-ESI-Q-TOF-MS^E: Phenolic profile and other compounds

The total ion current chromatogram of yacon is shown in Fig. 1. For the determination of the compounds, the retention time, mass fragmentation, accurate mass (chemical formula), and comparison with literature data resulted in 25 compounds putatively identified (Table 2). The main compounds identified were the caffeic acid derivatives, tryptophan, and CGA, in accord with the previous studies (Takenaka et al., 2003), besides organic acids, esters, and other polar compounds.

The mass spectra of compound **1** exhibited a deprotonated molecular ion $[M-H]^-$ at *m/z* 191.0197 ($C_6H_7O_7^-$) and a product ion at *m/z* 111.0078 $[M\text{-}H\text{-}CO_2\text{-}2H_2O]^-$, putatively identified as citric acid (Baskaran, Pullencheri, & Somasundaram, 2016). This organic acid was expected since it was used in the first step of yacon processing, as discussed previously.

Compounds **2** and **6** gave the same deprotonated molecule $[M-H]^-$ at *m/z* 397.0785, 397.0764 ($C_{17}H_{17}O_{11}^-$) with product ion at *m/z* 235.0 $[M\text{-}C_9H_7O_3$ (caffeoyl moiety)$]^-$. These compounds were analogous to previously published octulosonic acid derivatives isolated in *Smallanthus sonchifolius* (Takenaka & Ono, 2003). Thus, **2** and **6** were putatively identified as 4-*O*-caffeoyl-2,7-anhydro-D-glycero-*β*-D-galacto-oct-2-ulopyranosonic acid isomers.

Compound **15** showed a deprotonated molecule $[M-H]^-$ at *m/z* 559.1083 ($C_{26}H_{23}O_{14}^-$) and produced fragment ions at *m/z* 397.0 $[M\text{-}C_9H_7O_3]^-$ and *m/z* 235.0 $[M\text{-}C_{18}H_{13}O_6]^-$, corresponding to an additional caffeoyl group in the structure of compound **2**. Thus, **15** was putatively identified as 4,5-di-*O*-caffeoyl-2,7-anhydro-D-glycero-*β*-D-galacto-oct-2-ulopyranosonic acid, previously isolated in *Smallanthus sonchifolius* (Takenaka & Ono, 2003). Octulosonic acid derivatives, a rare class of natural products, are important regulators of lipid and carbohydrate metabolism and inflammatory signaling. They can regulate the inflammation process by mediating multiple targets involved in various biochemical and metabolic pathways (Zhao et al., 2014).

Compounds **3**, **8**, **9** and **19**, **21**, **24** gave the same $[M-H]^-$ ions at *m/z* 353.0872, 353.0873, 353.0874 ($C_{16}H_{17}O_9^-$) and *m/z* 515.1198, 515.1185, 515.1182 ($C_{25}H_{23}O_{12}^-$), respectively. The product ions at

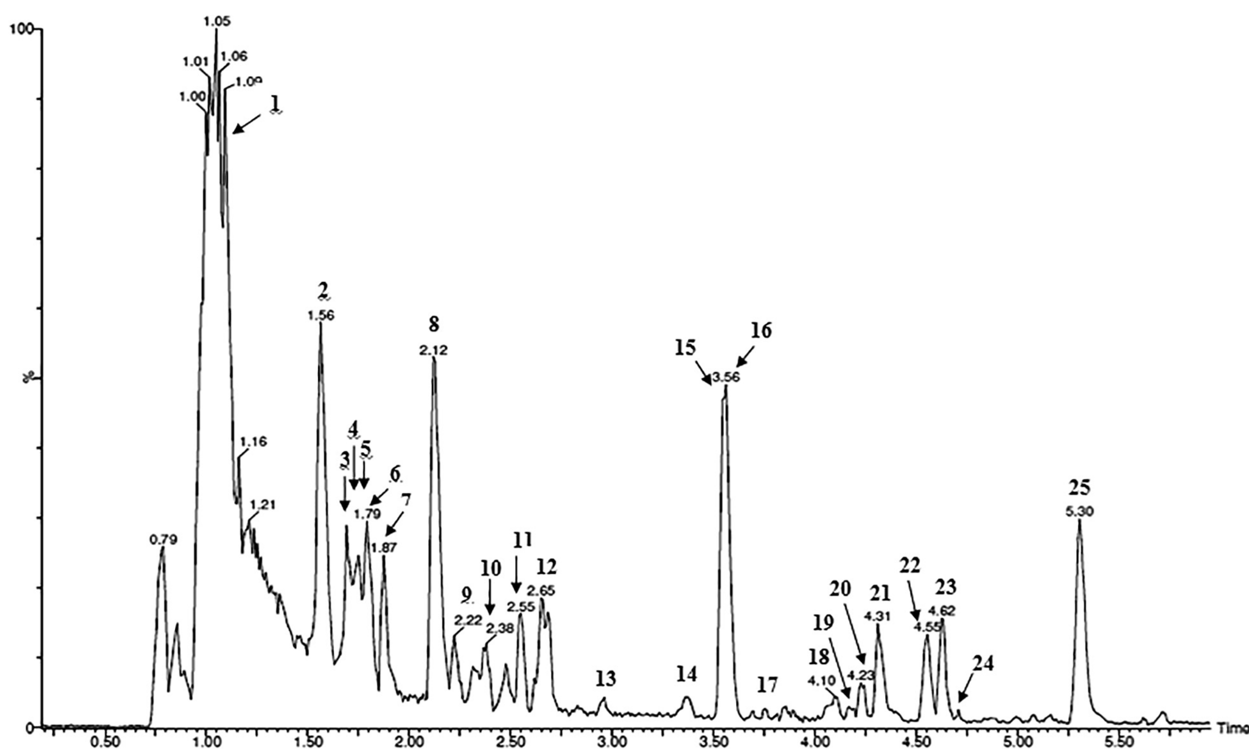

Fig. 1. Total ion current chromatogram of yacon syrup in negative electrospray ionization mode.

Table 2
Constituents tentatively identified in the yacon roots syrup using ESI negative mode.

Peak No.	RT (min)	Compounds	Formula	$[M-H]^-$ *m/z* (exp)	$[M-H]^-$ *m/z* (cal)	Error (ppm)	Fragments ions (intensity) *m/z* (%)	References
1	1.02	Citric acid	$[C_6H_7O_7]^-$	191.0197	191.0192	2.6	111.0078 (100)	Baskaran, Pullencheri, and Somasundaram (2016)
2	1.56	4-*O*-caffeoyl-2,7-anhydro-D-glycero-*β*-D-galacto-oct-2-ulopyranosonic acid isomer 1	$[C_{17}H_{17}O_{11}]^-$	397.0785	397.0771	0.3	235.0433 (40)	Takenaka and Ono (2003)
3	1.67	3-caffeoylquinic acid (Neochlorogenic acid)	$[C_{16}H_{17}O_9]^-$	353.0872	353.0873	0.1	191.0202 (100), 179.0427 (60), 135.0452 (42)	Clifford, Knight, and Kuhnert (2005)
4	1.70	Caffeoyl glucarate isomer 1	$[C_{15}H_{15}O_{11}]^-$	371.0606	371.0614	2.2	353.0725 (80), 209.0272 (100), 191.0193 (80)	Bazylko, Boruc, Borzym, and Kiss (2015);
5	1.74	Caffeoyl glucarate isomer 2	$[C_{15}H_{15}O_{11}]^-$	371.0613	371.0614	0.3	353.0643 (8), 209.0260 (95), 191.0155 (100)	Bazylko, Boruc, Borzym, and Kiss (2015);
6	1.79	4-*O*-caffeoyl-2,7-anhydro-D-glycero-*β*-D-galacto-oct-2-ulopyranosonic acid isomer 2	$[C_{17}H_{17}O_{11}]^-$	397.0764	397.0771	1.8	235.0498 (40)	Takenaka and Ono (2003)
7	1.88	Tryptophan	$[C_{11}H_{11}N_2O_2]^-$	203.0823	203.0821	1.0	116.0504 (22), 74.0230 (22)	Gómez-Romero, Segura-Carretero, and Fernández-Gutiérrez (2010)
8	2.12	Chlorogenic acid	$[C_{16}H_{17}O_9]^-$	353.0873	353.0873	0.0	191.0496 (100)	Clifford, Knight, and Kuhnert (2005)
9	2.22	4-caffeoylquinic acid (Cryptochlorogenic acid)	$[C_{16}H_{17}O_9]^-$	353.0874	353.0873	0.3	191.0184 (100), 179.0410 (100), 173.0455 (60), 135.0451 (40)	Clifford, Knight, and Kuhnert (2005)
10	2.38	Isopropyl malic acid	$[C_7H_{11}O_5]^-$	175.0608	175.0606	1.1	157.0495 (5), 131.0243 (10), 115.0380 (70), 113.0245 (35)	Gómez-Romero, Segura-Carretero, and Fernández-Gutiérrez (2010);
11	2.54	Not identified	$[C_{13}H_{15}O_8]^-$	299.0763	299.0767	1,3	191.0185 (40), 152.0092 (41), 108.0193 (70)	
12	2.65	Not identified	$[C_{18}H_{27}O_9]^-$	387.1664	387.1655	2,3	191.0166 (55)	
13	2.96	Dicaffeoylaltraric Acid Isomer 1	$[C_{24}H_{21}O_{14}]^-$	533.0929	533.0931	0,1	371.0579 (50), 209.0270 (100)	Takenaka et al. (2003)
14	3.30	Dicaffeoylaltraric Acid Isomer 2	$[C_{24}H_{21}O_{14}]^-$	533.0930	533.0931	0.2	371.0568 (30), 209.0269 (100)	Takenaka et al. (2003)
15	3.56	4,5-di-*O*-caffeoyl-2,7-anhydro-D-glycero-*O*-*β*-galacto-oct-2-ulopyranosonic acid	$[C_{26}H_{23}O_{14}]^-$	559.1083	559.1088	0.9	397.0822 (22), 235.0446 (40)	Takenaka and Ono (2003)
16	3.56	Dicaffeoylaltraric acid isomer 3	$[C_{24}H_{21}O_{14}]^-$	533.0922	533.0931	1.7	371.00573 (48), 209.0273 (100)	Takenaka et al. (2003)
17	3.86	Dicaffeoylaltraric acid isomer 4	$[C_{24}H_{21}O_{14}]^-$	533.0937	533.0931	1.1	371.0594 (22), 209.0292 (100)	Takenaka et al. (2003)
18	4.10	Dicaffeoylaltraric acid isomer 5	$[C_{24}H_{21}O_{14}]^-$	533.0923	533.0931	1.5	371.0607 (31), 209.0273 (100)	Takenaka et al. (2003)
19	4.17	3,4-dicaffeoylquinic acid	$[C_{25}H_{23}O_{12}]^-$	515.1198	515.1190	1.6	353.0861 (30), 191.0229 (62), 179.0469 (63), 173.0390 (59)	Clifford, Knight, and Kuhnert (2005)
20	4.23	Not identified	$[C_{26}H_{35}O_{11}]^-$	523.2166	523.2179	2,5	361.1678 (62), 191.0166 (22)	
21	4.31	3,5-dicaffeoylquinic acid	$[C_{25}H_{23}O_{12}]^-$	515.1185	515.1190	1,0	353.0854 (100), 191.0518 (100), 179.0325 (60), 135.0407 (15)	Clifford, Knight, and Kuhnert (2005)
22	4.56	Butanedioldiacetate	$[C_8H_{13}O_4]^-$	173.0809	173.0814	2.9	131.0 685 (100)	Rodríguez-Pérez, Quirantes-Piné, Fernández-Gutiérrez, and Segura-Carretero (2013)
23	4.62	Leeaoside	$[C_{24}H_{39}O_{11}]^-$	503.2470	503.2492	4.4	371.2050 (30), 209.1558 (2)	Zhang, Lv, Li, Jiang, and Lee (2015)
24	4.66	4,5-dicaffeoylquinic acid/3,4-dicaffeoylquinic acid	$[C_{25}H_{23}O_{12}]^-$	515.1182	515.1190	1.6	353.0812 (80), 191.0353 (50), 179.0385 (66), 173.0434 (93)	Clifford, Knight, and Kuhnert (2005)
25	5.32	2,3,5- or 2,4,5-tricaffeoylaltraric acid	$[C_{33}H_{27}O_{17}]^-$	695.1252	695.1248	0.6	533.0893 (100), 371.0563 (81), 209.0290 (22)	Takenaka et al. (2003)

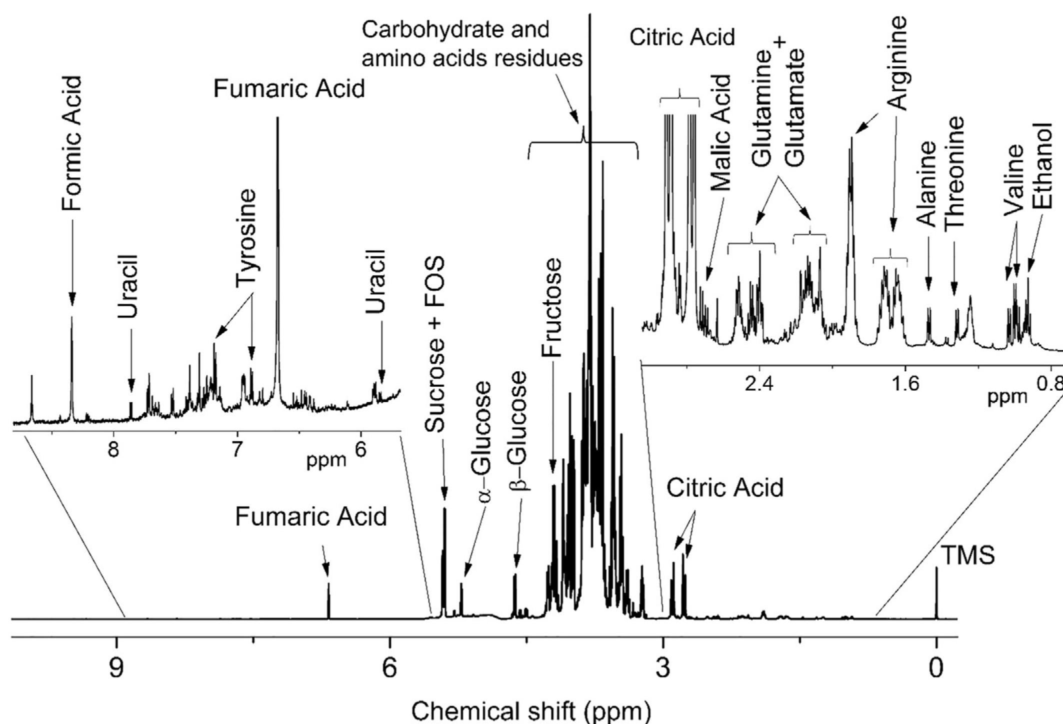

Fig. 2. ^{1}H NMR spectrum of yacon syrup showing signals of the identified compounds.

m/z 191.0, 179.0, 173, and 135.0, are characteristics of quinic or caffeic acid derivatives according to their characteristic fragmentation pattern (Clifford, Knight, & Kuhnert, 2005). Based on the approach developed by Clifford et al., 2005, where the relative abundance of the ion fragments in MS^2 was evaluated, compounds **3**, **9**, **19**, **21**, **24** were putatively identified as 3-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic or 3,4-dicaffeoylquinic acid, respectively. The compound **8** was identified as chlorogenic acid (5-caffeoylquinic acid, CGA) by comparing the retention time and MS^2 spectral data with an authentic standard. CGA is the most abundant hydroxycinnamic acid present in yacon (Russo et al., 2015). CGA has a strong antioxidant activity (Benzie & Choi, 2014) and also has a hypoglycemic effect, due to the modulation of the plasma insulin concentration and inhibition of hepatic gluconeogenesis (Genta et al., 2009).

Compounds **4** and **5** yielded the same deprotonated molecule $[M-H]^-$ at *m/z* 371.0606 and 371.0613 ($C_{15}H_{15}O_{11}^-$) with fragmentation ions at *m/z* 353.0 $[M\text{-}H_2O]^-$, 209.0 $[M\text{-}H_2O\text{-}C_9H_7O_3]^-$, 191.0 $[M\text{-}C_9H_7O_3\text{-}2H_2O]^-$. The product ion at *m/z* 209.0 reveals the presence of glucaric acid in agreement with those described in the literature (Bazylko, Boruc, Borzym, & Kiss, 2015). Therefore, **4** and **5** were putatively assigned as caffeoylglucaric acid isomers. Glucaric acid has been investigated for a wide variety of therapeutic and commercial uses, including cholesterol reduction, diabetes treatment and cancer therapy (Shiue & Prather, 2014).

Compound **7** was characterized as L-tryptophan based on the deprotonated molecule $[M-H]^-$ at *m/z* 203.0823 ($C_{11}H_{11}N_2O_2^-$) and the product ion at *m/z* 116.0 $[M\text{-}C_3H_6NO_2]^-$ of indole nucleus ion (Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2010). Tryptophan is an essential amino acid that should be provided in the diet and has an important role in intestinal microbiota and brain regulation. Also, tryptophan has the function of normalizing tolerance to hydrocarbons, increasing the level of insulin and as a consequence, decreasing hyperglycemia, also exerting positive effects on carbohydrate metabolism in hepatocytes due to increased activity of glycokinase, hexokinase and glucose-6-phosphate dehydrogenase (Khokhla et al., 2016).

Compound **10** had a molecular ion $[M-H]^-$ at *m/z* 175.0608 ($C_7H_{12}O_5^-$) and fragment ions at *m/z* 115.0 $[M\text{-}H\text{-}2CH_3]^-$, which proved to be isopropylmalic acid (Gómez-Romero et al., 2010). This compound was previously identified in members of the *Asteraceae* family.

Compound **13**, **14**, **16–18** showed an $[M-H]^-$ ion at *m/z* 533.0929, 533.0930, 533.0922, 533.0937, 533.0923 ($C_{24}H_{21}O_{14}^-$) with fragmentation ions of successive losses of two caffeoyl groups at 371.0 $[M\text{-}C_9H_7O_3]^-$ and 209.0 $[M\text{-}C_{18}H_{13}O_6]^-$. According to the fragmentation pattern, the compounds were putatively identified as dicaffeoylaltraric acid isomers in agreement to the literature (Takenaka et al., 2003). Compound **25** yielded the deprotonated molecule $[M-H]^-$ at *m/z* 695.1252 ($C_{33}H_{27}O_{17}^-$) and fragmentation ions at *m/z* 533.0 $[M\text{-}C_9H_7O_3]^-$, 371.0 $[M\text{-}C_{18}H_{13}O_6]^-$ and 209.0 $[M\text{-}C_{27}H_{19}O_9]^-$ corresponding to one caffeoyl group more than compound **13**. Thus, the compound **25** was putatively identified as 2,3,5- or 2,4,5-tricaffeoylaltraric acid (Takenaka et al., 2003). All these compounds have antioxidant activity and 2,3,5- or 2,4,5-tricafeoylaltrearic acid seems to be one of the most active compounds to inhibit the production of reactive oxygen species (Dudek, Dudkowski, Bazylko, Kaźmierski, & Kiss, 2016).

Compound **22** exhibited an $[M-H]^-$ ion at *m/z* 173.0809 ($C_8H_{13}O_4^-$) with an MS^2 predominant fragment ion at *m/z* 131.0 $[M\text{-}H\text{-}C_2H_3O]^-$, relative to the elimination of an acetyl group. A similar compound and its fragmentation was previously identified in *Cucumis melo* (Rodríguez-Pérez, Quirantes-Piné, Fernández-Gutiérrez, & Segura-Carretero, 2013). Therefore, compound **22** was putatively identified as butanedioldiacetate.

Compound **23** yielded a deprotonated molecule $[M-H]^-$ at *m/z* 503.2470 ($C_{24}H_{39}O_{11}^-$), fragment ions at *m/z* 371.2 $[M\text{-}H\text{-}C_5H_8O_4]^-$ and *m/z* 209.1 $[M\text{-}H\text{-}C_5H_8O_4\text{-}C_6H_{10}O_5]^-$ corresponding to the loss of pentoside and the successive losses of pentoside and glucoside, respectively. The observed molecular ion $[M\text{-}H]^-$ and fragmentation patterns were similar to the reported in *Ficus pandurata* aerial roots and *Leea thorelii* leaves (Zhang, Lv, Li, Jiang, & Lee, 2015). Thus, compound **23** was putatively identified as Leeaoside or its isomer.

The compounds **11**, **12** and **20**, with *m/z* 299.0763, 387.1664 and

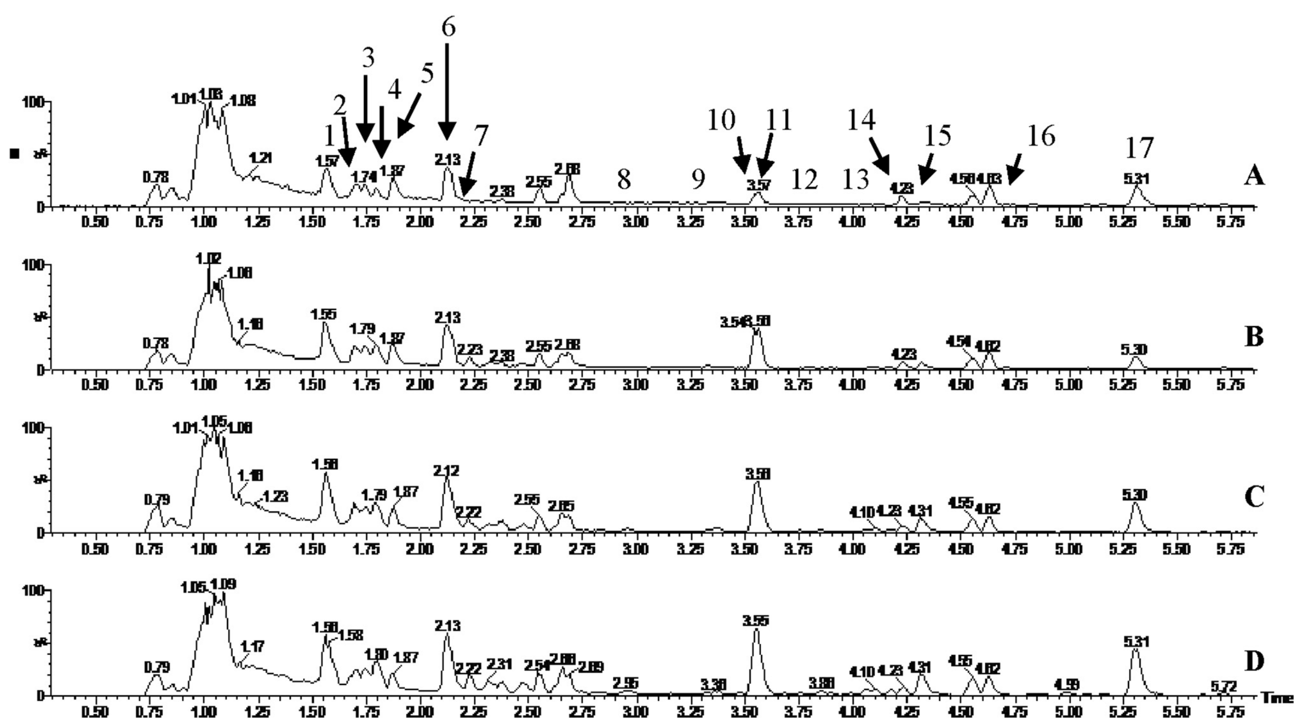

Fig. 3. Total ion current chromatogram in the different stages of yacon syrup production in negative electrospray ionization mode. pulping – (A); enzymatic maceration – (B); microfiltration – (C); vacuum concentration or syrup – (D); 1: 4-*O*-caffeoyl-2,7-anhydro-D-glycero-*β*-D-galacto-oct-2-ulopyranosonic acid isomer 1; 2: 3-caffeoylquinic acid (Neochlorogenic acid); 3:caffeoyl glucarate isomer 1; 4: Caffeoyl glucarate isomer 2; 5: 4-*O*-caffeoyl-2,7-anhydro-D-glycero-*β*-D-galacto-oct-2-ulopyranosonic acid isomer 2; 6: Chlorogenic acid; 7: 4-Caffeoylquinic acid (Cryptochlorogenic acid); 8: Dicaffeoylaltraric acid iomer 1; 9: Dicaffeoylaltraric acid isomer 2; 10: 4,5-di-*O*-caffeoyl-2,7-anhydro-D-glycero-*O*-*β*-galacto-oct-2-ulopyranosonic acid; 11: Dicaffeoylaltraric acid iomer 3; 12: Dicaffeoylaltraric acid iomer 4; 13: Dicaffeoylaltraric acid iomer 5; 14: 3,4-dicaffeoylquinic acid; 15: 3,5-dicaffeoylquinic acid; 16: 4,5-dicaffeoylquinic acid/3,4-dicaffeoylquinic acid; 17: 2,3,5- or 2,4,5-tricaffeoylaltraric acid.

Table 3
Chlorogenic acid (CGA) and fructooligosaccharides (FOS) contents in the different stages of yacon syrup production.

Yacon	CGA ($\mu g\ g^{-1}$)	FOS (%)	GF_6 (%)	GF_5 (%)	GF_4 (%)	GF_3 (%)	GF_2 (%)
Pulping	25.47 ± 0.00^b	3.34 ± 0.78^b	0.46 ± 0.11^b	0.36 ± 0.08^c	0.65 ± 0.15^b	1.08 ± 0.26^b	0.78 ± 0.18^b
Enzymatic maceration	25.60 ± 0.17^b	4.73 ± 0.29^b	0.61 ± 0.04^b	0.86 ± 0.05^b	0.98 ± 0.06^b	1.13 ± 0.07^b	1.14 ± 0.07^b
Microfiltration	23.92 ± 2.03^b	4.34 ± 0.42^b	0.58 ± 0.06^b	0.40 ± 0.04^c	0.71 ± 0.07^b	1.45 ± 0.14^b	1.20 ± 0.12^b
Syrup	175.13 ± 5.38^a	21.84 ± 1.31^a	3.47 ± 0.21^a	2.21 ± 0.14^a	3.04 ± 0.19^a	5.33 ± 0.33^a	7.80 ± 0.48^a

Values are given as the means ± standard deviation from triplicate determinations. Means in the same column followed with different superscript letters differ significantly ($p < .05$).

523.2166, respectively, were non-identified.

3.2.1. ^{1}H NMR: non-target analysis of metabolites

NMR is a spectroscopy technique that allows the unequivocal characterization of chemical structures in mixtures without extensive separation protocols or the use of analytical standards. Therefore, the ^{1}H NMR spectrum of the yacon syrup (Fig. 2) shows low molecular primary metabolites such as amino acids, sugars, and organic acids.

In the aliphatic region, were observed characteristic signals of metabolites such as valine (doublet at 1.00 and 1.03 ppm for both $-CH_3$); ethanol (triplet at 0.93 ppm for $-CH_3$); threonine (doublet at 1.32 ppm for $-CH_3$); alanine (doublet at 1.47 ppm for $-CH_3$); glutamine and glutamate (multiplets from 1.59 to 1.78 ppm for $-CH_2-$; multiplet from 1.84 to 1.95 ppm); malic acid (doublet at 2.70 and 2.72 of $-CH_2-$); and citric acid (doublet at 2.77 and 2.79 of $-CH_2-$). At the sugar region was observed the characteristic signal of malic acid (multiplet at 4.42 ppm for $-CH-$); fructose (overlapped multiplet at 4.2 ppm for $-CH-$); beta glucose (doublet at 4.65 ppm for $-CH-$); alpha glucose (doublet at 5.22 ppm for $-CH-$); sucrose (doublet at 5.42 ppm for $-CH-$) and the characteristic signal from FOS (broad singlet from 5.41 to 5.44 for $-CH-$). In the aromatic region, it was observed the uracil signals (doublets at 5.85 and 7.86 ppm); fumaric acid signals (singlet at 6.68 ppm); tyrosine (doublets at 6.89 and 7.18 ppm); and formic acid (singlet at 83.4 ppm).

In addition to FOS and other sugars, ^{1}H NMR spectrum of the yacon syrup shows essential amino acids such as threonine and valine, which presents important roles in human metabolism. Threonine has several functions, among them the synthesis of the mucin, that is necessary to maintain the integrity and intestinal function, immune function, phosphorylation and glycosylation of proteins and glycine synthesis. On the other hand, valine participates, in different biochemical reactions in the brain, such as protein synthesis, energy production and neurotransmitter serotonin synthesis (Brestenský, Nitrayová, Patras, Heger, & Nitray, 2015).

3.3. Effects of the different processing steps on phenolic profile, FOS and CGA contents

Total ion current chromatograms of samples obtained after the unit operations for yacon syrup production [namely, pulping (a); enzymatic maceration (b); microfiltration (c) and vacuum concentration or syrup (d)] are given in Fig. 3. The peaks were numbered according to their elution order previously shown in Table 2. These results show an increase in the intensity of the peaks after each processing step, showing that these components probably become more available due to the processing (see the peaks correspondent to the compounds 10, 11 and

17, for instance).

As discussed previously, FOS is one of the most important bioactive compounds in yacon syrup. Due to its prebiotic activity, FOS present positive biological effects (Silva et al., 2017; Dionísio et al., 2015). The prebiotic effectiveness of inulin-type fructans not only depends on the dietary dosage, but also on the carbohydrate degree of polymerization (DP), which can be affected by the processing conditions, such as temperature or pH (Matusek, Merész, Le, & Örsi, 2009). L'Homme, Puigserver, and Biagini (2003) showed that the FOS hydrolysis decreased at increasing pH values, and increased with temperature. The authors report a quick and complete degradation at 120 °C under acidic conditions. Low values of pH might affect the FOS stability due to FOS linkage resistance to acid conditions, resulting in the carbohydrate hydrolysis and the DP decrease. Thus, the FOS quantification along with its DP is an important information.

The results of FOS (total), GF_6, GF_5, GF_4 (1F-β-fructofuranosylnystose), GF_3 (nystose), GF_2 (1-kestose) and chlorogenic acid – CGA content are shown in Table 3. In general, the results showed no statistical differences ($P > .05$) among pulping (a), enzymatic maceration at 45° C (b) and microfiltration (c). The production steps used herein did not negatively affect the FOS content (including the DP). The final production step was the vacuum concentration (60 °C) to obtain the syrup (d). Thus, all functional compounds increased significantly ($P < .05$). Previous studies reported that at neutral pH, the FOS composition and structure were preserved in temperatures up to 170 °C (Forgo, Kiss, Korózs, & Rapi, 2013). Moreover, under acid conditions, the FOS hydrolysis was easier than at neutral or basic pH values, ant the FOS degradation occurred at 120 °C (L'Homme, Puigserver, & Biagini, 2003), which is much higher than that used in the present study.

As discussed previously, phenolic compounds of yacon, especially the CGA, are an important class of compounds responsible for its biological properties. Fortunately, the process used to obtain yacon syrup (steps a, b and c) did not affect CGA concentration (Table 3). In fact, Genta et al. (2009), Geyer et al. (2008), and Manrique, Párraga, and Hermann (2005) produced yacon syrup using a high temperature (up to 120 °C). However, these authors did not report the phenolics composition and antioxidant activity of the product obtained.

The use of microfiltration and mild conditions of temperature to obtain yacon syrup may be responsible for preserving the phenolic compounds and, specifically CGA. When high temperatures were used, the thermal processing may cause complex physical and chemical reactions affecting the phenolic composition, which include the release of phenolic compounds from their bonded forms, degradation of polyphenols or its transformation (Chen, Yu, & Rupasinghe, 2013; Rodríguez-Roque et al., 2015), resulting in a modification of their functional properties. For acidic matrices and low pH conditions, hydroxylation, esterification, and depolymerization are favorable (Tembo, Holmes, & Marshall, 2017). However, as discussed previously, no modification on the composition and quantification of phenolic compounds were observed.

4. Conclusion

In the present work, the assessment of nutritional and functional compounds was obtained for yacon syrup. The product presents high levels of FOS, and phenolic compounds, such as CGA. The product also presents mineral elements, such as K, Ca and P, and essential amino acids detected by RMN, such as tryptophan, valine, and threonine. Moreover, UPLC-ESI-Q-TOF-MS^E was confirmed as being an efficient analytical technique for the separation and detection of phenolic compounds in yacon syrup, where 25 phenolic compounds were putatively identified. In addition, no other work evaluated the FOS and CGA in all different processing steps. The results indicate that the technological process preserves its compounds, and shows that the yacon syrup proposed in this route is an innovative and promising product in the area of functional foods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.11.092.

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