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Nutritional and functional characterisation of Andean chicuru (*Stangea rhizanta*)

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ABSTRACT

A thorough functional and nutritional characterisation of Andean chicuru (*Stangean rhizanta*) root demonstrated its potential as an alternative source of fructooligosacharides (FOS), phenolic compounds, natural antioxidants and minerals. FOS of the GFn type with a degree of polymerisation (DP) of 3 (1-kestose) and 4 (nystose) were present. Important phenolic content and antioxidant activity values were obtained. The main phenolic compounds revealed by HPLC-DAD were caffeic and chlorogenic acids and their respective derivatives as well as flavan-3-ol derivatives. The nutritional analysis revealed high calcium and iron values, as well as considerable amounts of soluble and insoluble dietary fibre. The presence of low DP FOS, together with the high calcium and iron contents in chicuru, might favour its use for industrial applications.

1. Introduction

The Andean region is characterised by its enormous genetic diversity. The existing crops, even though traditionally and successfully used by the native population due to their healing and nutritional properties, unfortunately have been poorly studied. Variability, not only in colours, forms but in primary nutrient constituents and bioactive compounds (Campos et al., 2006) has recently been reported. The health-related properties of Andean crops claimed by folklore use could be partially attributed to the presence of bioactive compounds (Campos et al., 2006). Within this framework, the sub-exploited Andean root chicuru (*Stangean rhizanta*), cultivated since the Incas times and attributed with different therapeutic properties is at high risk of extinction. Chicuru (*Stangea rhizanta*) belongs to the Valerianaceae family, its evolutionary centre being the South American region at altitudes higher than 4000 m.a.s.l. This crop is an herbaceous perennial plant, with annual aerial organs about two centimetres from the ground (Weberbauer, 1945). Chicuru's main root is white, fusiform, spheroidal and 5–12 cm long. Chicuru is consumed by the poor Andean communities either raw or dehydrated (Antunez de Mayolo, 1991). There are no investigations about the medicinal applications of chicuru; however, folklore recounts healing properties against anxiety, insomnia and nervous diseases, as well as beneficial effects for kidney treatment, asthma, osteoporosis, obesity and stomach diseases.

A preliminary study conducted by our research group revealed the presence of a non-starch reserve carbohydrate, leading us to suspect that this crop could be an alternative source of fructans, besides other bioactive compounds.

There is growing interest in natural sources of nutrients and health-promoting compounds. Within these compounds, fructans and phenolics have aroused attention. Fructans are carbohydrate polymers composed of a sucrose molecule that is elongated by a chain of fructosyl units connected through β-(2→1) or β-(2→6) linkages. Depending on the linkage type, they receive different names, such as inulin and levan, respectively (Delzenne & Roberfroid, 1994). Fructooligosaccharides (FOS) are inulin-type fructans with a degree of polymerisation (DP) lower than 9 (average DP = 4.8). Short-chain fructooligosaccharides (sc-FOS), with a DP ranging from 1 to 5 (Bornet, Brouns, Tashiro, & Duvillier, 2002), and inulin, with a DP ranging from 10 to 60, fit within this FOS category (Biedrzycka & Bielecka, 2004).

FOS are considered as prebiotics because they improve the intestinal microflora balance and promote the growth of beneficial organisms (Delzenne & Roberfroid, 1994; Pedreschi, Campos, Noratto, Chirinos, & Cisneros-Zevallos, 2003). However, the prebiotic effect and functional activity of fructans is dose-dependent and chain length-related (Roberfroid, Van Loo, & Gibson, 1998). For instance, sc-FOS have a higher ability than have long chain

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fructans to specifically stimulate bifidobacterial growth (Biedrzycka & Bielecka, 2004). In addition, sc-FOS stimulate the absorption of minerals, such as Ca, Mg and Fe, in rats (Ohta et al., 1995) and have a stronger effect than has inulin in promoting recovery from post-gastrectomy anaemia in rats (Sakai, Ohta, Takasaki, & Tokunaga, 2000). FOS are involved in the decrease of blood glucose levels, health-related serum lipids in humans and animal models (Fiordaliso et al., 1995) and in the enhancement of mineral absorption, counteracting the deleterious effects of phytic acid on mineral homeostasis (Lopez et al., 2000). Sc-FOS are present in onions, Jerusalem artichokes, asparagus, garlic (Bornet et al., 2002) and Andean yacon root (Pedreschi et al., 2003). Sc-FOS can also be commercially produced from sucrose, using a food grade fungal fructosyltransferase (ACTILIGHT®), or from inulin, by partial hydrolysis, using endo-inulinase (Orafti) (Bornet et al., 2002).

The growing interest in the role of phenolic compounds from natural sources in human health has prompted research in the fields of horticulture and food science. Phenolic compounds constitute one of the main classes of secondary metabolites in plants with a large range of structures and functions. Thus, they are classified into different groups as a function of the number of phenol rings that they contain and based on the structural elements that bind these rings to one another. Distinctions are made among the phenolic acids (hydroxybenzoic and hydroxycinnamic acids), stilbenes, lignins (nonflavonoids) and flavonoids (anthocyanins, flavan-3-ols, flavones, flavanones and flavonols). Plant phenolics have been reported as potential protective and preventive molecules against chronic diseases, such as atherosclerosis and cardiovascular diseases, anti-allergic, anti-thrombotic, anti-inflammatory and anti-mutagenic processes, cancer, osteoporosis and vasodilatory activities (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004).

Chicuru is an under estimated crop, as previously mentioned, with healing properties recognised by the indigenous communities of the Andes. We believe that scientific evidence would arouse interest in this crop. Thus, to our knowledge, this is the first study focused, not only on the nature and/or concentration of FOS and phenolic compounds from chicuru root, but also on a proximal and mineral composition. The objectives of this study were: (1) to provide a complete proximal composition of the crop, (2) to characterise and quantify the fructans and sugars present and (3) to quantify and characterise the main phenolic compounds present in chicuru.

2. Materials and methods

2.1. Sample material

Chicuru roots were grown in the rural community of Tinca (Ayacucho, Peru) at approximately 4000 m.a.s.l. Subsequently, the roots were harvested, washed, dried and immediately packed in paper bags and sent to Universidad Nacional Agraria La Molina (Lima, Peru) to be stored at −20 °C prior to being analysed. Yacon (*Smallanthus sonchifolius* Poepp. Endl) syrup was obtained from the Instituto de Biotecnología, Universidad Nacional Agraria – La Molina (Peru).

2.2. Reagents and standards

Glucose and fructose were purchased from Merck (Darmstadt, Germany). Inulinase (I-2017) was purchased from Sigma Chemicals Co. (St. Louis, MO). 1-Kestose (GF_2) and nystose (GF_3) standards were generously given by Dr. K. Fukai (Japan). Phenolic acids (*p*-coumaric, *o*-coumaric, chlorogenic, caffeic, ferulic, protocatechuic and gallic), flavonols (quercetin and rutin), flavones (chrysin) and flavanones (naringenin) were purchased from Sigma Chemicals Co. (St. Louis, MO). Flavan-3-ols (catechin and epicatechin) were purchased from ChromaDex™ (Santa Clara, CA). Diphenylamine and phosphoric acid were purchased from Riedel de Haën (Seelze, Germany) and aniline was purchased from Carlo Erba (Milan, Italy). Silica gel 60 F_{254} plates, HPLC-grade acetonitrile, as well as other solvents and reagents, were purchased from Merck (Darmstadt, Germany).

2.3. Proximal composition

Moisture, ash, lipid, protein ($N \times 6.25$), crude fibre and total carbohydrates were determined according to the AOAC (1998) methods. Total dietary fibre was determined according to the 991.43 AOAC method (1995). Results were expressed as g/100 g of DM. Potassium, calcium, magnesium, iron, sodium, zinc, copper and manganese were estimated according to the method recommended by Perkin (1982) in a 3100 atomic absorption spectrophotometer (Perkin Elmer, Norwalk, CT). Phosphorus and sulphur contents were colorimetrically estimated (Chapman & Pratt, 1973). Mineral contents were expressed in mg/100 g or ppm of dry matter (DM). Starch was screened with the iodine reaction. Thus, 1.5 g of the blended sample was mixed with 20 ml of distiled water and the mixture was rapidly heated at 100 °C for 5 min and then cooled in an ice bath. Finally three drops of an aqueous iodine solution (1% v/v) were added to the mixture and the produced colour was observed. A positive control (appearance of blue colour) with pure starch was also tested under the same above-described conditions.

2.4. Sugars and fructooligosaccharides analysis

2.4.1. General

Sugars and FOS were extracted from chicuru roots following the procedure proposed by Jaime, Martin, Mollá, López-Andreú, and Esteban (2001) with slight modifications. Five grams of chicuru were cut into small pieces, and homogenised in an Ultra-turrax homogenizer (IKA, Germany) with 50 ml of 70% ethanol (v/v) and immediately heated at 100 °C for 10 min. The mixture was centrifuged at 4000 rpm for 15 min and the supernatant was collected. The chicuru residues were re-extracted four more times under the same conditions. The supernatants were combined and evaporated in a rotary evaporator at ~50 °C. The residue was re-dissolved in 50 ml of deionized water, and this chicuru aqueous extract was kept for further determination of sugars and FOS. Yacon syrup was threefold diluted in deionized water prior to analysis.

2.4.2. HPLC-IR quantification of glucose, fructose and sucrose

Glucose, fructose and sucrose, from chicuru extracts and diluted yacon syrup, were analysed using a Waters 2695 Separation Module (Waters, Milford, MA) equipped with an autoinjector, a refraction index detector (IR 410) and the Empower software. A carbohydrate analysis (10 μm, 300 × 3.9 mm) column (Waters, Ireland) and an HP carbohydrate (4 μm, 3.9 mm × 20 mm) guard column (Waters, Ireland) were used. The mobile phase was composed of acetonitrile:water (75:25, v/v) at a flow rate of 1.0 ml/min. Samples were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Twenty microlitres of sample were injected and run for 35 min at 30 °C. Sugars were identified and quantified by comparing their retention times to known previously injected standards (glucose, fructose and sucrose).

2.4.3. Quantification of total fructans (TF)

TF were determined according to the Jaime et al. (2001) and Pedreschi et al. (2003) protocols with slight modifications. An enzymatic hydrolysis of the sample with inulinase (Novo Nordisk Ferment Ltd., Bagsvaerd, Denmark) was carried out to liberate sugars. A 0.1 ml inulinase solution in acetate buffer 50 mM, pH 5 (1/10, v/v) was added to 0.9 ml of extract. The solution was mixed and incubated at 60 °C for 60 min, and the total glucose and fructose released was determined by HPLC-IR as described above. The initial amounts of glucose, fructose and sucrose in the samples were previously determined by HPLC-IR, and the obtained amounts were subtracted from the released sugars by the enzymatic hydrolysis. The concentration of TF was calculated according to Prosky and Hoebregs (1999) and Pedreschi et al. (2003).

$$G = G_t - S/1.9 - G_f$$
$$F = F_t - S/1.9 - F_f$$

where G = glucose from FOS or inulin, G_t = total glucose, F = fructose from FOS or inulin, F_t = total fructose, S/1.9 = glucose or fructose from initial sucrose, G_f = initial free glucose and F_f = initial free fructose. The total FOS or inulin content is the sum of G and F and corrected for the water loss during hydrolysis. Thus,

$$\text{Total FOS or inulin} = k(G + F)$$

where k = 0.925, for FOS (average DP = 4) and k = 0.91, for inulin, (average DP = 10). Results were expressed as g of fructans per 100 g of DM.

2.4.4. FOS identification and degree of polymerisation (DP)

FOS were separated by gel filtration chromatography (GFC) on Bio-Gel P-2 (Bio-Rad, California), following the methodology reported by Pedreschi et al. (2003). Half a millilitre of chicuru or threefold diluted yacon extract was applied to the column (210 × 1.2 cm). The elution was carried out with 20 mM phosphate buffer at pH 7.0, maintaining a constant 0.1 ml/min flow with a Masterflex L/S peristaltic pump (Cole Parmer Instrument Co., Vernon-Hills, IL). In total, 190 fractions of 1 ml each were collected using a fraction collector (Eldex Laboratories Inc., Napa, CA). Subsequently, each fraction was analysed in order to determine the presence of FOS. Because FOS do not absorb at a specific wavelength, 450 μl of each fraction were hydrolysed with 50 μl of inulinase solution at 50 °C for 60 min. Then the amount of released reducing sugars was determined by Miller's method (Miller, 1959). The amount of FOS is directly proportional to the amount of produced reducing sugars.

The average degree of polymerisation (DP) was calculated as proposed by Moerman, Van Leeuwen, and Delcour (2004). For each eluted peak, the collected fractions were pooled and the glucose and fructose contents were calculated.

$$DP = F/G + 1.$$

Additionally, the identification of the corresponding FOS peaks was achieved by TLC. A small amount (5 μl) of sample was applied to the silica gel 60 F_{254} plates (Merck). An ascending two development chromatography was performed, using propanol:water (85:15 v/v) as the solvent and diphenylamine–aniline–phosphate as the revealing agent. The plates were submerged in the revealing solution in order to identify the FOS according to Toba and Adachi (1978). Results were compared with standards of glucose, fructose and sucrose. Also, the retention factor (R_f) values, with respect to fructose standard (R_{fruc}), were determined for each peak. All assays were conducted in duplicate.

2.4.5. FOS quantification

Chicuru extract and diluted yacon syrups were analysed by HPLC-IR. A NH2P-50 4E (5 μm, 250 × 4.6 mm) column (Shodex Japan) and a NH2P-50G 4A (4.6 × 10 mm) guard column were used for FOS separation at 30 °C. The mobile phase was composed of water: acetonitrile (30:70, v/v) at a flow rate of 1 ml/min. Twenty millilitres of sample was injected. Samples, standards and mobile phases were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Chicuru FOS compounds were identified and quantified by comparing their retention time data to known previously injected standards.

2.5. Total phenolics and antioxidant activity

Phenolic compounds from chicuru were extracted, following the methodology proposed by André et al. (2007) with slight modifications. Approximately 5.0 g of chicuru sample were mixed with 100 ml of 80% methanol (v/v), wrapped with aluminium foil and homogenised in an Ultra-turrax macerator. The mixture was flushed with nitrogen for 10 min and allowed to stand with intermittent shaking for 2 h at room temperature. After centrifugation at 4000 rpm for 15 min, the supernatant was collected and evaporated to dryness at ⩽38 °C. Phenolic compounds were re-suspended in 5 ml of HPLC-grade methanol and stored at −20 °C under a nitrogen atmosphere.

Total phenolics in chicuru methanolic extract were determined with the Folin–Ciocalteu reagent by the method proposed by Swain and Hillis (1959), using chlorogenic acid as a standard. Absorbance was measured at 725 nm and the result was expressed as mg chlorogenic acid equivalents (CAE)/g of DM. Antioxidant activity was determined by the ABTS procedure proposed by Arnao, Cano, and Acosta (2003). Samples (150 μl) were mixed with 2850 μl of $ABTS^{+\cdot}$ solution. This mixture was reacted at 20 °C until a steady absorbance was reached. Methanol was used as a control. The Genesys-5 UV/vis spectrophotometer (Milton Roy, NY) was blanked with methanol, and the decrease in absorbance due to antioxidants was recorded at 734 nm. The antioxidant activity was calculated as micromoles of trolox equivalents (μmol TE)/ g of DM from a standard curve developed with trolox.

2.6. HPLC-DAD analysis of phenolic compounds

Phenolic compound profiles were obtained, following the procedure of Tsao and Yang (2003) with slight modifications. The chicuru methanolic extract was separated using a reversed-phase HPLC column on a Waters 2695 Separation Module (Waters, Milford, MA) equipped with an auto injector, a 996 photodiode array detector (DAD) and the Empower software. Spectral data were recorded from 200 to 700 nm during the whole run. An X-terra RP_{18} (5 μm, 250 × 4.6 mm) column (Waters, Milford, MA) and a 4.6 mm × 2.0 mm guard column were used for phenolic separation at 30 °C. The mobile phase was composed of solvent (A) water: acetic acid (94:6, v/v, pH 2.27) and solvent (B) acetonitrile. The solvent gradient was as follows: 0–15 B in 40 min, 15–40 B in 45 min and 45–100 B in 10 min. A flow rate of 0.5 ml/min was used and 20 μl of sample were injected. Samples, standards and mobile phases were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Phenolic compounds were identified and quantified by comparing their retention times and UV–visible spectral data to known previously injected standards.

2.7. Statistical analysis

Quantitative data are presented as means ± standard deviation. Glucose, fructose, sucrose, 1-kestose, nystose, FOS and mineral content were processed by the one-way analysis of variance (ANOVA). A Duncan test was used to determine significant differences. Differences at $p < 0.05$ were considered as significant. SPSS for

Windows 14.0 (SPSS, Chicago, IL, USA) was used for all statistical tests.

3. Results and discussion

3.1. Proximal composition

A complete proximal composition for chicuru root is provided in Table 1. Surprisingly, the protein content was higher (5.1 g/100 g of DM) than those reported for other Andean roots and tubers, such as yacon (2.6–2.7 g/100 g of DM), mashua (5.0 g/100 g of DM), oca (3.3 g/100 g of DM) and olluco (3.7 g/100 g of DM), respectively (Collazos et al., 1993), but lower than that for maca (10.2 g/100 g DM) (Dini, Migliuolo, Rastrelli, Saturnino, & Schettino, 1994). The main constituent of the dry matter in chicuru was represented by the carbohydrate content (82.8 g/100 g of DM). A negative starch screening convinced us that fructans and free sugars might be part of this carbohydrate content.

A total dietary fibre (TDF) content of 26.6 g/100 g of DM (or 3.6 g/100 g expressed in FM) was encountered and mainly composed of insoluble fibre (65.4%). This TDF content was higher than those reported for carrots (2.4 g/100 g of FM), potatoes (2.5 g/100 g of FM), and turnip (2.0 g/100 g of FM) (Dreher, 2001). From a nutritional point of view, this high value of TDF might be helpful in terms of maintaining positive effects on intestine and colon physiology, besides other homeostatic and therapeutic functions in human nutrition (McPherson, 1982). Insoluble fibre not only shortens bowel transit time but increases faecal bulk and renders faeces softer. On the other hand, soluble fibre, consisting of non-cellulosic polysaccharides such as pectin, gums and mucilages, mainly present in fruits, oats, barley and legumes, delays gastric emptying, slows glucose absorption, enhances immune function and lowers serum cholesterol levels. Soluble fibre, in addition, is largely fermented in the colon, resulting in short-chain fatty acid production which may inhibit hepatic cholesterol synthesis (Dreher, 2001). This compositional analysis of chicuru reveals that Andean people consuming this crop as part of their basic diet are nutritionally benefited due to the total amounts of carbohydrates, TDF and protein.

The mineral content analysis of chicuru root is presented separately in Table 2. Potassium, calcium, magnesium, sulphur, phosphorus and iron were the minerals present in highest amounts in chicuru roots. A high potassium amount (1960 mg/100 g of DM), close to the value reported for yacon (1980 mg/100 g of DM) (Hermann, Freire, & Pazos, 1999) and maca (2050 mg/100 g, DM) (Dini et al., 1994), was revealed. A higher calcium content (660 mg/100 g of DM), compared to those reported for mashua, oca, ulluco (Flores, Walker, Guimarães, Bais, & Vivanco, 2003), potato (*Solanum* sp.) (André et al., 2007; Flores et al., 2003), maca (Dini et al., 1994) and yacon (Hermann et al., 1999), was found. Calcium plays a crucial role in providing rigidity to the skeleton besides its involvement in neuromuscular function, blood clotting, and many other metabolic processes. A considerably high amount of iron was also present (148 mg/100 g of DM). Reported iron contents for mashua, oca, ulluco and potatoes ranged from 0.2 to 8.5; 0.8 to 4.9; 0.7 to 5.8 and 0.5 to 12.8 mg/100 g of DM, respectively (Flores et al., 2003).

3.2. Sugars and FOS analysis

3.2.1. Free sugar and total fructan (TF) contents

The glucose, fructose, sucrose and TF contents for chicuru root and yacon syrup are shown in Table 3. The amounts of glucose, fructose and sucrose for chicuru root and yacon syrup were 0.7, 1.1 and 24.9 g/100 g of DM, and 6.9, 15 and 6.6 g/100 g of DM, respectively. For yacon roots, reported contents of glucose, fructose and sucrose ranged from 1.1 to 15.8; 10.5 to 35.0 and 7.4 to 17.1 g/100 g of DM, respectively (Asami et al., 1991; Graefe, Hermann, Manrique, Golombek, & Buerkert, 2004; Lachman, Fernández, & Orsák, 2003). For other well-recognised sources of fructans, such as onion (Jaime et al., 2001) and chicory root (Beirão-da-Costa, Januário, Leitão, & Simão, 2005), contents of glucose, fructose and sucrose of ~10.9, 6.6 and 8.1 g/100 g of DM, and 0.1, 0.8 and 2.1 g/100 g of DM have been reported. Chicuru roots, presented lower glucose and fructose contents than did yacon syrup, yacon root and onion but values similar to chicory. However, the sucrose amount was higher than those reported for yacon root, onion and chicory.

In addition, our results revealed that TF (FOS + inulin) was the main carbohydrate in chicuru root (37.6 g/100 g of DM). The TF content for chicuru was comparable to the values reported for onion (29–45 g/100 g of DM) (Jaime et al., 2001) but lower than reported for different yacon cultivars (49.8–53.8 g/100 g of DM) (Graefe et al., 2004), and chicory (51–65 g/100 g of DM) (Beirão-da-Costa et al., 2005). The significant content of fructans displayed by chicuru and, thus, as an alternative source of prebiotics,

Table 1
Proximal analysis of chicuru roots[a]

		Content
Moisture (g/100 g FM)		86.2 ± 0.51
Protein (g/100 g DM)		5.1 ± 0.16
Lipids (g/100 g DM)		1.2 ± 0.06
Crude fibre (g/100 g DM)		6.5 ± 0.05
Ash (g/100 g DM)		4.3 ± 0.10
Carbohydrates (g/100 g DM)		82.8
Starch (g/100 g DM)		ND
Soluble solids (%)		11.6 ± 0.21
Acidity (% ascorbic acid equivalents)		1.2 ± 0.11
Dietary fibre	Soluble (g/100 g DM)	9.2 ± 0.50
	Insoluble (g/100 g DM)	17.4 ± 2.17

ND, not detected.
[a] Mean ± standard deviation of three determinations.

Table 2
Mineral content of chicuru root[a]

Mineral element	Content (DM)
Potassium (mg/100 g)	1960
Calcium (mg/100 g)	660
Phosphorus (mg/100 g)	150
Magnesium (mg/100 g)	300
Sulphur (mg/100 g)	200
Iron (mg/100 g)	148
Sodium (ppm)	900
Zinc (ppm)	16
Copper (ppm)	13
Manganese (ppm)	49

[a] Mean of three repetitions.

Table 3
Glucose, fructose, sucrose and total fructooligosaccharide contents[a] for chicuru root and yacon syrup

Sugar/FOS	Chicuru root (g/100 g of DM)	Yacon syrup (g/100 g of DM)
Glucose	0.74 ± 0.1^{x}	6.9 ± 0.3^{x}
Fructose	1.1 ± 0.2^{x}	15.0 ± 0.4^{y}
Sucrose	24.9 ± 2.6^{y}	6.6 ± 0.3^{x}
Total FOS	37.6 ± 2.3^{z}	49.5 ± 2.2^{z}

Means in the same column with different letters are significantly ($p < 0.05$) different.
[a] Means ± standard deviation of three determinations.

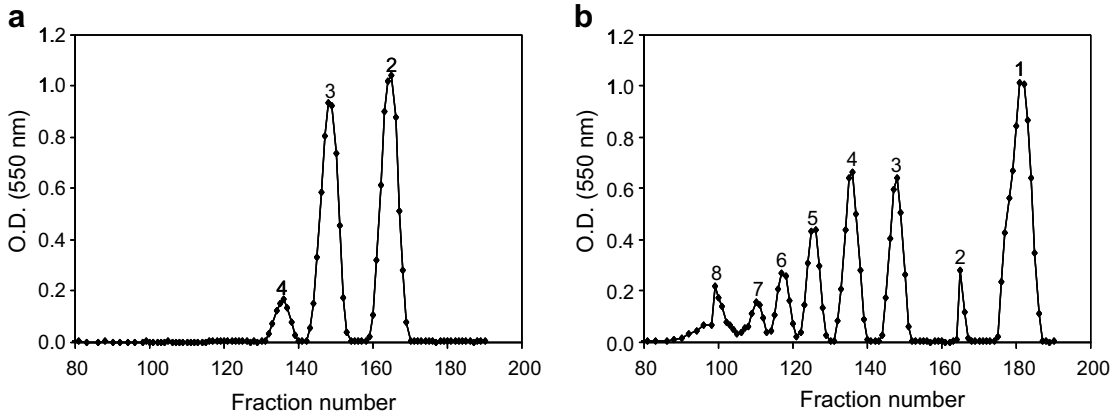

Fig. 1. Fructooligosaccharide profiles for chicuru roots (a) and yacon syrup (b) obtained by gel filtration chromatography (Biogel P-2®).

Table 4
Mean DP values[a] of fructooligosacharides present in chicuru root and yacon syrup

Peak	GF_n[b]	Chicuru root[c] (mean calculated DP)	Yacon syrup[c] (mean calculated DP)	DP values (assigned)
8	GF_7	–	7.88	8
7	GF_6	–	7.00	7
6	GF_5	–	5.45	6
5	GF_4	–	4.98	5
4	GF_3	3.76	4.02	4
3	GF_2	3.02	2.96	3
2	S	2.03	2.03	
1	*G*, F	–	–	–

[a] DP was calculated as $(F/G) + 1$.
[b] GF_n was calculated as F/G.
[c] Mean of two repetitions.

deserved further analysis to elucidate the type of fructans (FOS and/or inulin) present.

3.2.2. FOS identification and degree of polymerisation (DP)

Fructan profiles for chicuru and yacon syrup were determined by gel filtration chromatography, using Biogel P-2® as described above. The obtained fructan profiles for both samples are presented in Fig. 1. In total, three and eight peaks were obtained for chicuru (Fig. 1a) and yacon syrup (Fig. 1b), respectively.

Hydrolysis of the individual peaks, followed by HPLC-IR, was used to calculate the average DP. The molar ratios indicated that the fructans consisted of one glucose molecule and one or more fructose molecules. Similar results were found for yacon roots in a study reported by Goto, Fukai, Hikida, Nanjo, and Hara (1995). The average DP for each individual peak is shown in Table 4. Peaks 2, 3, 4 and 5 displayed mean DP values of 2.03, 2.96–3.02, 3.76–4.02 and 4.98, respectively. These values corresponded to DPs for sucrose and sc-FOS: 1-kestose (GF_2), nystose (GF_3) and 1^F-fructosylnystose (GF_4), respectively. Peaks 6, 7 and 8 presented mean DP values of 5.45, 7.00 and 7.88, respectively, corresponding to GF_5, GF_6 and GF_7, respectively. Additionally, the qualitative TLC analysis for the chicuru (Fig. 2a), yacon syrup peaks (Fig. 2b) yielded R_f values (R_{fruc}) of 1.00, 0.96, 0.73, 0.62, 0.40, 0.24, 0.18 and 0.08 for peaks 1 (glucose/fructose), 2 (sucrose), 3 (1-kestose, GF_2), 4 (nystose, GF_3), 5 (GF_4), 6 (GF_5), 7 (GF_6) and 8 (GF_7), respectively. The presence of sucrose and /or glucose/fructose in chicuru and yacon was confirmed because similar R_{fruc} were obtained for sucrose, glucose and fructose standards.

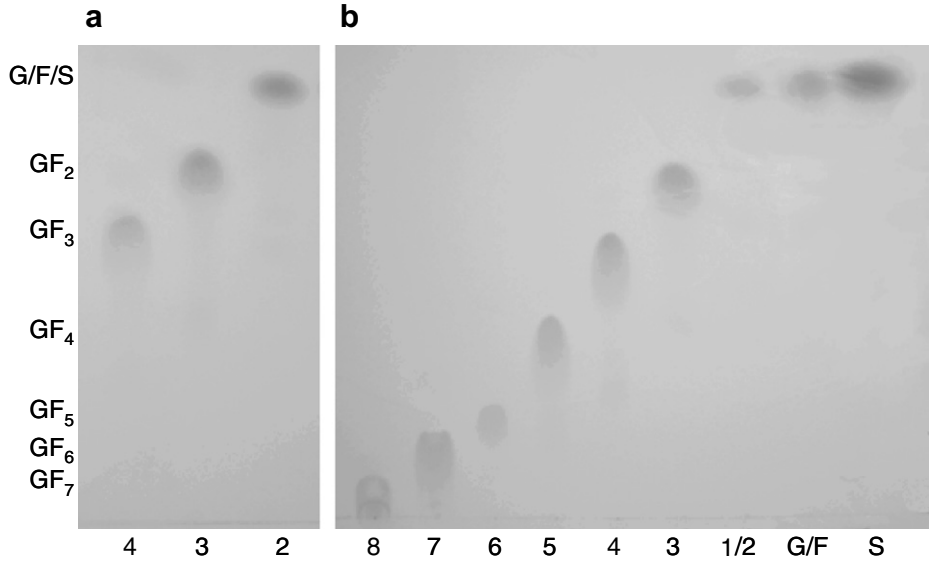

Fig. 2. Thin-layer chromatography (TLC) of fructooligosaccharides for chicuru (a) and yacon (b) peaks obtained by gel filtration chromatography. S, G, F for sucrose, glucose and fructose standards. Numbers 1–8 correspond to the peaks in Fig. 1.

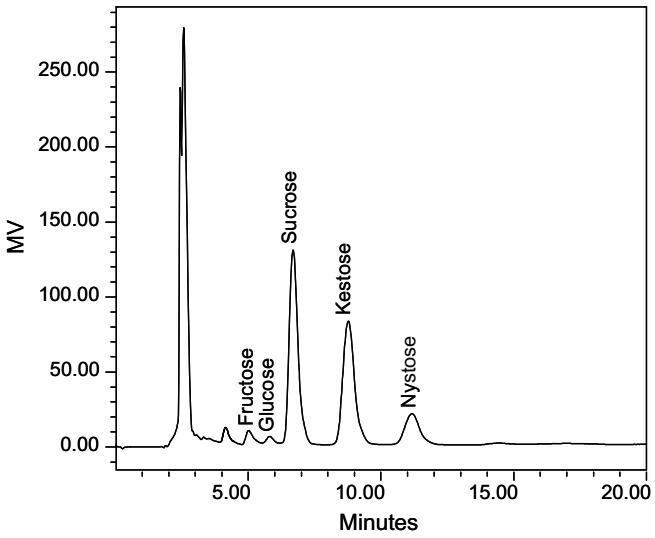

Fig. 3. HPLC-IR for glucose, fructose, sucrose, 1-kestose and nystose for chicuru roots.

It is noteworthy that the main fructans present in chicuru were of the sc-FOS type, 1-kestose (GF_2) and nystose (GF_3) being the main ones and sucrose the most representative sugar (Fig. 1a). The yacon syrup, however, displayed a more complex mixture of FOS that ranged from GF_2 to GF_7, as well as glucose, fructose and sucrose (Fig. 1b). FOS within the range GF_2 to GF_{10} have been reported in yacon (Asami et al., 1991). The absence of GF_8, GF_9 and GF_{10} in the evaluated yacon syrup might be the result of hydrolysis during the processing of the syrup, the type of cultivar, maturity stage and/or growing conditions (Graefe et al., 2004).

The absolute quantification of the sc-FOS present in chicuru (Fig. 3) yielded values of 25.6 and 11.2 g/100 g of DM of 1-kestose and nystose, respectively. The sum of both sc-FOS reached the value of 36.8 g/100 g of DM (or 5.08 g/100 g FM), similar to the value obtained by the TF analysis (37.6 g/100 g of DM). The content of sc-FOS (GF_2 + GF_3) in chicuru is comparable to that reported for onion (21.5–34.4 g/100 g of DM) but higher than that for chicory (1.35–1.80 g/100 g of DM) (Bornet et al., 2002).

Chicuru root can be considered as an alternative source of sc-FOS. Sc-FOS have received special attention in recent decades due to their functional and technological properties. Regarding their functional properties, the specific stimulation of bifidobacterial growth (Biedrzycka & Bielecka, 2004), while suppressing the growth of potentially harmful species, such as, for example, *Clostridium perfringes* in the colon (Bornet et al., 2002), has been highlighted. This property is associated with a decrease in faecal pH, an increase in faecal or colonic organic acids, a decrease in the production of nitrogenous products in urine and stools, a decrease in faecal bacterial enzymatic activities and a modification in faecal neutral sterols. Besides, the indigestibility of sc-FOS in the small intestine has been reported in several studies (Delzenne & Roberfroid, 1994; Molis et al., 1996). Ninety percent of the ingested sc-FOS were recovered in the ileum (Molis et al., 1996) and the percentages of the constitutive sc-FOS (GF_2, GF_3 and GF_4) remained identical to the ingested ones.

Furthermore, sc-FOS stimulate the absorption of Ca, Mg and Fe in rats (Ohta et al., 1995). It is noteworthy that the mineral composition of chicuru revealed significantly high amounts of calcium and iron. The absorption of these minerals might be facilitated when ingesting the sc-FOS also present in the root and might also explain why the Andean population that consumes chicuru roots has used this crop against osteoporosis and anaemia. From a technological point of view, the taste profile of the sc-FOS mixture is similar to sucrose, without any cooling effect but with a 30% lower

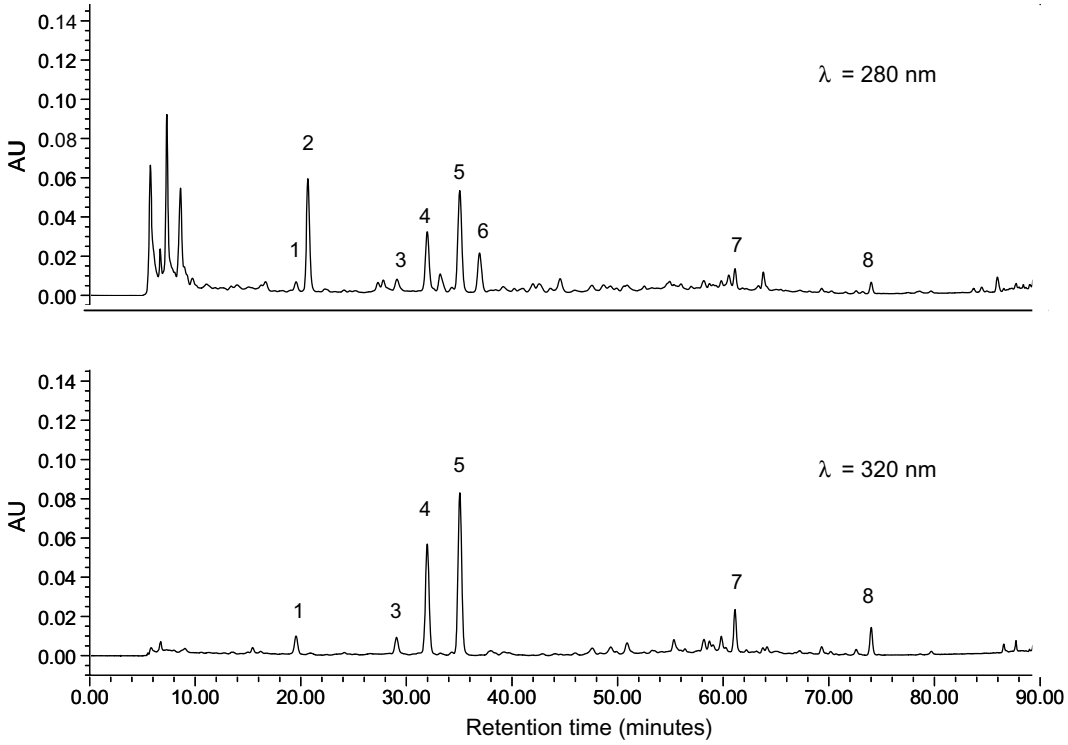

Fig. 4. HPLC-DAD phenolics profile for chicuru roots.

Table 5
Chromatographic and spectral characteristics of the main phenolic compounds detected in chicuru[a] by HPLC-DAD

Peak number	Retention time (min)	λ_{max} (nm)	Phenolic compound assignment	Relative area$_{280\ nm}$ (%)[a]
1	19.5	235.8, 327.1	Hydroxycinnamic acid derivative	2.1
2	20.6	235.8, 279.6	Flavan-3-ol derivative	27.7
3	29.1	243.5, 325.1	Hydroxycinnamic acid derivative	3.1
4	31.9	241.7, 327.1	Chlorogenic acid	17.7
5	35.0	242.9, 324.7	Caffeic acid	30.9
6	36.9	235.4, 278.4	Flavan-3-ol derivative	11.9
7	61.1	241.5, 325.1	Hydroxycinnamic acid derivative	4.2
8	74.0	247.6, 327.1	Hydroxycinnamic acid derivative	2.4

[a] Mean of two repetitions.

sweetness. Thus sc-FOS can be used in calorie-free sweeteners and other low caloric products. They are stable at pH > 3 and temperatures up to 130 °C (Bornet et al., 2002).

3.3. Antioxidant activity, total phenolics and HPLC-DAD phenolics profile

An antioxidant activity of 3.9 μmol TE/g of FM (or 26.0 μmol TE/g of DM) was found and in the range reported for potato and ulluco (3.4–15.1 and 1.9–6.1 μmol TE/g of FM, respectively) (Campos et al., 2006). The total phenolic content for chicuru was 0.98 mg/g of FM and is in the same range as displayed by potatoes, oca and ulluco (0.64–2.32; 0.71–1.32 and 0.41–0.77 mg/g of FM, respectively) (Campos et al., 2006).

To elucidate the phenolic profile of chicuru, an HPLC-DAD analysis was carried out. The HPLC chromatograms recorded at 280 nm and 320 nm for chicuru root are shown in Fig. 4. In total, eight and six representative peaks were observed at 280 and 320 nm, respectively. The main families of phenolic compounds present in this root were the hydroxycinnamic acids and flavan-3-ols (Table 5). Peaks identified in chicuru corresponded to chlorogenic acid (peak 4) and caffeic acid (peak 5). Identification was based on retention times and UV–vis spectral data compared to previously known injected standards. The non-identified phenolics in this root showed UV–vis spectral characteristics similar to those of chlorogenic or caffeic acids (hydroxycinnamic acids) and catechin (flavan-3-ol). On the basis of their spectral characteristics, they were tentatively identified as hydroxycinnamic acid derivatives (peaks 1, 3, 7 and 8) and flavan-3-ol derivatives (peaks 2 and 6). In terms of total percentage area at 280 nm, the hydroxycinnamic acid family represented 60.4%, followed by the flavan-3-ol family (39.6%). Chlorogenic and caffeic acids represented ~48% of the total. A considerable amount of chlorogenic and caffeic, among other hydroxycinnamic acids, has been reported in other Andean crops, such as yacon (Yan, Suzuki, O-Kameyama, Sada, & Nakanishi, 1999) and potato (Lewis, Walker, Lancaster, & Sutton, 1998). Phenolic acids, such as hydroxycinnamic acids, constitute a large group of organic compounds widely distributed in nature and with a broad spectrum of pharmacological activity, such as antioxidant, antimutagenic, antitumor and anticarcinogenic properties (Chun & Kim, 2004).

4. Conclusions

This study has for the first time, focused on the nutritional and functional characterisation of Andean chicuru root, which is at high risk of extinction. The main short-chain FOS present were 1-kestose and nystose. The HPLC-DAD analysis revealed caffeic and chlorogenic acids and derivatives and flavan-3-ol derivatives as the main phenolic families. In addition, high potassium, calcium and iron contents were present. The sc-FOS, together with the high calcium and iron contents present in chicuru root, might favour the availability of minerals. These last findings provide, for the first time, scientific evidence that supports the folklore use of chicuru by the Andean communities against anaemia and osteoporosis.

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