Andean Yacon Root (Smallanthus sonchifolius Poepp. Endl) Fructooligosaccharides as a Potential Novel Source of Prebiotics

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · SEPTEMBER 2003

Impact Factor: 2.91 · DOI: 10.1021/jf0344744 · Source: PubMed

Food Chemistry 135 (2012) 1592–1599

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Prebiotic effects of yacon (*Smallanthus sonchifolius* Poepp. & Endl), a source of fructooligosaccharides and phenolic compounds with antioxidant activity

David Campos [a,*], Indira Betalleluz-Pallardel [a], Rosana Chirinos [a], Ana Aguilar-Galvez [a], Giuliana Noratto [b], Romina Pedreschi [c]

[a] Instituto de Biotecnología (IBT), Universidad Nacional Agraria La Molina – UNALM, Av. La Molina s/n, Lima, Peru
[b] Department of Nutrition and Food Science, Texas A&M University, College Station, Texas 77843, United States Institute for Obesity Research and Program Evaluation, Texas A&M AgriLife Research, College Station, TX 77843, United States
[c] Food & Biobased Research, Wageningen UR. Bornse Weilanden 9, 6708WG, The Netherlands

ARTICLE INFO

Article history:
Received 22 February 2012
Received in revised form 25 April 2012
Accepted 23 May 2012
Available online 30 May 2012

Keywords:
Yacon
Smallanthus sonchifolius
Accessions
Phenolic compounds
Antioxidant activity
Fructooligosaccharides

ABSTRACT

Thirty-five different yacon (*Smallanthus sonchifolius* Poepp. & Endl) accessions were evaluated as potential alternative sources of fructooligosaccharides (FOS) and phenolic type natural antioxidants. FOS, total phenolics (TPC) and antioxidant capacity (AC) contents in the ranges of 6.4–65 g/100 g of dry mater (DM), 7.9–30.8 mg chlorogenic acid (CAE)/g of DM and 23–136 μmol trolox equivalente (TE)/g DM were found. Accession AJC 5189 sparked attention for its high FOS content while DPA 07011 for its high TPC and AC. In addition, the prebiotic effect of yacon FOS was tested *in vivo* with a guinea pig model. A diet rich in yacon FOS promoted the growth of bifidobacteria and lactobacilli, resulting in high levels of short chain fatty acids (SCFAs) in the cecal material and enhancement of cell density and crypt formation in caecum tissue, being indicative of colon health benefits. This study allowed identification of yacon cultivars rich in FOS, AC and/or FOS and AC for nutraceutical applications.

1. Introduction

Yacon (*Smallanthus sonchifolius* Poepp. & Endl), an Andean crop that grows at altitudes of 1000–3200 m above sea level, is particularly known as an abundant source of β-(2 → 1) fructooligosaccharides (FOS) (Goto, Fukai, Hikida, Nanjo, & Hara, 1995). FOS are considered as prebiotics and yacon FOS prebiotic effects have been demonstrated *in vitro* showing that they were selectively fermented by bifidobacteria and lactobacilli (Pedreschi, Campos, Noratto, Chirinos, & Cisneros-Zevallos, 2003). In addition, yacon roots are rich in phenolic compounds, mainly chlorogenic (caffeoyl-quinic) acid and other caffeic acid derivatives (Takenaka et al., 2003; Yan et al., 1999).

Yacon roots have a long history of safe use in South America and elsewhere with potential health-promoting properties, including prebiotic, antidiabetic, antioxidative and antimicrobial effects (Ojansivu, Ferreira, & Salminen, 2011). Yacon cultivation has been expanded to several countries such as New Zealand, Japan, and Brazil in the last decades, and the production in the Andean region and other countries have increased due to the presumed medicinal properties of both roots and leaves (Genta, Cabrera, Grau, & Sánchez, 2005). The antidiabetic effects of yacon root hydroalcoholic extract in streptozotocin (STZ)-induced diabetic rats have been attributed to its antioxidant activity and content in phenolic compounds, mainly chlorogenic acid (Park, Yang, Hwang, Yoo, & Han, 2009). Daily intake of yacon syrup decreased body weight, waist circumference and body mass index suggesting a role in obesity management. In addition, beneficial effects have been reported on insulin resistance and serum LDL-cholesterol levels suggesting a role on metabolic syndrome and diabetes (Genta et al., 2009). Stimulatory effects of yacon FOS on Ca intestinal absorption, bone mineral retention and structural properties in the femoral midshaft of Wistar rats fed *ad libitum* with diets supplemented with yacon flour, have also been reported (Lobo, Colli, Alvares, & Filisetti, 2007). Most of the beneficial effects of yacon consumption have been attributed to its content of phenolic compounds, antioxidants and prebiotics (FOS).

The largest diversity of yacon germoplasm is mainly found in the eastern Andean slopes of Peru and Bolivia (Grau & Rea, 1997). Up to date, only a few studies have related the biodiversity to the physical and chemical characteristics of yacon. Large differences in FOS and sugar contents have been reported for ten different yacon accessions (Hermann, Freire, & Pazos, 1997). There are also differences in tuber shape, weight, content of oligofructans, as well as in leaf isozymes, phenolics, and relative DNA contents reported for four yacon accessions cultivated under field conditions (Valentová et al., 2006). In an

* Corresponding author. Tel./fax: +511 3495764.
E-mail address: dcampos@lamolina.edu.pe (D. Campos).

0308-8146/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2012.05.088

effort to investigate the intra-specific genetic variability in *S. sonchifolius* for prediction of its total phenolic content, molecular markers have recently been investigated (Milella et al., 2011).

The objectives of this study are: (i) to evaluate total phenolic compounds (TPC), antioxidant capacity (AC), reducing sugars (RS), sucrose and FOS in 35 yacon accessions to identify accessions with potential to be used as sources of prebiotics and other bioactive compounds and (ii) to investigate *in vivo* the prebiotic effects of yacon FOS compared to the gold standard 'inulin'.

2. Materials and methods

2.1. Plant material and chemicals

Thirty-five accessions of yacon roots were kindly supplied by the International Potato Center (CIP) located in Lima (Peru). The roots were grown in the region of Huancayo (Peru) at approximately 3200 m above sea level, at ~80% relative humidity and 17 °C (average temperature). Three independent samples of ~0.5 kg were collected for each accession. Samples were collected at optimal harvest date (8 months of cultivation) and stored at −20 °C for further use. Moisture content was determined in yacon flesh and DM was calculated by difference (AOAC, 1995).

The standards used for analysis of phenolic acids (*p*-coumaric, *o*-coumaric, protocatechuic, ferulic, gallic, caffeic, chlorogenic and *p*-hydroxybenzoic acids), flavonols (quercetin, rutin, myricetin and kaempherol), flavones (luteolin and apigenin) and flavanones (naringenin) were purchased from Sigma Chemical Co. (St. Louis, MO). Flavan-3-ols (cathechin and epicathechin) were purchased from ChromaDexTM (Santa Clara, CA). Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman- 2-carboxylic acid) and 2N Folin–Ciocalteu reagent, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemicals Co. (St. Louis, MO). HPLC grade acetonitrile and other solvents and reagents were purchased from Merck (Darmstadt, Germany).

2.2. Chemical analysis

2.2.1. Antioxidant capacity (AC) and total phenolic compounds (TPC)

Yacon flesh (~5.0 g) was homogenized with 100 ml of acidified 80% methanol (formic acid, pH 2.0). The mixture was vortexed for 30 s and flushed with nitrogen for 2 min. After 60 min of intermittent shaking (200 rpm) at room temperature, the extract was centrifuged at 6000*g* for 10 min at 4 °C, and the supernatant was collected. The pellet was submitted to a second extraction for 30 min with 50 ml of solvent. The supernatants were combined and evaporated on a rotary evaporator at 40 °C for further AC, TPC, and HPLC-PAD (photodiode array detection) analysis.

AC was determined using the ABTS assay (Arnao, Cano, & Acosta, 2001) and expressed as μmol of trolox equivalents (TE)/g of DM from a standard curve developed with trolox. TPC were determined using a standard curve of chlorogenic acid (CA) according to Singleton and Rossi (1965) and the results were expressed as milligram of chlorogenic acid equivalents (CAE)/g of DM.

2.2.2. HPLC-PAD analysis of phenolic compounds

Phenolic compound profiles were analyzed by HPLC-PAD as previously reported (Chirinos et al., 2008). Briefly, phenolics were separated using a X-terra RP-C_{18} (5 μm, 250 × 4.6 mm) column (Waters, Milford, MA) and a 2.0 × 4.6 mm guard column at 30 °C on a Waters 2695 Separation Module (Waters, Milford, MA) equipped with an auto-injector, a 2996 photodiode array detector (PDA) and the Empower software. Spectral data were recorded from 200 to 700 nm. The mobile phase was solvent (A) water: acetic acid (94:6, v/v, pH 2.2) and solvent (B) acetonitrile. The solvent gradient was: 0–15% B in 40 min, 15–45% B in 40 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 and mobile phases were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Each sample was analyzed in duplicate. Phenolic compounds were identified and quantified by comparing their retention time and UV–Vis spectral data to standards.

2.2.3. Quantitate of reducing sugars, FOS and sucrose

Sugars and FOS were extracted from yacon flesh according to the method reported by Jaime, Martín-Cabrejas, Mollá, López-Andréu, & Esteban, 2001 with some modifications (Jaime et al., 2001). Briefly, 5 g of yacon was homogenized 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 6000*g* for 15 min and the supernatant was collected. The yacon cakes were re-extracted two more times under the same conditions.

The supernatants were combined and vacuum evaporated in a rotary evaporator at 50 °C. The residue was re-dissolved in 50 ml of deionized water, and this aqueous extract was kept for further analysis of reducing sugars (RS), sucrose (S) and fructooligosaccharides (FOS). RS were determined according to the method reported by Miller (1959) using dinitrosalicilic acid as reagent and fructose as standard, absorbance was measured at 550 nm. FOS content was determined by HPLC-IR as reported by Campos, Betalleluz, Tauquino, Chirinos and Pedreschi (2009). Briefly, a NH_2P-50 4E (5 μm, 250 × 4.6 mm) column (Shodex, Japan) and a NH_2P-50G 4A (4.6 × 10 mm) guard column were used. The mobile phase was composed of water: acetonitrile (30:70, v/v) at a flow rate of 1 ml/min and 30 °C. Twenty ml of sample was injected. Samples, standards (glucose, fructose and sucrose) and mobile phase were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. The results were expressed as g of fructans per 100 g of DM.

2.3. Prebiotic effects of yacon FOS using an in vivo guinea pig model

Yacon roots were obtained from a local market in Lima, Peru. Roots were boiled (99.5 °C for 25 min) to inactive enzymes, cut into slices and dried (~7% moisture) using a hot-air tunnel (relative humidity of ~22% and air flow rate of 2.5 m/s) at 65 °C. Dried yacon slices were milled to obtain yacon flour composed of FOS, 42%; RS 33% and sucrose 4.9% for further use in the diet formulation presented in Table 1. Pellets were obtained using a Hessen Boxtel–Holland (Robinson Milling Systems, Lima, Peru) machine.

Male guinea pigs (*Cavia porcellus*) of 14 ± 2 days age and 230 ± 30 g weight were purchased from a commercial guinea pig

Table 1
Formulation of experimental diets (g/100 g).

Ingredients	Diet control	Diet inulin[a]	Diet yacon flour[a]
Yellow maize	28.06	27.97	25.28
Wheat by-product	34.95	34.79	30.84
Rice husk	5.00	0.00	0.00
Inulin (Orafti®P95)	0.00	5.26	0.00
Yacon flour	0.00	0.00	11.9
Alfalfa hey	6.1	6.1	6.1
Soya cake	20.62	20.61	20.61
Vegetal oil	2.35	2.35	2.35
Mix (vitamins + minerals)	2.76	2.76	2.76
Anti-fungal	0.1	0.1	0.1
Vitamin C	0.06	0.06	0.06

Diet Composition: protein 18.0 (%), fibre 9.0 (%), fat 5.0 (%), lysine 0.84 (%), methionine + cysteine 0.79 (%), arginine 1.39 (%), tryptophan 0.28 (%), treonine 0.69 (%), calcium 0.80 (%), phosphorus 0.80 (%), sodium 0.20 (%) vitamin C 200 (mg/100 g). Gross energy 12.14 (Mj/kg).

[a] The content of inulin or FOS in the diet was 5 %.

Table 2
Total phenolic compounds, antioxidant capacity, dry matter, reducing sugars, sucrose and fructooligosaccharides in 35 yacon accessions.

Cultivar	TPC (mg CAE/g DM)	AC (μmol TE/g DM)	D M (g/100 g)	R S (g/100 g DM)	S (g/100 g DM)	FOS (g/100 g DM)
ARV 5073	9.0 ± 0.3	43.3 ± 2.4	15.1 ± 1.3	32.8 ± 0.8	7.1 ± 0.2	29.7 ± 2.3
ARB 5564	8.9 ± 1.2	42.5 ± 5.0	11.0 ± 0.2	32.3 ± 0.1	5.1 ± 0.9	41.5 ± 3.6
ARB 5185	10.3 ± 1.0	43.5 ± 6.5	14.2 ± 1.4	36.8 ± 3.2	4.8 ± 0.1	32.4 ± 4.7
AJC 5189	7.9 ± 0.8	45.4 ± 6.0	19.1 ± 1.2	19.7 ± 0.3	2.6 ± 0.2	65.0 ± 2.0
ARB 5184	12.0 ± 0.4	40.4 ± 4.7	9.1 ± 0.4	39.0 ± 2.8	6.4 ± 0.4	30.2 ± 2.8
DPA 07008	11.7 ± 0.5	39.5 ± 1.3	11.5 ± 0.8	43.1 ± 6.1	4.4 ± 0.2	47.2 ± 4.3
AMM 5163	10.9 ± 0.7	52.6 ± 4.4	18.6 ± 1.2	26.3 ± 2.6	4.0 ± 0.0	49.7 ± 7.5
ARB 5027	18.2 ± 1.2	69.8 ± 0.1	10.3 ± 0.9	43.3 ± 1.1	7.2 ± 0.8	37.5 ± 2.5
DPA 07010	11.8 ± 1.3	88.4 ± 1.2	7.5 ± 0.2	49.1 ± 3.9	8.9 ± 0.4	38.5 ± 0.2
DPA 07004	14.9 ± 0.8	57.3 ± 1.8	10.2 ± 0.1	43.1 ± 2.5	4.8 ± 0.6	28.4 ± 0.3
ARB 5382	13.0 ± 0.9	38.7 ± 5.1	11.3 ± 0.7	52.7 ± 2.2	2.2 ± 0.6	21.4 ± 2.8
DPA 07007	10.0 ± 0.8	59.6 ± 0.1	12.5 ± 1.4	45.8 ± 1.4	3.7 ± 0.1	38.6 ± 1.1
ARB 5125	10.3 ± 0.4	23.3 ± 2.5	13.6 ± 0.2	27.3 ± 3.2	2.0 ± 0.1	47.1 ± 4.5
AKW 5075	11.0 ± 0.4	38.1 ± 2.3	17.1 ± 0.7	30.8 ± 0.8	3.3 ± 0.7	51.3 ± 3.6
ARB 5124	11.3 ± 1.3	60.4 ± 3.4	17.0 ± 0.6	28.4 ± 3.4	5.9 ± 1.6	47.0 ± 2.0
DPA 7001	26.3 ± 0.3	122.0 ± 0.1	10.6 ± 0.0	56.1 ± 3.1	16.8 ± 0.5	12.2 ± 0.5
DPA 7005	25.8 ± 0.1	133.8 ± 2.9	12.2 ± 0.2	59.9 ± 1.7	6.1 ± 0.5	20.0 ± 0.2
DPA 7006	24.0 ± 0.1	110.7 ± 1.7	13.0 ± 0.5	55.4 ± 1.0	5.9 ± 0.1	22.6 ± 0.0
DPA 7002	24.5 ± 0.0	112.3 ± 0.1	12.7 ± 0.3	45.7 ± 0.4	14.1 ± 0.3	17.0 ± 0.6
ARB 5563	22.3 ± 0.1	136.0 ± 6.1	14.8 ± 0.0	42.7 ± 0.5	7.2 ± 1.4	34.4 ± 0.0
P 1385	14.3 ± 0.4	72.5 ± 5.4	16.2 ± 0.1	45.6 ± 0.7	2.0 ± 0.1	32.9 ± 0.5
AMM 5129	12.4 ± 0.2	57.9 ± 6.4	15.7 ± 0.6	33.2 ± 0.4	2.1 ± 0.0	45.1 ± 1.3
DPA 7009	26.0 ± 0.0	118.4 ± 0.2	12.0 ± 1.3	59.1 ± 0.4	4.2 ± 0.3	29.3 ± 1.3
SAL 136	14.9 ± 0.5	73.3 ± 2.8	15.3 ± 0.2	45.2 ± 1.0	5.8 ± 0.2	38.2 ± 4.3
Y. MORA.	11.2 ± 0.3	67.8 ± 0.1	13.1 ± 0.0	48.2 ± 0.0	6.9 ± 0.4	27.9 ± 2.1
ARB 5537	22.6 ± 0.2	83.2 ± 2.6	13.6 ± 0.1	54.9 ± 0.7	7.9 ± 1.2	26.4 ± 1.5
P 1185	20.6 ± 0.0	83.8 ± 6.1	13.0 ± 0.0	58.7 ± 0.5	5.0 ± 0.3	20.4 ± 0.9
GOM 130	19.7 ± 0.2	91.8 ± 4.6	14.6 ± 0.0	48.7 ± 0.7	7.3 ± 0.1	32.8 ± 0.2
AMM 5135	19.7 ± 0.2	99.2 ± 7.2	16.3 ± 0.1	45.3 ± 0.0	4.9 ± 0.1	42.0 ± 0.6
Y. BLANCO	28.3 ± 0.1	135.7 ± 4.5	11.1 ± 0.1	67.5 ± 0.0	5.2 ± 0.0	19.3 ± 0.6
AME 5186	25.6 ± 0.0	95.9 ± 7.2	11.0 ± 0.0	75.9 ± 4.3	6.6 ± 1.5	6.4 ± 2.5
AMM 5150	18.9 ± 0.1	92.2 ± 6.1	15.5 ± 0.0	51.6 ± 0.4	6.5 ± 0.5	31.3 ± 0.3
AMM 5136	22.4 ± 0.0	111.6 ± 4.3	13.6 ± 0.0	75.1 ± 1.6	8.3 ± 0.2	23.8 ± 0.1
DPA 07011	30.8 ± 0.1	135.1 ± 0.1	10.1 ± 0.0	66.0 ± 3.5	8.0 ± 1.2	6.9 ± 0.7
DPA 7003	23.3 ± 0.1	124.0 ± 4.4	13.3 ± 0.0	56.1 ± 0.1	4.8 ± 0.0	25.8 ± 0.1

Values are mean (n = 3) ± SD.

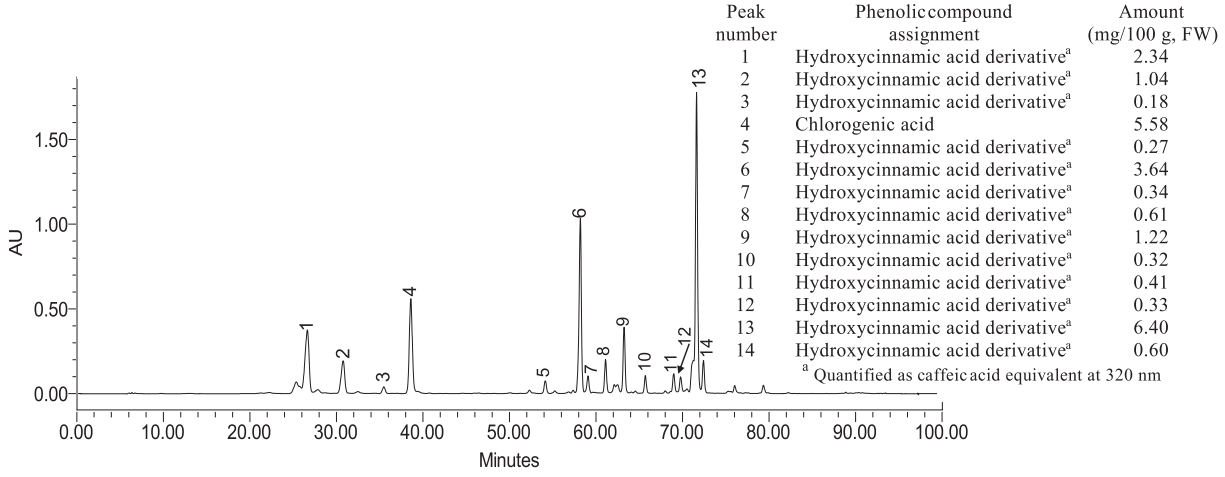

Fig. 1. HPLC-PAD phenolic compounds profile for yacon root accession DPA 07011 at 320 nm.

farm (Universidad Nacional Agraria La Molina, Cieneguilla, Perú). The guinea pigs were kept in ventilated cages covered with rice peel. Food and water were provided *ad libitum*. Animals were allowed to adjust to their environment for 4 days before initiation of the experiments and randomly assigned to three experimental groups (n = 16). Experimental groups received a basal diet (control group), a diet with inulin Orafti® P95 (positive control group) or a diet with yacon flour (Table 1). Food intake and body weight were recorded weekly during eight weeks. Body weight gain and feed efficiency (ratio of weight gain to food consumption) were calculated. At the end of the study (8 weeks), animals were humanely sacrificed using ether anesthesia following institutional regulations. Caecum was removed and the cecal content collected and frozen in liquid nitrogen. Cecal material and caecum tissues were stored at −80 °C for further analysis.

2.3.1. Bacteriological analysis of cecal material

One gram of cecal material was transferred into a sterile tube and mixed with 9 ml of sterile saline phosphate solution (PBS, Sigma Aldrich) containing 1% of hydrochloride L-cysteine (Scharlau Chemie,

Barcelona, Spain) and then serially diluted (from 10^{-1} to 10^{-7}). Bifidobacteria were quantified using Beerens medium (brain heart infusion agar Difco™, glucose, citrate of iron III, L-cysteine, sodium hydroxide and propionic acid) (Beerens, 1990). Lactobacilli and enterobacteria were quantified in MRS agar (Merck, Frankfurt, Germany) and McConkey agar (Difco™), respectively. Incubation was performed at 37 °C under anaerobic conditions using the anaerobic jar with Anaerogen® for bifidobacteria or CO_2 Gen® sachet for lactobacilli and enterobacteria. Number of cells was recorded as cfu/g of cecal material after 24 h incubation for lactobacilli or 72 h for enterobacteria and bifidobacteria.

2.3.2. Histological analysis of caecum tissues

Caecum tissues were fixed in 10 % formalin. Tissue fragments were imbibed in paraffin and stained with haematoxylin and eosin (H&E) for histological examination.

2.3.3. Short-chain fatty acid (SCFAs) analysis

Propionic, butyric and acetic acids (SCFAs) were quantified in cecal material as previously reported (Tzortzis, Goulas, Gee, & Gibson, 2005). Briefly, cecal samples were mixed with MilliQ water in a proportion of 1:1.5 (v:v), centrifuged at 12000*g* for 10 min. Supernatants were then filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) and analyzed by HPLC, using a prepacked Aminex HPX-87 H strong cation-exchange resin column (300 × 7.8 mm i.d.), fitted with an ion exclusion microguard refill cartridge (Bio-Rad Laboratories, Richmond, Calif.) in a Waters 2695 Separation Module (Waters, Milford, MA) equipped with an autoinjector, a 2996 photodiode array detector (PAD) and the Empower software were used for HPLC analysis. A sample of 20 μl was eluted with 0.005 mol/L sulfuric acid at 0.6 ml/min and 50 °C. SCFAs were identified and quantified by comparing retention times and UV–visible spectral data to known standards.

2.4. Statistical analysis

Quantitative data are mean ± standard deviation (SD) values. Data were analyzed using SPSS for Windows 14.0 (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by pairwise comparisons post hoc Duncan test ($p < 0.05$) were performed. For multivariate statistical analysis, principal component analysis (PCA) was performed on mean-centered and standardized data using Unscrambler 9.8 (CAMO A/S, Trondheim, Norway).

3. Results and discussion

3.1. Antioxidant capacity (AC) and total phenolic compounds (TPC)

AC values for the 35 yacon accessions ranged from 23 to 136 μmol TE/g DM or 3.2–20.1 μmol TE/g fresh weigh (FW) (Table 2). The highest values were found in ARB 5563, Y. BLANCO and DPA 07011 accessions, while the lowest values were found in ARB 5125, AKW 5075 and ARB 5382. Previous studies have reported AC values of 1.25 μmol TE/g FW for yacon roots quantified by the DPPH assay (Mikami, Yamaguchi, Shinmoto, & Tsushida, 2009). In general, the AC values found in the 35 yacon accessions were within the range reported for other Andean tuber crops, such as potato (*Solanum* sp.), mashua (*Tropaeolum tuberosum*), oca (*Oxalis tuberosa*) and olluco (*Ullucus tuberosus*) (3.4–15.1, 3.8–39.2, 6.5–19.8 and 1.9–6.1 μmol TE/g FW, respectively) (Campos et al., 2006), and similar to chicuru (*Stangea rhizantha*) (3.9 μmol TE/g FW), another FOS rich Andean crop (Campos et al., 2009).

TPC values varied from 7.9 to 30.8 mg CAE/g DM or 0.9–3.3 mg of CAE/g FW. The highest values were found in DPA 07011, Y. BLANCO, and DPA 7001; while the lowest values were found in AJC 5189, ARB 5564 and ARV 5073 (Table 2). These findings are consistent with previous studies showing that TPC for yacon roots are around 38 mg CAE/g DM (Yan et al., 1999), and 5.7–3.5 mg of gallic acid equivalent/g DM (Simonovska, Vovk, Andrenšek, Valentová &

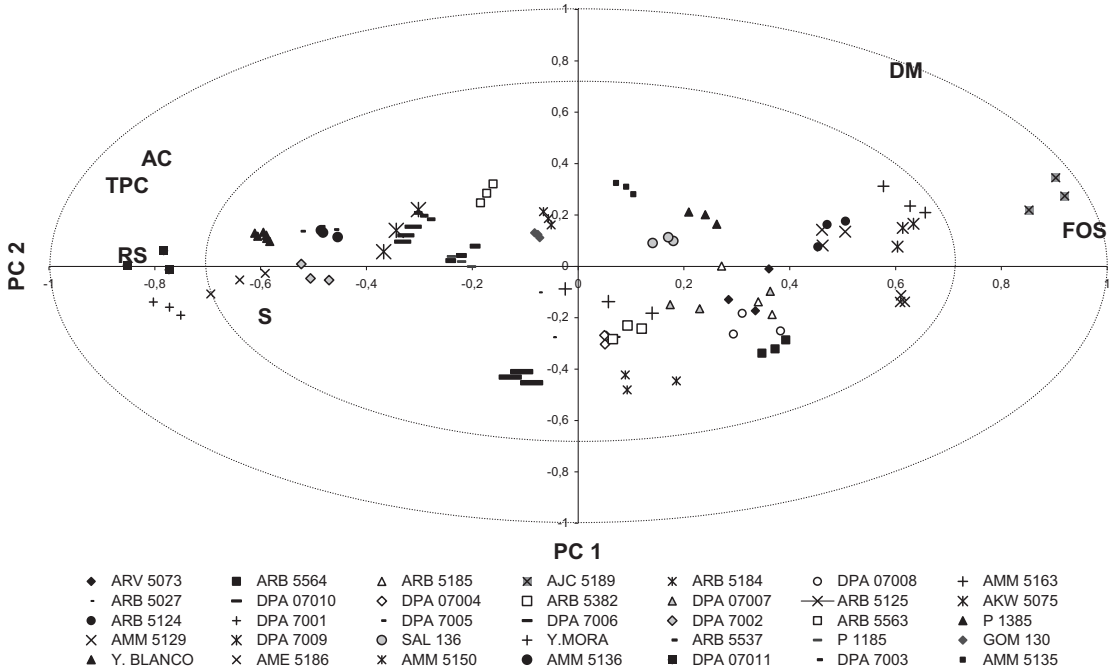

Fig. 2. Principal component analysis (PCA) biplot. Score and loading plots are superimposed. Samples (score plot) correspond to the different yacon accessions. Variables measured (correlation loading plot) correspond to fructooligosaccharides (FOS), dry matter (DM), RS (reducing sugars), S (sucrose), TPC (total phenolic content) and AC (antioxidant capacity). The scores representing each accession (different labels used) indicate differences for the 35 yacon accessions. The variables (FOS, DM, RS, S, TPC and AC) that are important in the discrimination are characterized by large loadings. Thus, the further the variable from the origin, the more influential is that variable in the discrimination among the different accessions. The closer an accession is to a particular variable is indicative of high positive correlation to that variable.

Ulrichová, 2003). Likewise, values reported for other Andean crops were within the range 0.47–3.31 mg CAE/g FW (Campos et al., 2006). Anthocyanins were not detected in the yacon accessions that presented either purple peel and/or purple spots in the flesh by using a spectrophotometric method (Giusti & Wrolstad, 2001), most likely due to the low concentrations. Similarly, the yellow or orange flesh accessions contained very low and non detectable amounts of carotenoids, quantified as previously described (Talcott & Howard, 1999). This finding was related to the low lipophilic AC (0.05–0.35 μmol TE/g FM) content quantified by the ABTS assay (Arnao et al., 2001). In general, a high correlation between AC and TPC ($r = 0.89$, $p < 0.01$) was found, being indicative that phenolic compounds are mainly responsible for the AC of yacon roots.

HPLC-PAD analysis performed for 5 yacon accessions showed similar phenolic profiles. Chromatograms at 320 nm showed 14 peaks of hydroxycinamic acid derivatives, being one of them chlorogenic acid (5-O-caffeoylquinic acid) (Fig. 1). The chlorogenic acid content for the five accessions varied from 1.8 to 7.5 mg/100 g FW (15–24% of total phenolics quantified by HPLC-PDA). This was consistent with previous investigations that reported chlorogenic acid (4.9 ± 1.3 mg/100 g FW) (Yan et al., 1999), and caffeic acid derivatives, mainly esters of caffeic acid with the hydroxy groups of aldaric acid (Takenaka et al., 2003) as main phenolics identified in yacon roots. In addition, tryptophan was detected within the range 0.5–2.8 mg/100 g. These values were consistent with previously reported values for yacon roots (1.46 ± 0.07 mg/100 g FW) (Yan et al., 1999).

3.2. Dry mater (DM), Reducing sugars (RS), sucrose (S), and fructooligosacharides (FOS)

The content of RS, S, and FOS based on DM are presented in Table 2 Results showed that DM varied from 7.5 to 19.1 g/100 g; accordingly RS varied from 19.7 to 75.9 g/100 g DM (3.5 to 8.5 g/100 g FW), S varied from 2 to 16.8 g/100 g DM (0.3 to 1.8 g/100 g FW), and FOS varied from 6.4 to 65.0 g/100 g DM (0.7 to 12.3 g/100 g FW). The highest FOS contents were found in AJC 5189 followed by AKW 5075 and AMM 5163 with values of 65.0, 51.3 and 49.7 g FOS/100 g DM respectively (Table 2). The content of RS in yacon accessions was inversely correlated to the FOS content

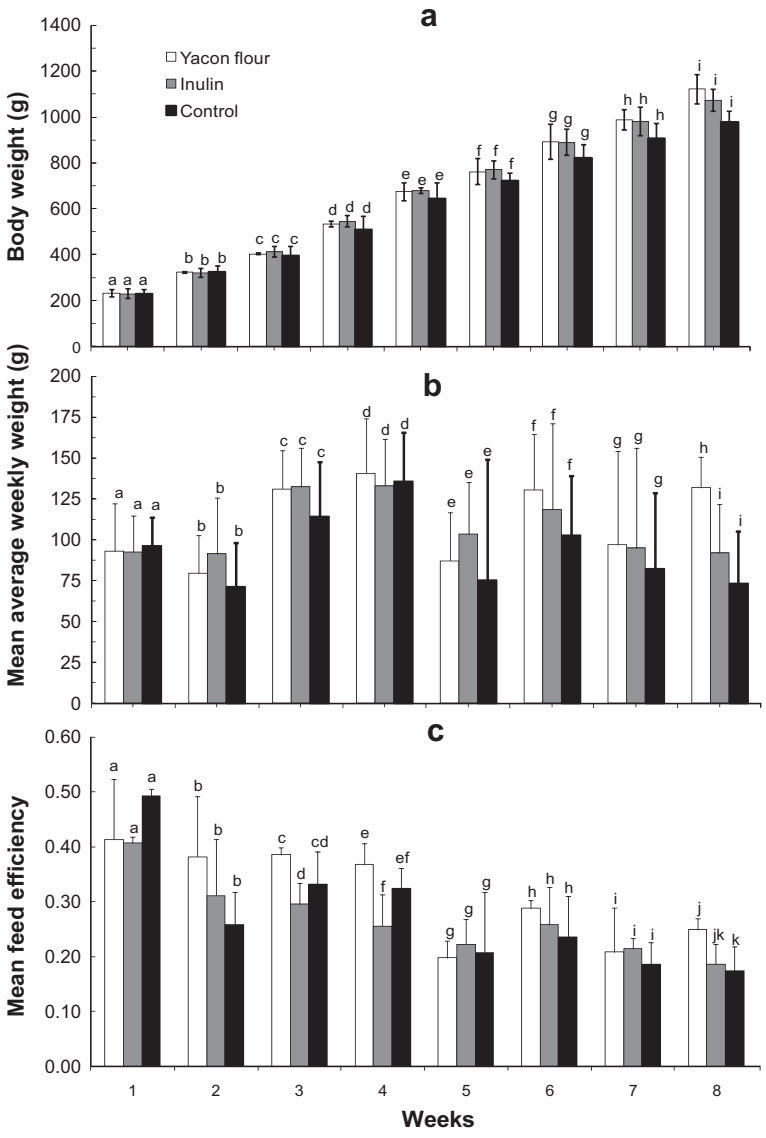

Fig. 3. Effects of yacon flour diet on (a) body weight, (b) body weight gain, and (c) feed efficiency (weight gain/food consumption). Data are mean values ($n = 16 \pm SD$), different letters at each time point indicate statistically significant differences ($p < 0.05$).

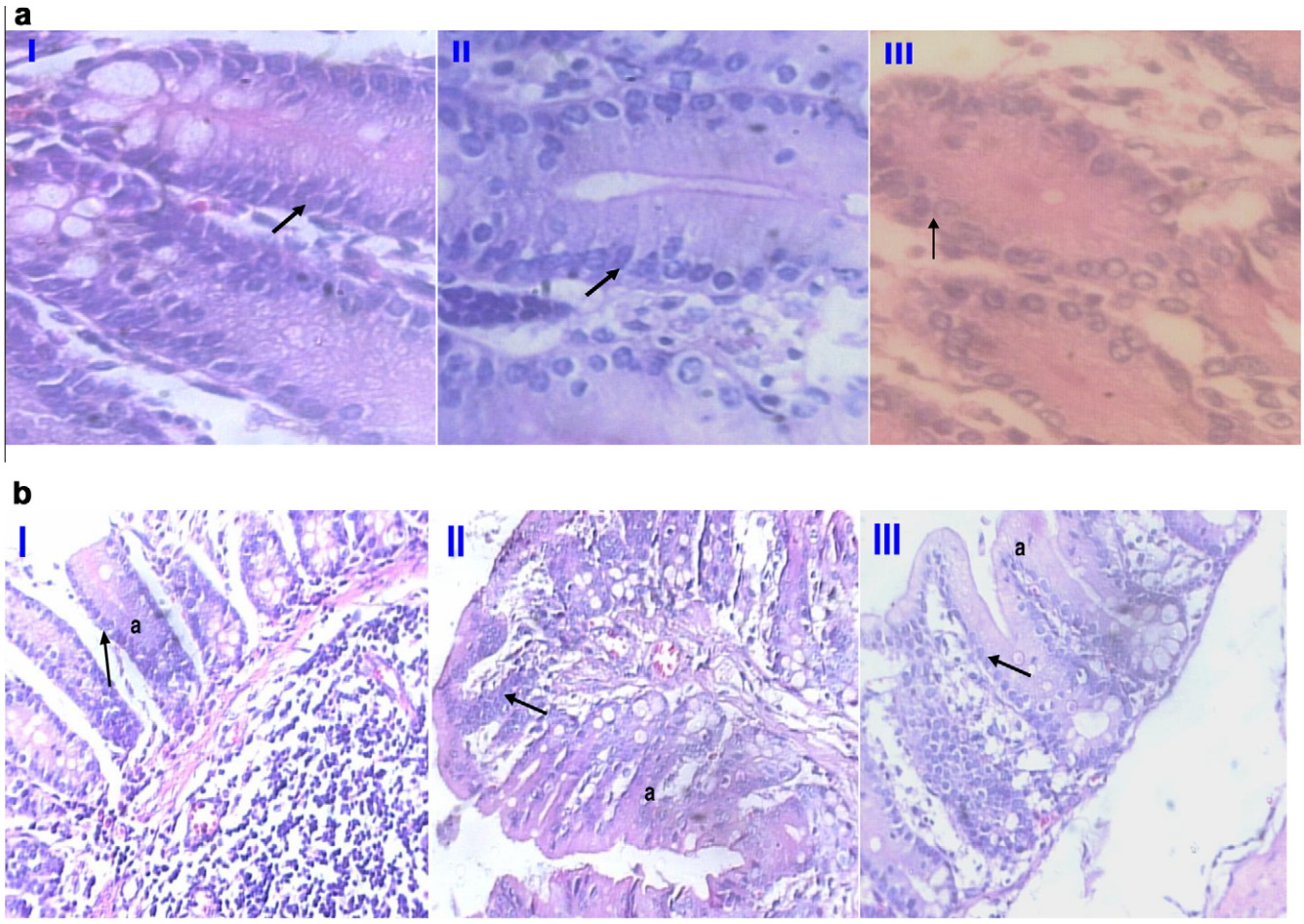

Fig. 4. Photographs from histological sections of caecum from guinea pigs fed with experimental diets. (a) Increased cell density as demonstrated by the higher number of cells with blue stained nucleus in the yacon flour diet (I), compared to the inulin (II) or the control diets (III). (b) Cross sections of caecum tissue showing the relatively higher number of crypts in yacon flour diet (I) compared to inulin (II) or control (III) diets. Sections stained with H&E were observed with light microscopy using a Carl Zeiss microscope (100× magnification).

($r = -0.81$, $p < 0.01$). Likewise, accessions with high content of FOS presented high DM contents ($r = 0.75$, $p < 0.01$).

RS and sucrose were within the 22.3–88.7 g/100 g DM range; these values were consistent with previously reported values for 10 greenhouse cultivated yacon cultivars collected from Peru, Ecuador, Argentina and Bolivia (Hermann et al., 1997).

In general, our results showed higher variability on RS, sucrose and FOS contents compared to previous studies (Lachman, Havrland, Fernández, & Dudjak, 2004; Ohyama et al., 1990; Valentová et al., 2006). This finding might be attributed to the larger number of accessions analyzed in this study. Variability in FOS content has been related to the activity of enzymes involved in synthesis and hydrolysis of FOS such as sucrose: sucrose 1-fructosyl transferase (1-SST), fructan: fructan 1-fructosyl transferase (1-FFT) and fructan 1-exohydrolase (1-FEH). Synthesizing enzyme activities (1-SST and 1-FFT) were always higher in rhizophores than in the tuberous roots, while hydrolysing activity (1-FEH) predominated in the latter (Itaya, de Carvalho, & Figueiredo-Ribeiro, 2002).

A good visualization multivariate tool is principal component analysis (PCA). The 35 accessions are displayed on a biplot (Fig. 2) and correlations can be directly assessed for the different variables evaluated (RS, S, FOS, DM, TPC, AC). This biplot showed that yacon accessions with high FOS content were mostly high in DM and low in RS and S. Likewise, accessions with high TPC were also high in AC. Similarly, there seems to be a trend, yacon accessions with very high FOS content displayed low AC values. This analysis is useful to identify accessions with potential as source of bioactive compounds that can help to prevent chronic diseases involving oxidative stress and glucose metabolism disorders.

3.3. Evaluation of in vivo prebiotic effect of yacon FOS

3.3.1. Effects of yacon flour on body weight and feed efficiency

In general, guinea pigs equally accepted the diets rich in yacon flour, inulin and control. No diarrhea symptoms and/or any other health problems were observed during this experiment. Weight gain and average body weight are presented in Fig. 3(a and b). In general body weight was similar among the three experimental groups; however the yacon flour diet favoured weight gain at week 8th. These results are consistent with previous studies reporting that yacon flour diet did not inhibit the growth and weight gain of male Wistar rats (Lobo et al., 2007) or female Wistar rats fed with FOS or galactooligosaccharides (GOS) (Anthony, Merriman, & Heimbach, 2006) when compared to control groups. Results of feed efficiency (ratio of weight gain to food consumption, Fig. 3c) showed that the yacon flour group was higher ($p < 0.05$) than the inulin group at the third and fourth week, while at the eighth week it was higher than the control group ($p < 0.05$).

3.3.2. Effects of yacon flour on caecum histology

The histological analysis of caecum tissue showed that yacon flour promoted caecum cell growth (Fig. 4a), and increased depth

Table 3
Microbiota (log_{10} CFU/g sample) and short chain fatty acids (SCFAs) (μmol/g) in cecal material from guinea pigs fed with different experimental diets.

	Yacon flour	Inulin	Control
Microbiota			
Bifidobacteria	8.7 ± 0.26[a]	8.2 ± 0.68[a]	5.8 ± 0.73[b]
Lactobacilli	5.9 ± 0.05[a]	6.1 ± 0.39[a]	4.3 ± 0.41[b]
Enterobacteria	2.5 ± 0.06[a]	3.0 ± 0.53[a]	3.2 ± 0.84[a]
SCFAs			
Acetate	77.67 ± 13.98[a]	62.33 ± 9.52[ab]	51.41 ± 8.94[b]
Propionate	19.42 ± 2.45[a]	18.33 ± 4.38[a]	13.75 ± 3.06[b]
Butyrate	9.75 ± 2.91[a]	8.33 ± 1.63[a]	0.7 ± 0.11[b]
Total SCFAs	110.83 ± 40.17[a]	87.08 ± 20.27[ab]	62.08 ± 17.49[b]

Data are mean (n = 16) ± SD. Different letters within each microbiota group or SCFA group indicate significant differences ($p < 0.05$).

and number of bifurcated crypts (Fig. 4b). These findings are consistent with a study that showed that a diet supplemented with 5 or 7.5% FOS from yacon flour enhanced the enlargement of the absorbing surface in the large intestine and the caecum wall with increased number of bifurcating crypts in male Wistar rats (Lobo et al., 2007). The stimulatory effects of yacon FOS on enlargement of caecum and caecum wall crypts might contribute to mineral absorption with implications in bone density and maintenance of healthy bones (Lobo et al., 2007).

3.3.3. Effects of yacon flour on bacterial population and short-chain fatty acids (SCFAs) in cecal material

Results from bacteriological analysis of cecal material are presented in Table 3. Concentrations ($\log_{10}$ CFU/g wet sample) of bifidobacteria and lactobacilli were significantly higher ($p < 0.05$) in samples obtained from animals fed with yacon flour and inulin compared to the control group, while no significant differences were observed for enterobacteria for the three experimental groups ($p > 0.05$). These results are consistent with previous studies showing that FOS from yacon are efficiently metabolized by bifidobacteria and lactobacilli *in vitro* (Pedreschi et al., 2003) and *in vivo* using a mice model (Bibas Bonet et al., 2010). Both, inulin and yacon FOS are fructooligosacharides with different degree of polymerization (DP). In general, yacon FOS (DP = 2–10) (Pedreschi et al., 2003), and inulin (DP = 2–60) promoted the growth of bifidobacteria and lactobacilli and stimulated the intestinal immune system with T cell activation and induction of IL-10 and IFN (Bibas Bonet et al., 2010).

During the last decades, much emphasis has been given to the role of dietary fiber, prebiotics and production of SCFAs in gastrointestinal functions (Mortensen & Clausen, 1996; Wong, de Souza, Kendall, Emam, & Jenkins, 2006), and the onset of gastrointestinal disorders, cancer, and cardiovascular diseases (Wong et al., 2006). Dietary fibers including FOS are substrates for the gut microflora. End products of the fermentation of these substrates are SCFAs (primarily acetate, propionate, and butyrate). These end products help to maintain the colonic mucosa by providing around 70% of their metabolic requirements (Cummings, Pomare, Branch, Naylor, & Macfarlane, 1987).

Higher values of SCFAs in cecal samples (Table 3) were observed for the experimental groups fed with yacon flour and inulin diets compared to the control ($p < 0.05$) and it was correlated with a higher number of bifidobacteria and lactobacilli. Yacon flour diet increased the production of acetate, propionate, butyrate and total SCFAs by 51%, 41%, 1293%, and 78.5% compared to the control diet; while lower increases were observed for the experimental group fed with the inulin diet (21%, 33%, 1090%, and 40%, respectively). These results are supported by a previous study with Sprague–Dawley rats demonstrating that a FOS-containing diet resulted in higher cecal butyrate concentrations and lower pH compared with the control diet, and this was accompanied by higher cecal bifidobacteria and total anaerobes (Campbell, Fahey, & Wolf, 1997). Another study reported that differences in SCFAs profiles vary according to the FOS degree of polymerization (short-chain or medium-chain) and changes in gut microbiota (Sung, Choi, Cho, & Yun, 2006).

4. Conclusions

This study demonstrated the great variability in contents of bioactive compounds and antioxidant activity of 35 yacon accessions. Overall, among the 35 accessions of yacon, the FOS content was inversely correlated with reducing sugars (RS), total phenolic content (TPC) and AC and positively correlated with dry matter (DM). The accessions identified for their enhanced content of FOS (AJC 5189, AKW 5075 and AMM 5163) might be of interest for the FOS nutraceutical industry. Only a few accessions with reasonable amounts of FOS and TPC were found (AMM 5135, SAL 136 and ARB 5027). These accessions might be of interest as accessions of enhanced content of novel health promoting compounds.

The *in vivo* study demonstrated that a yacon flour diet enhanced caecum cell density and crypts, confirming the role of SCFAs as source of energy for colonic mucosa. The prebiotic effects of yacon flour were demonstrated by the FOS-stimulating effect on bifidobacteria and lactobacilli growth and increased concentrations of SCFAs. Overall, these results strongly suggest that yacon FOS consumption might play an important role in colonic health maintenance.

Acknowledgements

The authors thank Marco Ibarra-Castro, Ana Carrasco and Adelayda Pardo for their technical assistance. Dr. Carlos Arbizu from the International Potato Center (CIP), Lima - Perú, is kindly acknowledged for provision of the 35 yacon accessions. This work was supported by the Consejo Nacional de Ciencia y Tecnología (CONCYTEC), Perú.

References

Anthony, J. C., Merriman, T. N., & Heimbach, J. T. (2006). 90-day oral (gavage) study in rats with galactooligosaccharides syrup. *Food and Chemical Toxicology, 44*, 819–826.

AOAC (1995). Official methods of analysis Washington, DC: Association of Official Analytical Chemists.

Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry, 73*, 239–244.

Beerens, H. (1990). An elective and selective isolation medium for *Bifidobacterium* spp. *Letters in Applied Microbiology, 11*, 155–157.

Bibas Bonet, M. E., Meson, O., de Moreno de LeBlanc, A., Dogi, C. A., Chaves, S., Kortsarz, A., et al. (2010). Prebiotic effect of yacon (*Smallanthus sonchifolius*) on intestinal mucosa using a mouse model. *Food and Agricultural Immunology, 21*, 175–189.

Campbell, J. M., Fahey, G. C., & Wolf, B. W. (1997). Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. *Journal of Nutrition, 127*, 130–136.

Campos, D., Betalleluz, I., Tauquino, R., Chirinos, R., & Pedreschi, R. (2009). Nutritional and functional characterisation of Andean chicuru (*Stangea rhizanta*). *Food Chemistry, 112*, 63–70.

Campos, D., Noratto, G., Chirinos, R., Arbizu, C., Roca, W., & Cisneros-Zevallos, L. (2006). Antioxidant capacity and secondary metabolites in four species of Andean tuber crops: native potato (*Solanum sp.*), mashua (*Tropaeolum tuberosum* Ruiz & Pavon), oca (*Oxalis tuberosa* Molina) and ulluco (*Ullucus tuberosus* Caldas). *Journal of the Science of Food and Agriculture, 86*, 1481–1488.

Chirinos, R., Campos, D., Costa, N., Arbizu, C., Pedreschi, R., & Larondelle, Y. (2008). Phenolic profiles of andean mashua (*Tropaeolum tuberosum* Ruiz & Pavon) tubers: Identification by HPLC-DAD and evaluation of their antioxidant activity. *Food Chemistry, 106*, 1285–1298.

Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P. E., & Macfarlane, G. T. (1987). Short chain fatty-acids in human large-intestine, portal, hepatic and venous-blood. Gut, *28*, 1221–1227.

Genta, S. B., Cabrera, W. M., Grau, A., & Sánchez, S. S. (2005). Subchronic 4-month oral toxicity study of dried *Smallanthus sonchifolius* (yacon) roots as a diet supplement in rats. *Food and Chemical Toxicology, 43*, 1657–1665.

Genta, S., Cabrera, W., Habib, N., Pons, J., Carillo, I. M., Grau, A., et al. (2009). Yacon syrup: Beneficial effects on obesity and insulin resistance in humans. *Clinical Nutrition, 28*, 182–187.

Giusti, M. M., & Wrolstad, R. E. (2001). *Anthocyanin characterization and measurement with UV–Visible spectroscopy*. New York: John Wiley & Sons.

Goto, K., Fukai, K., Hikida, J., Nanjo, F., & Hara, Y. (1995). Isolation and structural analysis of oligosaccharides from yacon (*Polymnia sonchifolia*). *Bioscience, biotechnology, and biochemistry, 59*, 2346–2347.

Grau, A. & Rea, J. (1997). Yacon *Smallanthus sonchifolius*. In: Hermann, M., Heller (Eds.), Andean Root and Tubers: Ahipa, Arracacha, Maca and Yacon. IPGRI, Rome, pp. 199–240. Available from: <http://www.cipotato.org/market/ARTChermann/yacon.pdf>.

Hermann, M., Freire, I., & Pazos, C. (1997). Compositional diversity of the yacon storage root. CIP Program, Report 1997–98.

Itaya, N. M., de Carvalho, M. A. M., & Figueiredo-Ribeiro, R. D. L. (2002). Fructosyl transferase and hydrolase activities in rhizophores and tuberous roots upon growth of Polymnia sonchifolia (Asteraceae). *Physiologia Plantarum, 116*, 451–459.

Jaime, L., Martín-Cabrejas, M. A., Mollá, E., López-Andréu, F. J., & Esteban, R. M. (2001). Effect of storage on fructan and fructooligosaccharide of onion (*Allium cepa* L.). *Journal of Agricultural and Food Chemistry, 49*, 982–988.

Lachman, J., Havrland, B., Fernández, E. C., & Dudjak, J. (2004). Saccharides of yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] tubers and rhizomes and factors affecting their content. *Plant Soil and Environment, 50*, 383–390.

Lobo, A. R., Colli, C., Alvares, E. P., & Filisetti, T. (2007). Effects of fructans-containing yacon (*Smallanthus sonchifolius* Poepp & Endl.) flour on caecum mucosal morphometry, calcium and magnesium balance, and bone calcium retention in growing. *British Journal of Nutrition, 97*, 776–785.

Mikami, I., Yamaguchi, M., Shinmoto, H., & Tsushida, T. (2009). Development and validation of a microplate-based beta-carotene bleaching assay and comparison of antioxidant activity (AOA) in several crops measured by beta-carotene bleaching, DPPH and ORAC assays. *Food Science and Technology Research, 15*, 171–178.

Milella, L., Martelli, G., Salava, J., Fernández, E., Ovesná, J., & Greco, I. (2011). Total phenolic content, RAPDs, AFLPs and morphological traits for the analysis of variability in *Smallanthus sonchifolius*. *Genetic Resources and Crop Evolution, 58*, 545–551.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*, 426–428.

Mortensen, P. B., & Clausen, M. R. (1996). Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. *Scandinavian Journal of Gastroenterology – Supplement, 216*, 132–148.

Ohyama, T., Ito, O., Yasuyoshi, S., Ikarashi, T., Minamisawa, K., Kubota, M., et al. (1990). Composition of storage carbohydrate in tubers of yacon (*Polymnia-sonchifolia*). *Soil Science and Plant Nutrition, 36*, 167–171.

Ojansivu, I., Ferreira, C. L., & Salminen, S. (2011). Yacon, a new source of prebiotic oligosaccharides with a history of safe use. *Trends in Food Science & Technology, 22*, 40–46.

Park, J. S., Yang, J. S., Hwang, B. Y., Yoo, B. K., & Han, K. (2009). Hypoglycemic effect of yacon tuber extract and its constituent, chlorogenic acid, in streptozotocin-induced diabetic rats. *Biomolecules & Therapeutics, 17*, 256–262.

Pedreschi, R., Campos, D., Noratto, G., Chirinos, R., & Cisneros-Zevallos, L. (2003). Andean yacon root (*Smallanthus sonchifolius* Poepp. Endl) fructooligosaccharides as a potential novel source of prebiotics. *Journal of Agricultural and Food Chemistry, 51*, 5278–5284.

Simonovska, B., Vovk, I., Andrenšek, S., Valentová, K., & Ulrichová, J. (2003). Investigation of phenolic acids in yacon (*Smallanthus sonchifolius*) leaves and tubers. *Journal of Chromatography A, 1016*, 89–98.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Sung, H. Y., Choi, Y. S., Cho, S. H., & Yun, J. W. (2006). Fructooligosaccharides alter profiles of fecal short-chain fatty acids and bile acids in rats. *Food Science and Biotechnology, 15*, 51–56.

Takenaka, M., Yan, X. J., Ono, H., Yoshida, M., Nagata, T., & Nakanishi, T. (2003). Caffeic acid derivatives in the roots of yacon (*Smallanthus sonchifolius*). *Journal of Agricultural and Food Chemistry, 51*, 793–796.

Talcott, S. T., & Howard, L. R. (1999). Phenolic autoxidation is responsible for color degradation in processed carrot puree. *Journal of Agricultural and Food Chemistry, 47*, 2109–2115.

Tzortzis, G., Goulas, A. K., Gee, J. M., & Gibson, G. R. (2005). A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. *Journal of Nutrition, 135*, 1726–1731.

Valentová, K., Lebeda, A., Doležalová, I., Jirovský, D., Simonovska, B., Vovk, I., et al. (2006). The biological and chemical variability of yacon. *Journal of Agricultural and Food Chemistry, 54*, 1347–1352.

Wong, J. M., de Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology, 40*, 235–243.

Yan, X. J., Suzuki, M., Ohnishi-Kameyama, M., Sada, Y., Nakanishi, T., & Nagata, T. (1999). Extraction and identification of antioxidants in the roots of yacon (*Smallanthus sonchifolius*). *Journal of Agricultural and Food Chemistry, 47*, 4711–4713.