



Impact of yacon landraces cultivated in the Czech Republic and their ploidy on the short- and long-chain fructooligosaccharides content in tuberous roots

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ABSTRACT

Fructooligosaccharides (FOS) are important tuberous root constituents of yacon (*Smallanthus sonchifolius*) with beneficial nutritional and prebiotic effects on human health. That is why landraces originally cultivated in Andes are explored with the aim to obtain new ones with high FOS content. In this study eighteen octoploid and five dodecaploid landraces were for the first time evaluated in terms of their tuberous root contents of short-chain fructooligosaccharides GF3–GF10 (Sc-FOS) and long-chain fructooligosaccharides >GF10 (Lc-FOS) by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD). Significant differences between individual landraces were found; eleven of them contained high Sc-FOS and Lc-FOS, whilst twelve showed low Lc-FOS contents. Comparison of octoploid and dodecaploid groups showed that degree of ploidy level can affect FOS content and the distribution according to degree of polymerisation. High correlations between the contents of Sc-FOS, Lc-FOS and total carbohydrates ($r^2 = 0.97$, $r^2 = 0.98$ and $r^2 = 0.95$, respectively) have been found.

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1. Introduction

Yacon originates from the Andean region, whence it has spread to New Zealand, Japan and Brazil; currently yacon is also grown in the U.S.A. and Russia. First report of its using in Europe comes from Italy, San Remo 1927. It was recommended for a dietetic nutrition, a feeding crop and a material for sugar industry. In the region of Central Europe (the Czech Republic) yacon was studied and grown since 1994 (Fernández, Viehmannová, Lachman, & Milella, 2006; Ojansivu, Ferreira, & Salminen, 2011).

In contrast with most edible roots, yacon stores its carbohydrates in the form of fructooligosaccharides (FOS). FOS are fructose oligosaccharides joined by β -(2→1) or β -(2→6) linkages and terminated with a glucose molecule linked to fructose by an α -(1→2) bond as seen in sucrose. FOS pass through the stomach and

small intestine without being absorbed or degraded and reach the colon intact. FOS naturally exists in many of plants, but the concentration is lower than those in yacon root. FOS are able to resist the hydrolysis of enzymes in the upper part of the human gastrointestinal tract. For this reason, they have a low caloric value for humans. FOS have been shown to exert health benefits during digestion and can relieve the constipation. They have also been shown to reduce blood lipid and glucose levels in animals and in diabetic subjects. Yacon FOS are completely fermented in the colon by a group of beneficial bacteria that form part of the intestinal microflora. These bacteria (especially of the genus *Bifidus* and *Lactobacillus*) improve the gastrointestinal fiction (Genta et al., 2009). Inulin-type fructans, FOS and inulin have been studied as prebiotic non digestible oligosaccharides because they modulate the composition and metabolic activity of the intestinal microbiota, favouring the growth of bifidogenic bacteria rather than other species considered to be pathogenic to the host (Charalampopoulos & Rastall, 2012). In addition to their effects on the gastrointestinal tract, they possess also other favourable effects on human health,

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such as hypolipidemic effect (Habib, Honoré, Genta, & Sánchez, 2011). They affect mineral bioavailability, esp. calcium and magnesium (Lobo, Filho, Alvares, Cocato & Colli, 2009). FOS contained in yacon flour positively affected iron bioavailability from ferric pyrophosphate in rats fed with fructan-containing yacon flour (Lobo et al., 2011). Similarly FOS content stimulates the absorption of magnesium from the hindgut as has been shown in rats (Baba et al., 1996). The main prebiotic components of yacon – FOS and inulin were safety evaluated *in vitro* mutagenicity Ames test and there were no consistent pathology responses (Boyle et al., 2008). Thus, FOS and inulin are appreciated as effective and safe prebiotics and yacon is one of their basic sources. However, individual landraces may be different in short chain (Sc-FOS) and long-chain (Lc-FOS) fructooligosaccharides contents and therefore their contribution to the effect on human health can distinguish. The effect of short chain fructooligosaccharides (Sc-FOS) in promoting recovery from post-gastrectomy anaemia is stronger than that of inulin (Sakai, Ohta, Takasaki & Tokunaga, 2000). In addition, in combination with silymarin yacon appeared to be promising as a nutraceutical in the prevention of diseases with a proatherogenic lipoprotein profile and liver steatosis (Valentová et al., 2008). Recently the protective effects of yacon intake on experimental colon carcinogenesis related to FOS content itself or with symbiotic effect in combination with probiotic *Lactobacillus casei* has been reported (De Moura et al., 2012).

The content of saccharides in yacon tuberous roots may be influenced by origin, landrace, climatic conditions and plant material (Cisneros-Zevallos et al., 2002; Hermann, Freire & Pazos, 1999; Lachman, Havrland, Fernández & Dudjak, 2004). The most of yacon cultivated plants are octoploid ($2n = 58$), but also dodecaploid ($2n = 87$) plants are cultivated. Up to date, only a few studies to the yacon germoplasm diversity related to chemical characteristics (Campos et al., 2012) that evaluated yacon accessions as potential alternative sources of FOS, but not distinguished between short-chain and long chain FOS.

Since so far no data have been published on the Sc-FOS (GF2–GF10) and Lc-FOS (>GF10) contents and their distribution in yacon landraces, the objective of this study focused on the study and evaluation of 23 landraces in terms of their ploidy and origin and their possible effect on total saccharides, Sc-FOS and Lc-FOS contents with the aim to identify landraces with high potential to be used as sources of prebiotics.

2. Materials and methods

2.1. Plant material

Carbohydrate content was analysed in 23 yacon landraces obtained from Bolivia (BOL), Ecuador (ECU), Germany (GER), Peru (PER) and New Zealand (NZL) with different ploidy level. Collection consisted of octoploid ($2n = 58$) – BOL 20, BOL 21, BOL 22, BOL 23, BOL 24, ECU 40, DEU 30, NZL 51, NZL 52, PER 01, PER 02, PER 03, PER 04, PER 06, PER 07, PER 08, PER 09, PER 10 – and dodecaploid landraces ($2n = 87$) – PER 05, PER 11, PER 12, PER 13, PER 14 (Fernández & Kučera, 1997; Fernández, Viehmannová, Meza, Klíma, & Robles, 2008; Viehmannová, Fernández, Bechyně, Vyvadilová, & Greplová, 2009).

Plants were grown under field conditions on experimental plots of the Czech University of Life Sciences in Prague – Institute of Tropics and Subtropics, which is located in the sugar – barley type of production with an average altitude of 286 m, 50° 04' north latitude and 14° 26' east length. Yacon root tubers were harvested after 156 days of cultivation (18 May – 20 October 2010). Yacon root tubers were harvested after 156 days of cultivation (18 May – 20 October 2010). The growing season in the Central Europe is

influenced by spring frosts (May) and the harvest season depends on first autumn frosts (October).

The average daily temperature during the vegetation was 15.8 °C and sum of rainfall 355.5 mm.

2.2. Reagents

All reagents were analytical grade: sodium hydroxide solution 50–52%, Sigma Aldrich, Steinheim Germany, sodium acetate anhydrous p.a., Lachner, Neratovice, Czech Republic, ethyl alcohol p.a., Lachner, Neratovice, Czech Republic, demineralised water (Milli Q quality) was used for the preparation of mobile phase, standard solutions and extraction of samples. As standards sucrose (>99.5%), Sigma Aldrich Chemie, Steinheim, Germany, D-(+)-glucose (minimum 99%), Sigma Aldrich, St. Louis, U.S.A., D-(-)-fructose (minimum 99%), Sigma Aldrich, St. Louis, USA, 1-kestose, (>98.0%), Sigma Aldrich, St. Louis, U.S.A., nystose, (>98.0%), Sigma Aldrich, St. Louis, USA were used.

2.3. Preparation of samples

Peeled tuberous roots were homogenized and then 20 g was extracted under boiling reflux with a mixture of ethanol/demi-water (80:20 v/v). The mixture was then filtered and the filtrate evaporated in a vacuum evaporator at 40 °C. The residue was dissolved in demineralised water and added in a volumetric flask of 50 ml. Before the measurements 3 ml of prepared extracts were diluted to 10 ml volumetric flask with 100 mmol l⁻¹ NaOH solution. From each cultivar an average sample of at least four tuberous roots was analysed. Samples were analysed immediately after harvest.

2.4. Determination of fructooligosaccharides (FOS) by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) and relative response factors by high performance liquid chromatography with refractive index detection (HPLC-RID)

HPAE-PAD analyses were performed according to Dionex Corporation Application (2005) on the ICS 3000 Ion Dionex Chromatograph with an automatic dosing device AS-1 (Dionex Corporation, Thermo Fisher Scientific Inc., Sunnyvale, California, USA). As a detector the amperometer with a gold working electrode (Dionex ED Electrode Gold (Au) and a silver reference electrode (Dionex pH – Ag/AgCl Reference Electrode) was used (Dionex Corporation, Thermo Fisher Scientific Inc., Sunnyvale, California, USA). Separation took place on the CarboPac®PA-100 4 × 250 mm Analytical column (Dionex Corporation, Thermo Fisher Scientific Inc., Sunnyvale, California, USA), with the precolumn CarboPac®PA-100 4 × 50 mm Analytical column (Dionex Corporation, Thermo Fisher Scientific Inc., Sunnyvale, California, USA). The column temperature was 30 °C and flow rate 1 ml min⁻¹. The injection onto the column was 11 µl. Separation took place according to the gradient elution with mobile phase A 100 mmol l⁻¹ NaOH and B 1 mol l⁻¹ CH₃COONa. The elution conditions were: 0–60 min, 0–55% B concave gradient; 60–75 min 0% B. The quantitative analysis of fructose, glucose, saccharose, 1-kestose and nystose was based on the external standard method.

The relative response factors of GF4 and GF5 were determined by analysis on HPLC-RID (NH₂ column). Demineralised water (Milli-Q quality) was used for preparation of a mobile phase, standard solutions and for extraction of samples. HPLC analyses were performed on the Dionex HPLC instrument (Dionex Corporation, Thermo Fisher Scientific Inc., Sunnyvale, California, USA) including a P680 pump and ASI 100 autosampler controlled by Chromeleon 6.80 software package. A Shodex, RI-101 Refractive Index Detector

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