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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.

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 hypolidemic 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., Lach-ner, 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/demiwater (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} CH_3COONa. 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 (NH_2 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

(Showa Denko Europe, Munich, Germany) was used. Chromatographic separation of carbohydrates was carried out on Nucleosil NH_2, 250 × 4.6 mm, 5 μm, (Macherey–Nagel, Düren, Germany). The column and refractive detector temperature was kept at 30 °C. The mobile phase consisted of acetonitrile and water: 85:15 (v/v). One analysis took 45 min. The determined concentrations of GF4 and GF5 fractions were used to calculate the PAD response factors. There was a negligible influence of degree of polymerisation of FOS on response of refractive detector. According to this fact, the concentration of GF4 and GF5 was possible to calculate on available standard.

Previous procedure was verified by collection and analysis of GF4 and GF5 fractions separated on a NH_2 column by HPAE-PAD. The fractions of FOS were collected after separation on column and evaporated by nitrogen at 40 °C. The residues were weighed and then dissolved in 100 μl of demineralised water. This way prepared standards were analysed by HPAE-PAD and results were used to confirm the response of the PAD detector. Response factors of FOS higher than GF6 were calculated according to the equation given in Fig. 1. The dependence of PAD relative detector response on degree of polymerisation (Fig. 1) correlates well with data of White, Hudson, & Adamson (2003). Sample chromatogram (PER 08) is given in Fig. 2. All analyses were carried out in three parallel measurements. The relative standard deviations ranged up to 5%.

2.5. Statistical analysis

Statistical analyses were performed using the software Statistica 7.0 (StatSoft). Effect of landrace was evaluated by one-way factorial ANOVA Tukey's Post Hoc HSD test separately for each ploidy group. Effect of ploidy level on analysed parameters was evaluated by two-sample *t*-test and principal component analysis. The least significant difference test was applied to determine differences among means $p \leq 0.05$.

3. Results and discussion

3.1. Content of fructooligosaccharides and total saccharides

Results obtained for 23 analysed yacon landraces showing the contents of Sc-FOS, Lc-FOS and total carbohydrates (TC) are summarised in Table 1. Sc-FOS were defined as GF3 –GF10 fraction, Lc-FOS as > GF10 fructans and TC was expressed and calculated as sum of glucose, fructose, sucrose and 1-kestose and all fructans > GF2. The contents are expressed in g per kg dry matter (g kg^{-1} DM). The differences in the contents of Sc-FOS, Lc-FOS and TC between individual landraces were significant (Fig. 3 and Table 1). In octoploid plants (Sc + Lc)-FOS ranged from 44.70 to 150.4 g kg^{-1} DM with average level 90.93 g kg^{-1} DM and in dodecanoploid plants from 60.6 to 178.3 g kg^{-1} DM with average level 120.4 g kg^{-1} DM. FOS levels are comparable to those of Campos et al. (2012) who analysed by HPLC-IR 35 yacon accessions, however their FOS levels ranged from 64 to 650 g kg^{-1} DM. The highest contents were typical for landraces obtained in Peru (PER 13, PER 05, PER 06 and PER 03) and Bolivia (BOL 20 and BOL 22). In addition, relatively high contents were also found in other landraces originated in Peru – PER 08, PER 04 and PER 12, in the landrace NZL 52 (origin in New Zealand), and the landrace originated in Ecuador – ECU 40. In these landraces high contents of all analysed saccharides – Sc-FOS, Lc-FOS and TC – were determined. Between individual categories of saccharides exist high positive correlations (between Sc-FOS and Lc-FOS $r^2 = 0.97$, Sc-FOS and TC $r^2 = 0.98$, and Lc-FOS and TC $r^2 = 0.95$, respectively). Landraces characterised with high Sc-FOS, Lc-FOS and TC levels contained mainly GF2 – GF19, whereas landraces with low Sc-FOS, Lc-FOS and TC levels contained mainly fractions of lower degree of polymerisation – GF2 – GF7. Analysed yacon landraces can be according to their content of saccharides divided into two groups. The first group is characterised by high contents of Sc-FOS, Lc-FOS and TC and high index Lc-FOS/Sc-FOS (Table 1, Figs. 3 and 4). This group involves landraces PER 13, PER 05, PER 06, PER 03, PER 08, PER 04, PER 12, BOL 20, BOL 22, ECU 40 and NZL 52. On contrary, the second group was characterised by low Lc-FOS and TC levels and low index Lc-FOS/Sc-FOS. The proper matrix was constructed on the basis of the original data set reflecting Sc-FOS, Lc-FOS and ploidy or origin of the landraces. The samples occurred in two major clusters (Fig. 3).

It was reported that yacon tuberous roots accumulate high amounts of FOS of the inulin type with DP < 10 (Goto, Fukai, Hikika, Nanjo, & Hara, 1995; Ohyama et al., 1990). The underground reserve organs of yacon accumulate more than 60%, on a DW basis, of inulin type β(2–1) fructans, mainly oligomers GF2–GF16 (Itaya, Carvalho, & Figueiredo-Riberio, 2002). However, our findings revealed also LC-FOS minor levels and that could be related to shorter yacon growth period in the Czech Republic (ca 5 months) due the spring and the first autumn frosts. The activities of FOS synthesising enzymes – sucrose:sucrose 1-fructosyltransferase and fructan:fructan 1-fructosyltransferase, responsible for the reversible elongation of acceptor fructans, at this growth stage are relatively high, and to the 10 month growth period they significantly decrease, while conversely of fructan 1-exohydrolase hydrolytic activity increases.

3.2. Effect of ploidy on short-chain and long-chain fructooligosaccharides and total saccharides levels

In term of ploidy, dodecaploid landraces ($2n = 87$) showed higher average values (60.35 g kg^{-1} DM Lc-FOS, 60.07 g kg^{-1} DM Sc-FOS and 168.6 g kg^{-1} DM TC) in comparison with octoploid landraces ($2n = 58$) with mean values 36.59 g kg^{-1} DM Lc-FOS, 54.34 g kg^{-1} DM Sc-FOS and 132.3 g kg^{-1} DM TC (Table 1). The highest values were found in the dodecaploid landraces (Fig. 3) PER 13 (66.14, 112.2 and 224.7 g kg^{-1} DM, respectively) and PER 05 (65.36, 100.7 and 224.5 g kg^{-1} DM, respectively). The results suggest positive effect of higher ploidy on the FOS content, however high contents were also found in some octoploid landraces. Among the analysed dodecaploid landraces, 60% can be included into the group of landraces characterised by high FOS content, while among octoploid landraces only 44.4% could be put in this group. Also degree of polymerisation of major FOS contained was higher in dodecaploid landraces (GF5 – GF12) as compared to octoploid landraces (GF4 – GF10).

From statistical analysis (Table 1) it is evident that octoploid and dodecaploid groups of landraces differed significantly. Octoploid

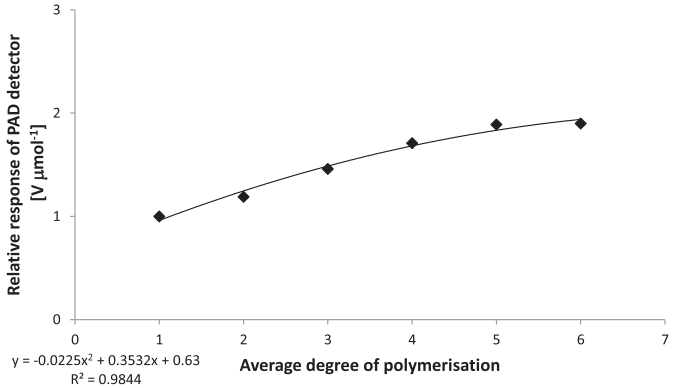

Fig. 1. Relative response of pulsed amperometric detector on average degree of oligofructans polymerisation [V μmol^{-1}].

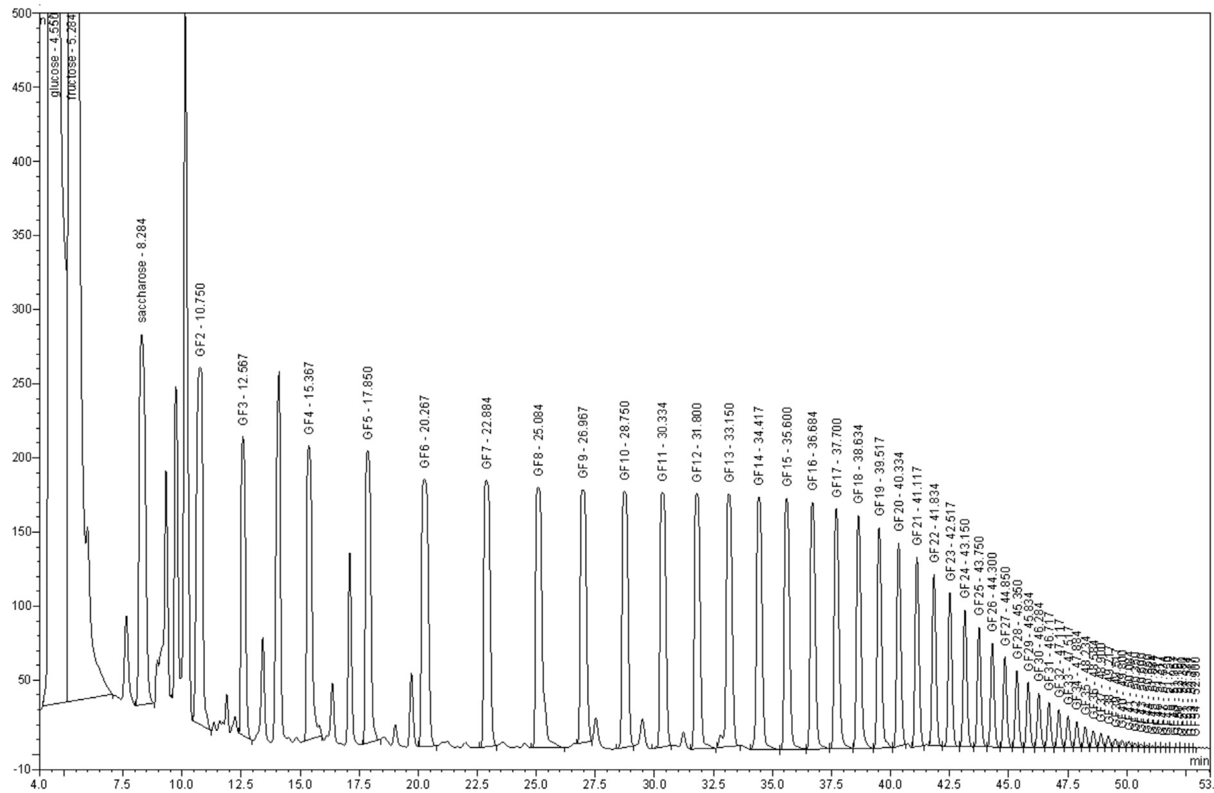

Fig. 2. Chromatogram of the PER 08 yacon landrace.

Table 1
Contents of short-chain (Sc-FOS) and long-chain fructooligosaccharides (Lc-FOS) and total carbohydrates (TC) in yacon landraces [g kg^{-1} DM].

Sample	Landrace	TC[a] (g kg^{-1} DM)	(Sc + Lc)-FOS > GF2 (g kg^{-1} DM)	Lc-FOS > GF10 (g kg^{-1} DM)	Sc-FOS GF3-GF10 (g kg^{-1} DM)	Main FOS fraction
Octoploid plants (2n = 58)						
1	BOL 20	179.3 ± 11.12 de	141.9 ± 7.52 ef	81.50 ± 11.00 bcd	60.44 ± 4.65 cd	GF6-GF12
2	BOL 21	92.39 ± 7.30 ab	51.84 ± 4.56 abc	3.15 ± 0.54 a	48.69 ± 3.75 ab	GF2-GF6
3	BOL 22	172.4 ± 10.69 de	131.9 ± 11.21 ef	74.76 ± 9.42 bcd	57.14 ± 3.77 bcd	GF6-GF15
4	BOL 23	90.56 ± 6.79 ab	65.34 ± 5.36 bc	6.73 ± 1.07 a	58.61 ± 3.93 bcd	GF2-GF6
5	BOL 24	98.30 ± 7.37 abc	61.81 ± 5.82 abc	4.62 ± 0.79 a	57.19 ± 3.83 bcd	GF3-GF6
6	ECU 40	171.2 ± 10.78 de	126.5 ± 9.61 de	70.13 ± 10.66 bcd	56.36 ± 3.61 bcd	GF6-GF14
7	DEU 30	86.98 ± 5.74 ab	47.49 ± 4.23 ab	2.43 ± 0.43 a	45.06 ± 3.47 a	GF3-GF6
8	NZL 51	113.6 ± 8.75 bc	57.03 ± 4.79 abc	4.66 ± 0.84 a	52.37 ± 3.20 abc	GF3-GF7
9	NZL 52	158.10 ± 9.64 d	123.2 ± 8.01 de	69.80 ± 10.82 bc	53.36 ± 3.46 abcd	GF4-GF16
10	PER 01	109.3 ± 8.09 bc	69.45 ± 5.63 c	5.34 ± 0.95 a	64.11 ± 3.53 d	GF3-GF6
11	PER 02	80.62 ± 4.92 a	44.70 ± 4.02 a	1.86 ± 0.34 a	42.84 ± 3.34 a	GF3-GF6
12	PER 03	171.9 ± 11.00 de	135.7 ± 8.01 ef	83.57 ± 12.37 cd	52.17 ± 3.08 abc	GF5-GF19
13	PER 04	151.9 ± 9.42 d	110.9 ± 7.54 d	59.60 ± 9.72 b	51.34 ± 3.49 abc	GF4-GF15
14	PER 06	197.8 ± 12.06 e	150.4 ± 7.82 f	92.21 ± 10.05 d	58.22 ± 3.61 bcd	GF6-GF16
15	PER 07	121.1 ± 8.96 c	66.48 ± 3.92 bc	17.12 ± 2.89 a	49.36 ± 3.75 abc	GF2-GF3
16	PER 08	174.5 ± 11.87 de	124.6 ± 7.35 de	71.93 ± 11.59 bcd	52.69 ± 3.74 abc	GF5-GF17
17	PER 09	102.8 ± 7.09 abc	65.29 ± 5.42 bc	5.56 ± 0.95 a	59.73 ± 3.94 bcd	GF3-GF7
18	PER 10	108.6 ± 8.14 bc	62.10 ± 5.22 abc	3.68 ± 0.68 a	58.42 ± 3.74 bcd	GF3-GF4
	Average	132.3 ± 8.87	90.93 ± 6.45	36.59 ± 5.28	54.34 ± 3.66	GF4-GF10
	Group 1	A	A	A	A	
Dodecaploid plants (2n = 87)						
19	PER 05	224.5 ± 13.02 c	166.0 ± 8.47 c	100.7 ± 10.27 c	65.36 ± 4.12 b	GF2-GF13
20	PER11	105.0 ± 7.67 a	60.55 ± 5.33 a	4.61 ± 0.77 a	55.94 ± 3.30 ab	GF2-GF6
21	PER 12	176.9 ± 11.50 b	125.8 ± 8.05 b	77.51 ± 10.54 b	48.30 ± 3.62 a	GF12-G17
22	PER 13	224.7 ± 13.03 c	178.3 ± 9.09 c	112.2 ± 10.96 c	66.14 ± 3.97 b	GF6-GF16
23	PER 14	111.7 ± 7.04 a	71.39 ± 5.78 a	6.78 ± 1.21 a	64.61 ± 4.33 b	GF3-GF6
	Average	168.6 ± 10.45	120.4 ± 7.34	60.35 ± 6.75	60.07 ± 3.87	GF5-GF12
	Group 2	B	B	B	B	

[a] Total carbohydrates were calculated as sum of glucose, fructose, sucrose, 1-kestose and (Sc + Lc)-FOS; values marked with different letters in columns are significantly different at $p \leq 0.05$; effect of landrace was evaluated by one-way factorial ANOVA Tukey's Post Hoc HSD test separately in each ploidy level group; effect of ploidy levels of both groups on analysed parameters was evaluated by two-sample *t*-test. All analyses were performed in three replicates.

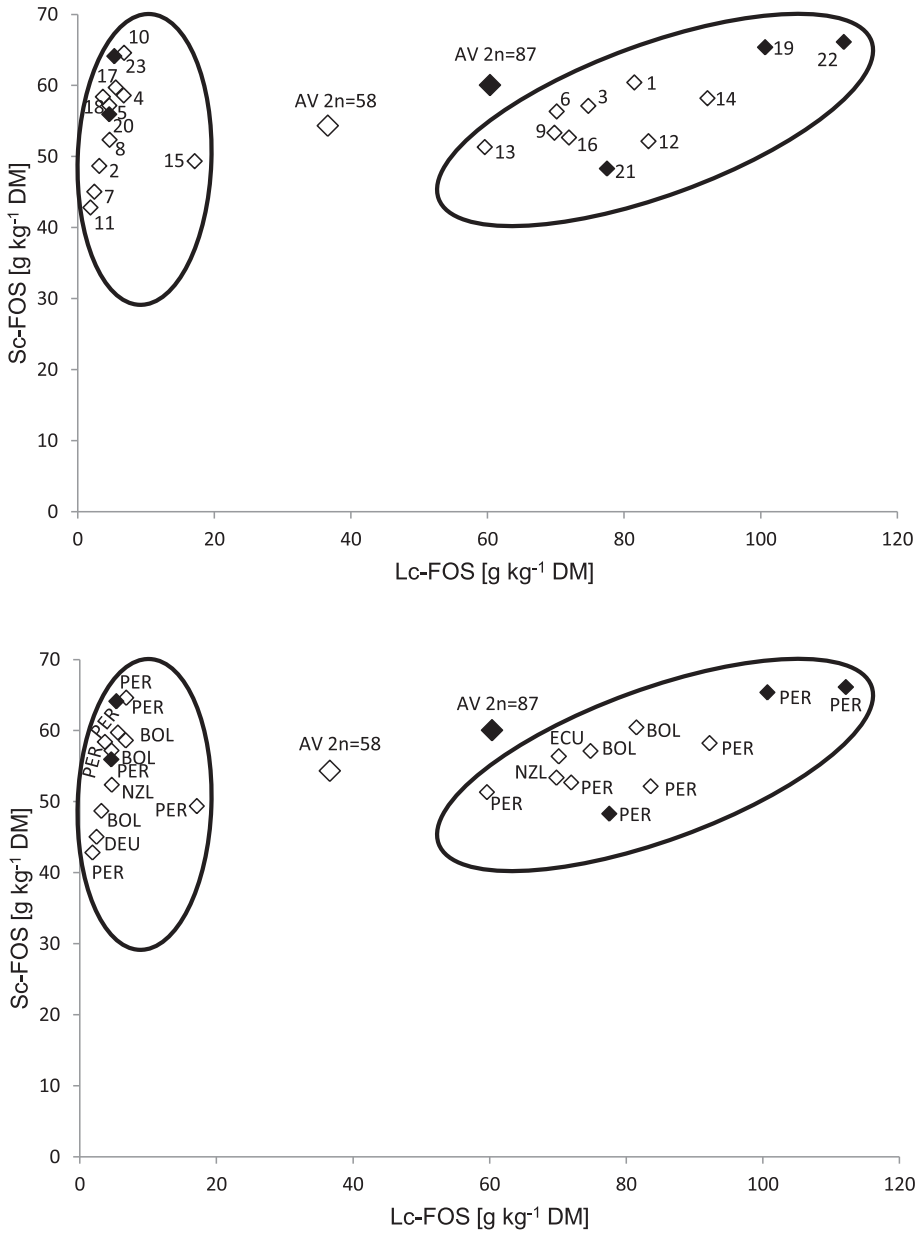

Fig. 3. Diagram of clusters of yacon landraces characterised by Lc-FOS and Sc-FOS contents related to their ploidy and origin.

landraces BOL 21, BOL 23, BOL 24, DEU 30, NZL 51, PER 01 and PER 02 were characterised by low Lc-FOS (GF3–GF10) contents (1.86–17.12 g kg^{-1} DM), whereas octoploid landraces BOL 20, BOL 22, ECU 40 and PER 08 distinguished with high Lc-FOS contents (70.13–81.50 g kg^{-1} DM). Low Sc-FOS (GF3 – GF10) contents showed BOL 21, DEU 30, NZL 51, NZL 52, PER 02, PER 03, PER 04, PER 07 and PER 08, whereas the BOL 22, BOL 23, BOL 24, ECU 40, PER 01, PER 09 and PER 10 landraces were characterised with high Sc-FOS contents (56.36–64.11 g kg^{-1} DM). In the dodecaploid landraces PER 05 and PER 13 differed with high Sc-FOS contents (100.66 and 111.17 g kg^{-1} DM, respectively) from PER 11 and PER 14 (4.61 and 6.78 g kg^{-1} DM, respectively). In terms of the Lc-FOS content only PER 12 differed significantly from other dodecaploid landraces.

Recently in the Czech Republic a set of 25 genotypes was studied for variation in morphotypes, weight, saccharide content of tubers, disease and pest occurrence, DNA content, isozyme polymorphism, and phenolic content in leaves with the aim of finding the most suitable genotype(s) for industrial processing. A significant variation in tuber shape, weight, and content of glucose, fructose, sucrose and phenolics was found. The same was concluded for DNA content and leaf isozyme polymorphism (Lebeda et al., 2008). It seems reasonable to expect that significant differences among landraces related to different Sc-FOS and Lc-FOS contents and their ratios can be based on hydrolase and fructosyltransferase activities implicated in the accumulation of different chain size fructans in different yacon landraces (Itaya, Asega, Carvalho, & Figueiredo-Riberio, 2007; Itaya et al., 2002). Two enzymes are involved in fructan synthesis: sucrose:sucrose 1-fructosyl transferase (EC 2.4.1.99), which produces 1-kestose by fructosyl transfer from donor to acceptor sucrose, and fructan:fructan 1-fructosyltransferase (EC 2.4.1.100), responsible for the reversible elongation of acceptor fructans by the transfer of fructosyl residues from donor fructans. Fructan mobilisation occurs primarily by the action of a fructan 1-exohydrolase (EC 3.2.1.153), which catalyses the release of free fructose. 1-Fructosyltransferase, together with fructan 1-exohydrolase, is believed to catalyse fructan depolymerisation in Asteraceae species.

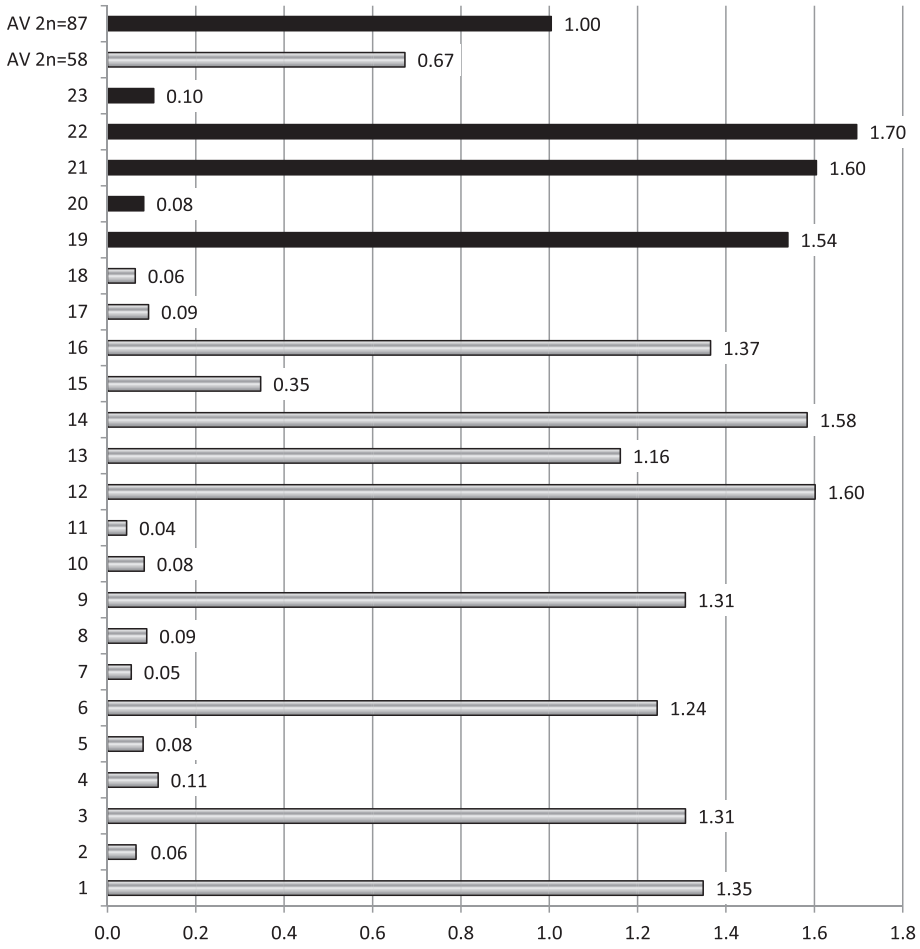

Fig. 4. Index Lc-FOS/Sc-FOS characterising individual yacon landraces and average values of their octoploid (AV $2n = 28$) and dodecaploid (AV $2n = 87$) groups.

In agreement with the finding that the higher fructan 1-exohydrolase activities were found in the species with lower DP fructans, such as *Smallanthus sonchifolius* and *Vinca herbacea* (Itaya et al., 2007), our results reinforce the hypothesis of fructan 1-exohydrolase activity participation in the composition of fructane profile and the variability between individual yacon landraces. Recent research has shown the largest diversity of their germoplasm and landraces and varietal differences and also effects of post-harvest treatments on the carbohydrate composition of yacon roots (Graefe, Hermann, Manrique, Golombek, & Buerkert, 2004).

Numerous factors can influence contents of these fructooligosaccharides. It may be involved enzymatic system biosynthesising and hydrolysing FOS (Itaya et al., 2007; Van Arkel et al., 2012), climatic conditions, ploidy level and other factors such as conditions during storage (Milella et al., 2011; Narai-Kanayama, Tokita, & Aso, 2007). Recent research suggests that high concentrations of FOS from yacon roots could be obtained by using suitable cooking conditions such as boiling, microwaving, baking and steaming (Miyaguchi, Inoue, & Tsukihashi, 2012). They found that FOS content dramatically decreased with deep-frying and was little influenced by heating by methods such as boiling, microwaving, baking and steaming. In recent study steam blanching reduced polyphenoloxidase and peroxidase activity by 84.62 and 83.76%, respectively, with colour loss, and with losses of inulin, glucose and fructose of 30.64, 39.40 and 15.82%, respectively (Fante, Scher, Noreña, & Rios, 2012).

Recently was reported that in hexadecaploid yacon significantly higher levels of saccharides were detected (FOS 13.9 g 100^{-1} g^{-1} FM, fructose 4.6 g 100^{-1} g^{-1} FM and glucose 2.1 g 100^{-1} g^{-1} FM) compared to the octoploid control (FOS 5.3 g 100^{-1} g^{-1} FM, fructose 2.9 g 100^{-1} g^{-1} FM and glucose 1.0 g 100^{-1} g^{-1} FM). These results indicated that *in vitro* treatment for chromosome doubling and the polyploidy breeding can increase the FOS content in the tuberous roots (Viehmannová et al., 2009).

Some studies have shown prebiotic potential of yacon and a positive correlation between yacon ingestion and the reduction of the glycaemic response, especially toward diabetic individuals and therefore the preparation of bread with the addition of yacon meal was suggested recently (Habib et al., 2011; Rolim et al., 2011). Yacon tuberous root flour is a natural product rich in FOS that could be well positioned as a nutraceutical product with beneficial effects on diabetes-associated hyperlipidaemia. FOS are prospective prebiotics as they are fermented by beneficial species of gut bacteria (Du Pont & Du Pont, 2011; Havenaar, 2011). They are also used as a source of natural sweeteners and syrups suitable for persons suffering from digestive problems (Charalampopoulos & Rastall, 2012). Oral treatment with yacon syrup markedly accelerated colonic transit time in healthy individuals (Geyer, Manrique, Degen, & Beglinger, 2008) and increased defecation frequency and satiety sensation in obese and slightly dyslipidemic pre-menopausal women (Genta et al., 2009).

The addition of yacon flour on bread rendered products of low to moderate glycaemic index, with prebiotic potential, low fat and high fibre contents. Yacon is considered a novel food and is currently sold in many health food stores in Europe, mainly as syrup or dried slices (Ojansivu, Ferreira, & Salminen, 2011). Recently was yacon also cultivated in North Mississippi as a specialty crop expected that in

the near future could be introduced as a functional food to supplement the diet of Mississippians (Sumiyanto et al., 2012). Thus, yacon landraces with high FOS content and particularly SC-FOS may be very valuable in healthy human nutrition. Yacon root β-(2→1) fructooligosaccharides appear to be a good candidate as a prebiotic supplement (Valentová et al., 2006) and, in addition, improving the immune parameters (Delgado, Thomé, Gabriel, Tamashiro, & Pastore, 2012) in human nutrition.

4. Conclusions

Significant differences in the content of long-chain fructooligosaccharides (>GF10), short-chain fructooligosaccharides (GF3-GF10) and total carbohydrates between yacon landraces experimentally introduced in the Czech Republic have been found. The highest contents and index Lc-FOS/Sc-FOS were typical for the landraces originated in Peru, Bolivia, and Ecuador. On average, dodecaploid landraces (2n = 87) showed higher average values (60.35 g kg^{-1} DM Lc-FOS, 60.07 g kg^{-1} DM Sc-FOS and 168.6 g kg^{-1} DM total saccharides) in comparison with octoploid landraces (2n = 58) with lower mean values (36.59 g kg^{-1} DM Lc-FOS, 54.34 g kg^{-1} DM Sc-FOS and 132.3 g kg^{-1} DM TC) and both, octoploid and dodecaploid clusters differed significantly. Thus, the selected landraces with higher ploidy levels could be useful for obtaining plants containing in tuberous roots high levels of fructooligosaccharides and particularly short-chain fructooligosaccharides with beneficial prebiotic and other nutritional effects on human health and protection against metabolic disorders.

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