CONTENT OF POLYPHENOLIC ANTIOXIDANTS AND PHENOLIC ACIDS IN SELECTED PARTS OF YACON [*SMALLANTHUS SONCHIFOLIUS* (POEPP. ET ENDL.) H. ROBINSON]*

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Yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson], a native Andean plant cultivated for its tuberous roots and medicinal infusions from leaves, is very rich in phenolic components with strong antioxidant activities. The major phenolic components are chlorogenic acid and caffeic acid, present both in the form of their esters and in the free form. Total polyphenolics were estimated spectrophotometrically with Folin-Ciocalteau's phenolic reagent in four parts of the plant: tuberous roots, rhizomes, stems and leaves from the harvests in the years 2002 and 2003. Rhizomes and leaves were observed to be the richest sources of polyphenolic antioxidants (31 600 and 14 300 mg.kg^{-1} DM, respectively). Polyphenol contents in different parts decreased in order rhizomes > leaves > stems ≥ tuberous roots. As compared with potato tuberous roots, the yacon tuberous roots are 3–10 times richer in polyphenolic antioxidants. These results could evaluate the yacon tuberous roots as a rich source of phenolic antioxidants in human nutrition as well as leaves as an active component in dietary supplements for the prevention of chronic diseases. Moreover, the yacon rhizomes could be used both as plant cultivation material, as well as a rich source of polyphenolic antioxidants. Phenolic acids determined using HPLC were chlorogenic acid, caffeic acid, 3,5-*O*-dicaffeoylquinic acid and ferulic acid. Phenolic acids are contained mainly in rhizomes and leaves; lesser contents are present in tuberous roots and stems. Rhizomes significantly differed from other plant parts regarding the highest levels of phenolic acids. Contents in stems and tuberous roots were nearly comparable. While chlorogenic acid is the major constituent in rhizomes and tuberous roots, its precursor caffeic acid in stems. There were found both, quantitative and qualitative differences in the content of polyphenols and individual phenolcarboxylic acids in the years 2002 and 2003. Phenolic acids derived from cinnamic acid contained in yacon are dominant yacon antioxidants.

yacon; rhizomes; leaves; stems; tuberous roots; polyphenols; phenolic acids; chlorogenic acid; caffeic acid; 3,5-*O*-dicaffeoylquinic acid; ferulic acid

INTRODUCTION

Natural antioxidants occurring in foods and other biological materials have attracted a great interest regarding their potential and therapeutic effects in the last period (Valentová et al., 2001). Antioxidants according to their chemical structure could be divided into polyphenols (flavonoids, anthocyanins, phenolic acids and coumarins), carotenoids (carotenes – precursors of vitamin A and xanthophylls) and tocopherols (vitamin E). Also ascorbic acid (vitamin C) and selenium posses high antioxidant activity. Antioxidants scavenge free radicals sooner, than these radicals could harm and protect by this effect against oxidative damage. It was found that those antioxidants weaken oxidative changes caused by free radicals in human body and its cells. From the data obtained in vitro and biochemical knowledge, flavonoids could be regarded as the constituents with preventive effects against some types of cancer, heart and vascular diseases and with radical scavenging effect. Moreover, they positively affect immunity system, have anti-inflammatory effect, influence permeability and fragility of blood capillaries and vessels, renew liver cells, posses hypoazotemic effect and many other healthy effects (Meltzer, Malterud, 1997). These compounds relate activity of enzymes (e.g. inhibition of lipoxygenase), have antiviral, antibacterial and antifungal properties. Free radicals attack biomolecules (lipids, proteins, DNA) or cell biomembranes and these processes are inhibited by antioxidants. Anti-inflammatory effects and liquidation of free radicals are in very close correlation.

Yacon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson] represents a significant source of phenolic antioxidants. Its polyphenolic complex is formed mainly by phenolic acids (Chen, Ho, 1997). These acids are de-

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rived from benzoic and cinnamic acids and could be present both free and bound as esters. Yacon tuberous roots contain polyphenolic compounds on the level of 2030 $mg.kg^{-1}$, with chlorogenic acid being the dominant constituent (48.5 ± 12.9 $mg.kg^{-1}$, Yan et al., 1999). Chlorogenic acid (3-*O*-caffeoylquinic acid) and 3,5-*O*-dicaffeoylquinic acid are usual phenolic compounds contained in plants of family Asteraceae (Takenaka et al., 2003). Valentová et al. (2003) found in two fractions from yacon leaves caffeic acid (14.7 and 0.09 $mg.g^{-1}$), chlorogenic acid (9.9 and 1.7 $mg.g^{-1}$), protocatechuic acid (2.5 and 0.12 $mg.g^{-1}$) and ferulic acid in trace levels. Takenaka et al. (2003) confirmed on the basis of hydrolysis and comparison of NMR spectra that caffeic acid was bound with its phenolic groups as esters with altraric acid as 2,4-, 2,5- or 3,5-dicaffeoylaltraric acids, and 2,3,5- or 2,4,5-tricaffeoylaltraric acids.

The aim of this work was to determine the content of total polyphenols and individual phenolic acids in selected parts of yacon plants.

MATERIAL AND METHODS

Material. Samples of different parts of yacon from the harvests in the years 2002 and 2003 (trial field of the Czech University of Agriculture in Prague) were used – rhizomes, leaves, stems and tuberous roots. Cultivated material was originally introduced to Czech Republic from Bolivia (natural habitat San Pedro – Potosí on the altitude 2800 m above sea) in the year 1995. Yacon was harvested in October 2002 and 2003 after average 160-days' vegetation periods. The plants were separated into tuberous roots, rhizomes and leaves and plant material was subsequently freeze-dried using a Lyovac GT 2 (Leybold-Heraeus, Germany) freeze-drier.

Reagents and chemical standards used for the determination of total phenolics and phenolic acids. Folin-Ciocalteau's reagent (PENTA Chrudim CZ), HPLC gradient grade methanol (Merck, Germany), HPLC standards of phenolic acids: chlorogenic acid ZZ97%, caffeic acid ZZ97% and ferulic acid > 98% (T), (Fluka Chemie, Switzerland).

Determination of dry matter (DM). 2 g of finely ground-homogenized sample was weighed up and dried to constant weight at 105 °C.

Extraction of samples. The individual freeze-dried yacon samples were ground in a laboratory mill and approximately 12 g was weighed into a cartridge of a Soxhlet extractor and extracted with 80% ethanol/water for 18 hours. Extract was then transferred into 250 mL volumetric flask and adjusted with extraction solvent mixture till the mark.

Determination of total phenolics. Determination of total phenolics (TP) was carried out by the methods described by Lachman et al. (1996). Aliquots of sample extract were pipetted into 50 mL volumetric flask. Approximately 25 mL of distilled water and 2.5 mL of Folin-Ciocalteau's reagent (PENTA Chrudim CZ) were added and the mixture was thoroughly mixed and left to stand for 3 minutes. Then 7.5 mL of 20% Na_2CO_3 solution were added and the volume was adjusted till the mark with distilled water. Samples were centrifuged using a Janetzki T 30 centrifuge to remove precipitate. Absorbance of blue-coloured solution was measured against blank using an UV-VIS spectrophotometer Heλios γ (Spectronic Unicam, UK) at wavelength 765 nm. Contents of total phenolics in μg in aliquots of the extract were obtained. These results were expressed as mg of gallic acid in kg of sample dry matter. Mean content of polyphenols was calculated from four parallel determinations. Standard deviation of the determination of TP was 2.63%.

HPLC of phenolic acids. High performance liquid chromatography (HPLC) with gradient elution Waters™ (pump Waters™ 600S, autosampler Waters™ 717 plus, detector Waters™ PDA 996 – UV-VIS, column Watrex 250 x 4 mm Sepharon SGX C18 7 μm) was used for the determination of phenolic acids (Fig. 1). Solution of methanol in water (5%) was used as mobile phase A and solution of methanol in water (40%) as mobile phase B. The both phases were adjusted by phosphoric acid to pH 2.5. Flow rate through the column was 1 $mL.min^{-1}$, elution time 56 minutes, sample injection 20 μL (Table 1), absorbance detection at wave length 280 nm, and UV-VIS spectra were measured simultaneously (Figs 2 and 3). Parameters of gradient elution are given in Table 1. Quantitation of the individual phenolic acids was made by a method of absolute calibration. Detection limit was 0.1 $\mu g.mL^{-1}$. Mean content of phenolic acids was calculated from four parallel determinations. Standard deviation of HPLC determination of individual phenolic acids was 13.0%, in majority of measurements it ranged from 9.2 to 14%.

Table 1. Parameters of gradient elution

Time (min)	% A (V/V)	% B (V/V)
0	100	0
30	15	85
35	15	85
55	0	100
56	0	100
60	100	0

Statistical evaluation. The results obtained (mean values of four parallel determinations) were statistically evaluated by ANOVA and Tukey's methods with Statgraphics program by the variance analysis with single grouping.

RESULTS AND DISCUSSION

Results of the determination of total polyphenols are given in Table 2. From the observed data it is apparent

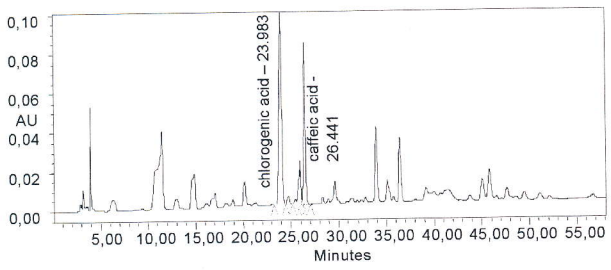

Fig. 1. Chromatogram of the extract from yacon tuberous roots

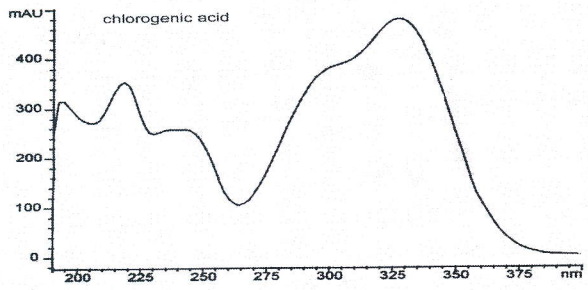

Fig. 2. UV spectrum of chlorogenic acid

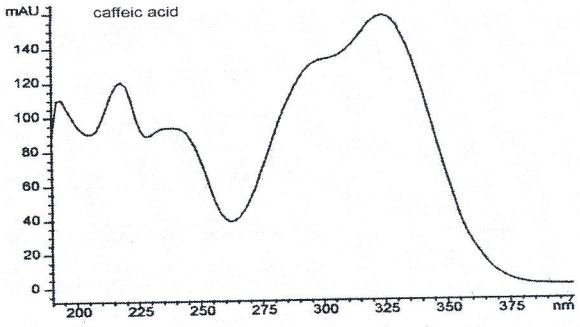

Fig. 3. UV spectrum of caffeic acid

that the highest content of total polyphenols was determined in yacon rhizomes. On the contrary, the lowest levels were determined in tuberous roots. Contents of total polyphenols in stems and tuberous roots of yacon are nearly comparable. Results of the determination of total polyphenols were evaluated statistically (Table 3). On the basis of more detailed evaluation of variance analysis by T-method (Table 4) it could be stated that there exists statistically significant difference ($p < 0.01$) in the content of total polyphenols between yacon rhizomes and other plant parts. With the same probability level, it was found that other parts of the plant (leaves, stems and tuberous roots) differed significantly each from other.

Only yacon stems and tuberous roots do not differ significantly each from other in the content of total polyphenols. The highest content of total polyphenols (42 200 mg.kg^{-1} DM in 2002, 21 000 mg.kg^{-1} DM in 2003) was found in rhizomes, lower levels were estimated in leaves (13 700 mg.kg^{-1} DM in the year 2002, 14 900 mg.kg^{-1} DM in the year 2003), stems (9000 mg.kg^{-1} DM in the year 2002, 6240 mg.kg^{-1} DM in the year 2003) and tuberous roots (6880 mg.kg^{-1} DM in the year 2002, 7680 mg.kg^{-1} DM in the year 2003) of yacon. The analysed parts could be thus arranged according to their polyphenol content in descending order: rhizomes > leaves > stems ≥ tuberous roots.

Polyphenolic compounds of yacon possess significant antioxidant effects as it was reported by Valentová et al. (2003) by DPPH and SOH tests. As it follows from the content and ratio of the individual phenolics, especially hydroxycinnamic acids contribute to the antioxidant properties of yacon. Among them, the most protective effect against lipid oxidation had caffeic acid in free and bound forms (esters) > chlorogenic acid > ferulic acid (Chen, Ho, 1997). Esterification of caffeic acid with quinic acid or methylation of hydroxy group (conversion to ferulic acid) leads to decrease of antioxidant activity, however, its value could be influenced also by synergic interactions.

As it follows from the comparison of the content of total polyphenolic antioxidants in the potato and yacon tuberous roots (Hamouz et al., 1997; Lachman et al., 2000), dried yacon tuberous roots contain approximately 3–10 times more polyphenolic antioxidants

Table 2. Content of total polyphenols (TP) in dry matter of the individual yacon parts

Part of plant	Average TP content 2002 (mg.kg^{-1} DM)	Average TP content 2003 (mg.kg^{-1} DM)	Average TP content 2002–2003 (mg.kg^{-1} DM)
Tuberous roots	6 880	7 680	7 280
Rhizomes	42 200	21 000	31 600
Stems	9 000	6 240	7 620
Leaves	13 700	14 900	14 300

Table 3. Results of variance analysis of single grouping

Source of variability	Degrees of freedom	Sum of squares	Variance	*F*-test	α
Part of plant	3	3.233E+09	1.078E+09	365.70	*0.00000
Residual	12	3.537E+07	2.947E+06		
Total	15	3.269E+09			

* – statistically significant effect

Table 4. Detail evaluation of variance analysis by *T*-method

Parts of plant	Tuberous roots	Rhizomes	Stems	Leaves
Tuberous roots		*0.0002	0.3415	*0.0007
Rhizomes	*0.0002		*0.0002	*0.0002
Stems	0.3415	*0.0002		*0.0099
Leaves	*0.0007	*0.0002	*0.0099	

* – Levels of significance, at which averages of TP content of the individual parts of plant, differ significantly

Table 5. Content of phenolic acids in dry matter of the individual yacon parts

Part of plant	CHA 2002 (mg.kg^{-1} DM)	CHA 2003 (mg.kg^{-1} DM)	CA 2002 (mg.kg^{-1} DM)	CA 2003 (mg.kg^{-1} DM)	3,5-D 2002 (mg.kg^{-1} DM)	3,5-D 2003 (mg.kg^{-1} DM)	FA 2002 (mg.kg^{-1} DM)	FA 2003 (mg.kg^{-1} DM)
Rhizomes	8040	6480	3050	2320	2850	< D.L.	183	< D.L.
Leaves	779	714	699	2090	9020	< D.L.	212	< D.L.
Stems	712	641	754	765	2330	< D.L.	214	< D.L.
Tuberous roots	942	1420	329	824	249	< D.L.	218	< D.L.

CHA – chlorogenic acid; CA – caffeic acid; 3,5-D – 3,5-*o*-dicaffeoylquinic acid, FA – ferulic acid, < D.L. – under detection limit

(2030–6880 mg.kg^{-1}) than potato tuberous roots (422–834 mg.kg^{-1}). The content of phenolic acids (chlorogenic, caffeic, 3,5-*O*-dicaffeoylquinic and ferulic acids) in dry matter of the individual parts of yacon plant was determined by HPLC (Fig. 1) and the results obtained are given in Table 5. Four phenolic acids derived from cinnamic acid were estimated in four analysed yacon parts: chlorogenic acid, caffeic acid, 3,5-*O*-dicaffeolyquinic acid and ferulic acid. It is in good agreement with the results obtained by Takenaka et al. (2003) and Valentová et al. (2003). The highest content of these acids was found in rhizomes (chlorogenic acid 8040 mg.kg^{-1} DM in the year 2002, caffeic acid 3050 mg.kg^{-1} DM in the year 2002 and 3,5-*O*-dicaffeoylquinic acid 2850 mg.kg^{-1} DM in the year 2002) and in the yacon leaves (3,5-*O*-dicaffeoylquinic acid 9020 mg.kg^{-1} DM in the year 2002, chlorogenic acid 779 mg.kg^{-1} DM in the year 2002 and caffeic acid 699 mg.kg^{-1} DM in the year 2002). Regarding the content of phenolic acids, the yacon parts could be compared in descending order: rhizomes > leaves > stems and tuberous roots. High content of phenolic acids in yacon leaves predetermines them for use in prevention and treatment of chronic diseases involving oxidative stress, as it was shown by Valentová et al. (2004). Contents in stems and tuberous roots were nearly comparable. In rhizomes and tuberous roots the highest content was found for chlorogenic acid (8040 mg.kg^{-1} DM in the year 2002 and 6480 mg.kg^{-1} DM in the year 2003 in rhizomes, 942 mg.kg^{-1} DM in the year 2002 and 1420 mg.kg^{-1} DM in the year 2003 in tuberous roots). There were found both, quantitative and qualitative differences in the content of polyphenols and individual phenolcarboxylic acids in the years 2002 and 2003. The average total polyphenol content was in the year 2003 by 30% less in comparison with the year 2002. Also differences in the contents of 3,5-*O*-dicaffeoylquinic acid and ferulic acid were found. Whereas in the year 2002 3,5-*O*-dicaffeoylquinic acid was the significant compound found in the leaves (9020 mg.kg^{-1} DM), in the year 2003 only trace amounts were found and 3,5-*O*-dicaffeoylquinic acid was evidently split into caffeic acid, which occurred in relatively higher amounts in the leaves (in contrary to rhizomes) as in 2002 in relation to total polyphenols. Also ferulic acid was found in 2003 in all yacon organs in difference to the year 2002, when it was present only in trace amounts. It seems that higher average temperature during vegetation period in the year 2003 and sun irradiation (Jaakola et al., 2003) as well as lesser mean precipitation in this year (Table 6) affected interconversions of phenolic acids. 3,5-*O*-dicaffeoylquinic acid could produce one mole of quinic acid and two moles of caffeic acid and that could by methylation transformed to ferulic acid, which is further involved in suberisation and polymers constitution (Mark et al., 1995; Li et al., 1997).

Caffeic acid is a precursor for biosynthesis of chlorogenic acid isomers, where it is attached to the molecule of quinic acid. The results are in accordance with conclusions of Valentová et al. (2003) that phenolic acids present in yacon leaves are chlorogenic acid and caffeic acid. In the extracts we refound only traces of free ferulic acid, which could be released by acid hydrolysis. Ferulic acid, isomers of dicaffeoylquinic acid and an unidentified derivative of chlorogenic acid in yacon leaves were reported in recent time by Simonovská et al. (2003). The compounds were identified on the basis of UV-VIS spectra by comparison with authentic samples.

CONCLUSION

Yacon as "once more appearing" crop (Michl, Valíček, 1996) represents a very rich source of antioxidants, above all of polyphenolic compounds. Whereas

Table 6. Mean air temperatures and mean precipitation during vegetation period of yacon in the years 2002 and 2003 compared with the long-term normal 1961–1990

Year		2002							2003						
Month		May	June	July	August	September	October	Average	May	June	July	August	September	October	Average
Prague and Central Bohemia	T (°C)	16.0	18.0	18.9	19.2	12.5	7.7	15.4	15.8	20.2	19.1	20.9	13.9	5.6	15.9
	N_T (°C)	12.9	16.1	17.5	17.0	13.4	8.5	14.2	13.0	16.3	17.8	17.2	13.6	8.6	14.4
	O (°C)	3.1	2.0	1.4	2.3	–0.8	–0.8	1.2	2.7	4.0	1.4	3.7	0.3	3.0	2.5
	S (mm)	56	87	87	162	57	71	86.7	72	38	73	30	25	38	46.0
	N_P (mm)	68	74	71	73	46	36	61.3	70	75	72	73	46	36	62.0
	% (mm)	82	118	122	224	124	196	144	103	51	102	41	55	106	76.3

T – temperature normal 1961–1990, O – deviation from long-term normal, S – mean precipitation amount, N_P – long-term precipitation normal 1961–1990, % – mean precipitation amount as percentage of the long-term normal

the yacon tuberous roots with low energetic potential could be used in human nutrition, infusions prepared from the leaves could be not only effective means against hyperglycaemia, but they also represent a relatively rich source of polyphenolic antioxidants. From this point of view high content of polyphenolic antioxidants in rhizomes is also interesting offering other use possibilities besides the seedling material. The main phenolic acids contained in yacon rhizomes and tuberous roots are chlorogenic acid and caffeic acid. Other phenolic acids, e.g. ferulic and other bound forms of caffeic acid are affected also by climatic conditions. Regarding the content of phenolics and phenolcarboxylic acids, the yacon parts could be arranged in descending order: rhizomes > leaves > stems ≥ tuberous roots. Rhizomes differ from other parts by apparently higher content of phenolic acids; their contents in stems and tuberous roots are nearly comparable. Phenolic acids derived from cinnamic acid, above all chlorogenic acid and its isomers, are significant yacon antioxidants.

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Obsah polyfenolických antioxidantů a fenolických kyselin ve vybraných částech jakonu [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson].

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Jakon [*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson], rostlina pocházející z And a pěstovaná pro své kořenové hlízy a léčivé nálevy z listů, je velmi bohatý na fenolické sloučeniny se silnými antioxidačními účinky. Hlavními fenolickými složkami jsou kyseliny chlorogenová a kávová, které jsou v jakonu obsaženy jak ve formě svých esterů, tak i jako volné. Obsah celkových polyfenolů byl stanoven spektrofotometricky s Folin-Ciocalteuovým fenolovým reagens ve čtyřech částech rostliny: kořenových hlízách, stonkových hlízách, stoncích a listech ze sklizní v roce 2002 a 2003. Nejbohatším zdrojem polyfenolických antioxidantů byly stonkové hlízy (průměrně 31 600 $mg.kg^{-1}$ sušiny) a listy (14 300 $mg.kg^{-1}$ sušiny). Části rostliny mohou být podle obsahu polyfenolických látek seřazeny v sestupném pořadí stonkové hlízy > listy > stonky ≥ kořenové hlízy. Ve srovnání s hlízami brambor jsou jakonové hlízy 3–10krát bohatší na polyfenolické antioxidanty. Z těchto výsledků vyplývá, že jakonové hlízy, resp. listy (příprava čajů) jsou v lidské výživě bohatým zdrojem fenolických antioxidantů jako aktivní složka dietetických doplňků při prevenci některých významných civilizačních chorob. Stonkové hlízy jakonu mohou být využity nejen jako sadba pro vegetativní rozmnožování, ale i jako bohatý zdroj polyfenolických antioxidantů. Fenolové kyseliny stanovené v jakonu pomocí HPLC byly chlorogenová, kávová, 3,5-*O*-dikávoylchinová a ferulová kyselina. Fenolové kyseliny byly zastoupeny převážně ve stonkových hlízách a listech, méně pak v kořenových hlízách a stoncích. Obsah těchto kyselin ve stonku a kořenových hlízách byl téměř srovnatelný. Zatímco chlorogenová kyselina byla hlavní složkou stonkových hlíz a kořenových hlíz, její prekurzor kávová kyselina byla významně zastoupena ve stoncích. Mezi sklizněmi v roce 2002 a 2003 byly nalezeny rozdíly jak v obsahu celkových polyfenolů, tak i v kvalitativním zastoupení jednotlivých fenolových kyselin. Fenolové kyseliny odvozené od skořicové kyseliny jsou dominantními antioxidanty jakonu.

jakon; stonkové hlízy; listy; stonky; kořenové hlízy; polyfenoly; fenolové kyseliny; chlorogenová kyselina; kávová kyselina; 3,5-*O*-dikávoylchinová kyselina; ferulová kyselina

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