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SHORT COMMUNICATION

The amino acid and carbohydrate composition of the herb and roots of *Smallanthus sonchifolius*

Složení aminokyselin a sacharidů v nati a kořenech *Smallanthus sonchifolius*

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Summary

The free and protein-bound amino acid composition of the herb and roots of *Smallanthus sonchifolius* was analyzed by HPLC method. Fourteen free and fifteen protein-bound amino acids were determined in yacon herb, and three free and fourteen protein-bound amino acids in the roots. Among the free amino acids, proline (0.44 μg/mg) and aspartic acid (0.12 μg/mg) were dominant in the herb and proline (0.28 μg/mg) in the roots. Among the protein-bound amino acids, aspartic acid (18.58 μg/mg), glutamic acid (16.33 μg/mg) and proline (14.52 μg/mg) prevailed in the herb, and proline (3.14 μg/mg) in the roots. Fructose, sucrose and arabinose were identified in free form in the herb of *S. sonchifolius* applying gas chromatography-mass spectrometry (GC-MS). The polysaccharide complex was obtained from yacon herb, its yield was 5.13 ± 0.09%. Fructose (3.11 μg/mg) was the only monosaccharide identified in the hydrolysate of the obtained complex.

Key words: *Smallanthus sonchifolius* (yacon) • amino acids • carbohydrates • GC-MS HPLC methods

Souhrn

Složení volných a v proteinech vázaných aminokyselin bylo analyzováno metodou HPLC v nati a kořenech *Smallanthus sonchifolius*. V nati jakonu bylo stanoveno čtrnáct volných a patnáct vázaných aminokyselin, v kořenu tři volné a čtrnáct v proteinech vázaných aminokyselin. V nati mezi volnými aminokyselinami dominoval prolin (0,44 μg/mg) a kyselina asparagová (0,12 μg/mg), v kořenech potom prolin (0,28 μg/mg). Mezi vázanými aminokyselinami v nati převažovala kyselina asparagová (18,58 μg/mg), kyselina glutamová (16,33 μg/mg) a prolin (14,52 μg/mg), v kořenech se jednalo zejména o prolin (3,14 μg/mg). V nati *S. sonchifoli* byly s použitím plynové chromatografie spojené s hmotnostní spektrometrií (GC-MS) identifikovány fruktosa, sacharosa a arabinosa ve volné formě. Z natě jakonu byl získán polysacharidový komplex a jeho výtěžek činil 5,13 ± 0,09 %. Fruktosa (3,11 μg/mg) byla jediným monosacharidem identifikovaným v hydrolyzátu získaného komplexu.

Klíčová slova: *Smallanthus sonchifolius* (jacon) • aminokyseliny • sacharidy • metody GC-MS HPLC.

Introduction

The rapidly increasing incidence of diabetes throughout the world[1)] has led to the expansion of the search for natural sources of biologically active substances to improve the quality of life and enrich the diet of this category of patients. One of the most promising plants in this case is *Smallanthus sonchifolius* (yacon). This species is the herbaceous perennial plant of the genus *Smallanthus* (*Polymnia*) in the Asteraceae family. Yacon, which is a distantly related species to the common sunflower (*Helianthus annus*) and Jerusalem artichoke (*Helianthus tuberosus*), was traditionally grown in Andean highland regions of Central and Western America. This vegetable and medicinal plant is introduced in many European countries[2)]. In recent years hypoglycemic and antioxidant activities of yacon[3–6)] have been investigated by scientists from different countries.

The tuberous roots of yacon have a sweet and crunchy flesh taste. Yacon roots sweetness is caused by free fructose that is predominant monosaccharide in the underground storage organs. The tuberous roots contain 70–80% saccharides, mainly fructooligosaccharides. The underground storage organs of yacon accumulate over 60% (on dry basis) of inulin type (2→1) fructans, mainly oligomers (GF2–GF16). The tuberous roots contain only 0.3–3.7% of protein. Also, the free amino acid

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composition of yacon roots was studied. The quantity of total free amino acids in the yacon ranged from 147.9 to 341.1 mg/100 g and it differed among the samples from different producing districts. The main free amino acids in yacon were arginine and glutamine in all cultivars[7]. The herb of *S. sonchifolius* is a by-product in the harvesting of yacon roots and additionally has a large phytomass that makes the phytochemical study of biologically active substances of this raw material promising. In contrast to the underground part, amino acids and carbohydrates of leaves and stems are not studied enough. In the literature, there are data that the aerial part of this plant contains 15.97% of protein (on dry basis)[8].

Previously, we reported about the carboxylic acid composition of the herb and roots of *S. sonchifolius*[9]. For further comprehensive research of the biologically active substances of yacon, it is expedient to study the amino acid and carbohydrate composition of these raw materials. It is important to note that amino acids and carbohydrates are substances of the primary biosynthesis of plants and influence the occurrence and development of the general pharmacological effect.

The aim of this study was to evaluate the content of free and protein-bound amino acids in the herb and roots of *S. sonchifolius* and to determine the qualitative and quantitative content of carbohydrates in the herb of this species.

Experimental part

Plant materials

The objects of the research were yacon herb and root crops harvested at the end of the growing season in the Merefa district of the Kharkiv region in the experimental farm of the Ukrainian Academy of Agrarian Sciences in 2018.

Determination of amino acids

Free protein-forming amino acids in the plant raw material were determined quantitatively after extraction of free amino acids from the plant raw material, and protein-bound amino acids were determined after acid hydrolysis of the preparations, followed by HPLC analysis of the hydrolysates, using pre-column derivatization by 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalaldehyde (OPA) and a fluorescence detector. Each analysis used five determinations.

Samples of the plant raw material were prepared and analyzed as follows:

Table 1. The amino acid content in the herb and roots of S. sonchifolius, μg/mg

№	Amino acid	TR, min	Herb			Roots		
			Free	Bound	Total	Free	Bound	Total
1	Asp	1.69	0.12 ± 0.05	18.58 ± 0.94	18.70 ± 0.98	–	0.96 ± 0.09	0.96 ± 0.08
2	Glu	3.09	0.07 ± 0.03	16.33 ± 0.58	16.40 ± 0.62	–	1.54 ± 0.17	1.54 ± 0.15
3	Ser	6.31	0.06 ± 0.04	6.12 ± 0.44	6.18 ± 0.51	–	0.60 ± 0.04	0.60 ± 0.03
4	His*	7.36	0.03 ± 0.01	2.74 ± 0.34	2.78 ± 0.42	–	–	–
5	Gly	7.79	0.01 ± 0.01	6.77 ± 0.37	6.78 ± 0.35	–	0.53 ± 0.07	0.53 ± 0.06
6	Thr*	8.01	0.03 ± 0.02	5.96 ± 0.28	5.99 ± 0.34	–	0.46 ± 0.06	0.46 ± 0.08
7	Arg*	8.91	0.08 ± 0.05	7.22 ± 0.56	7.30 ± 0.63	0.02 ± 0.04	0.83 ± 0.09	0.85 ± 0.08
8	Ala	9.47	0.02 ± 0.01	6.92 ± 0.47	6.94 ± 0.53	0.02 ± 0.05	0.74 ± 0.08	0.76 ± 0.09
9	Tyr	10.89	0.01 ± 0.01	3.81 ± 0.58	3.82 ± 0.49	–	0.27 ± 0.04	0.27 ± 0.02
10	Val*	13.12	0.03 ± 0.02	5.17 ± 0.39	5.21 ± 0.48	–	0.52 ± 0.06	0.53 ± 0.08
11	Met*	13.35	–	1.34 ± 0.12	1.34 ± 0.12	–	0.10 ± 0.01	0.10 ± 0.02
12	Phe*	14.83	0.03 ± 0.02	5.96 ± 0.78	6.00 ± 0.81	–	0.49 ± 0.03	0.49 ± 0.03
13	Iso*	15.06	0.02 ± 0.01	4.34 ± 0.75	4.36 ± 0.68	–	0.40 ± 0.03	0.41 ± 0.02
14	Leu*	15.82	0.01 ± 0.01	9.28 ± 1.36	9.29 ± 1.36	–	0.69 ± 0.07	0.69 ± 0.05
15	Pro	20.33	0.44 ± 0.05	14.52 ± 1.93	14.96 ± 1.95	0.28 ± 0.05	3.14 ± 0.16	3.42 ± 0.19
∑ essential		0.26	42.01	42.27	0.02	3.49	3.51	
∑ nessential		0.73	73.05	73.78	0.3	7.78	8.08	
∑		0.99	115.06	116.05	0.32	11.27	11.59	

* essential amino acids

a) *free amino acids:* 100 mg of powdered preparation was placed into a vial, treated with 2 ml of 1 N aqueous HCl solution, and held at 50 °C for 3 h in an ultrasonic bath.
b) *total amino acids:* 100 mg of preparation was placed into a vial, treated with 2 ml of 6 N aqueous HCl solution, and placed into a thermostatic chamber at 110 °C for 24 h.

Then, 0.5 ml of the centrifuged extract/hydrolysate was evaporated in a rotary vacuum evaporator, rinsed three times with distilled H_2O to remove HCl, re-suspended in 0.5 ml of distilled H_2O, and filtered through a 0.2 μm regenerated cellulose membrane. Amino acids were identified by comparing their retention times with a mixture of amino acid standards (Agilent 5061-3334). The contents of bound amino acids were determined by subtracting the contents of free amino acids from their total contents[10–12, 14].

Chromatographic separation was performed on an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) using a Zorbax AAA column (150 mm × 4.6 mm, 3 μm) and mobile phases A (Na_2HPO_4, 40 mm, pH 7.8) and B (AcCN– MeOH– H_2O, 45:45:10, v/v/v) in gradient mode at a constant flow rate of 1.5 ml/min. The column was thermostated at 40 °C. The pre-column derivatization was carried out in automated programmed mode using FMOC (Agilent 5061-3337) and OPA (Agilent 5061-3335). Derivatized amino acids were detected using a fluorescence detector. The results of studies of amino acids in the herb and roots of yacon are presented in Table 1.

Determination of the carbohydrates

Obtaining polysaccharides

20.0 g of powdered dry herb of *S. sonchifolius* was poured with 200 ml of water and heated under reflux in the water bath for 1 hour. The extraction was performed twice. The extracts were combined, concentrated to 20 ml and precipitated with four times the volume of 96% ethanol. The precipitate was separated, washed and dried.

Primary screening of monosaccharides and oligosaccharides, free monosaccharides and oligosaccharides

The vacuum dried supernatant obtained during the centrifugation of polysaccharides precipitate was used for identification of free mono- and oligosaccharides. The dry residue was dissolved in 0.5 ml of 96% ethanol and studied by descending paper chromatography in the acetone-n-butanol-water solvent system (7 : 2 : 1). The monosaccharides and oligosaccharides were detected by spraying the aniline hydrogen phthalate solution followed by heating in a drying cabinet up to 105–110 °C for 5–7 minutes. For identification of monosaccharides, reference solutions of standard samples were used.

The bound monosaccharides and oligosaccharides in polysaccharides

0.2 g of polysaccharides of yacon herb was dissolved in 0.72 ml of a mixture of water and ethanol (1 : 1) and hydrolyzed with the same volume of 20% sulfuric acid in a water bath, controlling the degree of hydrolysis by paper chromatography. The duration of the complete hydrolysis was 5 hours. The hydrolysate was neutralized, vacuum dried. The dry residue was dissolved in 0.5 ml of 96% ethanol and studied by the method described above.

HPLC conditions for qualitative and quantitative analysis

The qualitative and quantitative composition of sugars in plant material were determined by GC/MS based on the extraction of free sugars from plant material and full acid hydrolysis of herbal preparations to determine the total monosaccharide composition, followed by obtaining acetates of their aldonitrile derivatives and their analysis. Each analysis used five determinations.

Sample preparation and analysis of plant raw materials

a) *free monosaccharides:* 0.5 g of powdered dry plant material was placed in a vial and 5 ml of 80% ethanol was added. Extraction of free monosaccharides was performed in an ultrasonic bath at 80 °C for 4 h. Then, 2 ml of the extract were collected, evaporated to dryness, and re-suspended with 2 ml of an aqueous solution of the internal standard (2.5 mg per sample);
b) *monosaccharide composition after hydrolysis of plant raw material:* 5 ml of 2 M trifluoroacetic acid was added to 0.5 g of powdered dry raw material; hydrolysis held at 110 °C for 6 h. Then, 2 ml of hydrolyzate was collected, evaporated, and washed with water to remove trifluoroacetic acid. The hydrolysate was then re-suspended with 2 ml of an aqueous solution of the internal standard (2.5 mg per sample).

Chromatographic separation was performed on a chromatograph Agilent 6890N/5973inert (Agilent technologies, USA) using a capillary column HP-5 ms (3 mm × 0.25 mm × 0.25 μm, Agilent technologies, USA). Evaporator temperature was 250 °C, the interface temperature 280 °C. Separation was carried out in the programming mode of the temperature: initial temperature of 160 °C was maintained for 8 min, then raised with a gradient of 5 °C/min to 240 °C. The final temperature was held for 6 min. A sample of 1 μl was injected in a split flow mode 1 : 50. Detection was in the SCAN mode in a range of 38–400 m/z. The flow rate of carrier gas through the column was 1.2 ml/min. Identification was carried by retention time of monosaccharide standards and by the library of mass spectra NIST 02. Quantitative analysis was carried out by adding a solution of internal standard to the test sample.

The following mixture of standard samples was used: monosaccharides: Rhamnose, Arabinose, Xylose, Fucose, Mannose, Glucose, Galactose, Fructose; disaccharide:

Table 2. Carbohydrates of the herb of S. sonchifolius, mg/kg

Standard	Retention time, min	Free	Sum
Arabinose	5.90	16.47 ± 0.02	
Fructose	19.01	2.56 ± 0.01	3.11 ± 0.04
Sucrose	32.8640	18.28 ± 0.02	
Total		37.31	

Sucrose. For the internal standard solution Sorbitol was used[13–15]. The study of carbohydrates in the herb of the *S. sonchifolius* is presented in Table 2.

Results and discussion

As a result of the HPLC analysis, the content of 14 free and 15 protein-bound amino acids was determined in *S. sonchifolius* herb, and 3 free and 14 protein-bound amino acids were detected in roots. Among the free amino acids, Pro (0.44 μg/mg), Asp (0.12 μg/mg) were dominant in the herb. Asp (18.58 μg/mg), Glu (16.33 μg/mg), Pro (14.52 μg/mg) prevailed among the protein-bound amino acids in this raw material. In yacon roots, Pro was found in the highest quantity in free and bound forms.

Primary screening of the monosaccharides and oligosaccharides by paper chromatography showed the presence of fructose, sucrose and arabinose in the free form in the herb of *S. sonchifolius*. The polysaccharide complex was obtained from the yacon herb, its yield being 5.13 ± 0.09%. Fructose was the only monosaccharide identified during the pre-screening in the hydrolysate of the complex studied.

The chromatography-mass-spectrometry results confirmed the data obtained during primary screening. Sucrose, arabinose, and fructose were determined among free monosaccharides and oligosaccharides of the yacon herb. Sucrose was dominant in this raw material in the free form. In the hydrolysate of the polysaccharide complex, only fructose was detected (3.11 μg/mg).

Conflict of interest: none.

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