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Bioactive Substance and Free Radical Scavenging Activities of Flour from Jerusalem Artichoke (*Helianthus tuberosus* L.) Tubers – a Comparative Study

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Abstract

The Jerusalem artichoke (*Helianthus tuberosus* L.) known also as topinambour is plant with high importance for human and animal nutrition during the last decade. Its tubers were consumed fresh, stewed or they were added as flour in food products to improve their functional properties. In the current research a comparative study of bioactive substance and free radical scavenging activities of flour obtained from tubers of different varieties and wild populations of Jerusalem artichoke (*Helianthus tuberosus* L.) grown on territory of Bulgaria was done. The ultrasonic irradiation (42 kHz) was performed with 70 % ethanol and distilled water as solvents to accelerate extraction process. The total fructans, phenolic content and radical scavenging activities of the extracts were investigated. The 70 % ethanol extracts possessed the highest total phenolic content (6-17 mg GAE/g dry weight) and antioxidant activity defined by ABTS and CUPRAC methods. The water extracts characterized with higher fructan levels - 32 to 69 g/100 g dry weight. The flour obtained from tubers of Scorospelcu variety and wild population of *Helianthus tuberosus* L. were evaluated as a valuable source of total polyphenols and soluble dietary fibers, because of the rich fructan content. The results from our study also revealed the potential application of these flours as radical scavengers in human and animal nutrition for preparation of foods with improve health benefits.

Keywords: *Helianthus tuberosus* L., ultrasound-assisted extraction, total fructans, antioxidant activity

Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a perennial plant from Asteraceae family, originating from the United States. It has tall stem, large leaves, bright yellow sunflower-like flowers, and fleshy tubers (Yuan et al., 2013). As a most of the vegetables, the quality of Jerusalem artichoke tubers will depend of harvest conditions (Haytova 2013, Petkova et al., 2013).

The important feature of this plant is that its tubers are natural source of inulin. Inulin is a linear fructan, a plant reserve polysaccharide constituted by fructose molecules linked by $\beta(2\rightarrow1)$ bonds,with a terminal glucose unit linked by an α $(1\rightarrow2)$ bond (Kays & Nottingham, 2007). Its degree of polymerization (DP) typically ranges from 2 to 60 units,with number average values (DPn) of 10–12 units (Niness, 1999). Inulin is dietary fiber and as a part from this group it also have importance for human nutrition (Petrova, 2007).The tubers of Jerusalem artichoke (JA) possesses higher antioxidant activity, as the polyphenols were accomulated in higher concentrtion mainly in the epidermis (Seljåsen et al., 2005; Saikaew et al, 2010). Phenolics are secondary plant metabolites found in the majority of herbs, vegetables with well pronounced radical scavenging activity (Shao et al., 2008; Mihaylova et al., 2014; Georgieva and Mihaylova, 2014,)

JA tubers are rich source of biologically active substances including the naturally occurring isomers of caffeoylquinic acid namely neo-chlorogenic acid, chlorogenic acid and crypto-chlorogenic acid, 4 isomeric di-caffeoylquinic acids Caffeoylquinic derivatives represented minor amounts ranging from 2% for 5-caffeoylquinic, 4-caffeoylquinic and 4,5-dicaffeoylquinic to 18% for 1,3-dicaffeoylquinic acids (Kapusta et al., 2013). Previous phytochemical studies reported for the presence of coumarins, unsaturated fatty acids polyacetylenic derivatives and sesquiterpenes in H. tuberosus plants (Pan, 2009).

H. tuberosus has also been reported to have aperient, cholagogue, diuretic, spermatogenic, stomachic, and tonic effects and has been utilized as a folk medicine for the treatment of diabetes and rheumatism (Kays and Nottingham, 2007).

Because of its nutritional values and heathy effect Jerusalem artichoke has been used in food, pharmaceutical, feed, sugar, paper, cosmetics, and bioethanol industry, and in desert and tideland control (Cieślik et al., 2005; Petkova et al., 2012; Yuan et al., 2012; Vlaseva et al., 2014). The interest to application of *Helianthus tuberosus* tuber flour or extracts from then still increased due to the potential healthy effect. To the best of our knowledge the information about total phenols and radical scavenging activity of Jerusalem artichoke tubers especially for some varieties grown in Bulgaria were uncompleted or unavailable.

The aim of the current study was to determinate the content of biologically active substances (total phenols and fructans) and to evaluate antioxidant activities of extracts obtained from tubers of the different varieties and wild populations of Jerusalem artichoke.

Materials and Methods

The tubers of different varieties (Energina, Topstar, Scorospelcu) and wild populations *Helianthus tuberosus* L. were collected from Bulgaria. The Jerusalem artichoke tubers were washed with tab-water, sliced and air-dried at room temperature. Then plant samples were finely ground in a grinder with 1 mm sieve and prepared for further analysis.

Dry matter contents of tuber flour were determined by drying the samples at 105 ^{o}C until the constant weight (AOAC, 2005).

The extraction process of biologically active substances from flour from tubers of *Helianthus tuberosus* were carried out with two solvents – 70 % (v/v) ethanol and subsequent water extraction in ultrasonic bath (VWR, Malaysia) with frequency 45 kHz and 30 W power. In centrifuge tube from 15 mL with screw cap were weighed 0.5 g Jerusalem artichoke tuber flour. Ten mL solvent (70 % ethanol or water, respectively) were added to samples and the tubes were placed in ultrasonic bath at 45 °C for 15 min. The ultrasound-assisted extraction were performed in triplicate. Each extract was filtered and the combined extracts were used for further analysis.

Measurement of total phenolic content. The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent according to the described procedure (Stintzing et al., 2005) with slight modifications. Basically, 0.2 ml test sample was mixed with 1 ml Folin–Ciocalteu reagent diluted five times and 0.8 mL 7.5 % Na_2CO_3. The reaction was 20 min at room temperature in darkness. After reaction time, the absorption of sample was recorded at 765 nm against blank sample, developed the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (DW), according to calibration curve; build in range of 0.02 - 0.10 mg (Ivanov et al., 2014).

Antioxidant activity (AOA):

ABTS+ radical scavenging ability assay. ABTS radical was generated by mixing aliquot parts of 7.0 mM 2, 2`azinobis (3)ethylbenzthiazoline-6-sulfonic acid (ABTS, Sigma) in dd H2O and 2.45 mM potassium persulfate (Merck) in dd H2O. The reaction was performed for 16 h at ambient temperature in darkness and the generated ABTS radical is stable for several days. Before analyses, 2.0 mL of generated ABTS·+ solution was diluted with methanol at proportions 1:30 (v/v), so the obtained final absorbance of the working solution was about 1.0 ÷ 1.1 at 734 nm. For the assay, 2.85 mL of thisABTS·+ solution was mixed with 0.15 mL of obtained extracts. After 15 min at 37 °C in darkness the absorbance was measured at 734 nm against methanol. The antioxidant activity was expressed as mM (TE)/g DW by using calibration curve, build in range of 0.05-0.5 mM Trolox (Fluka) dissolved in methanol (Merck) (Ivanov et al., 2014).

CUPRAC assay: The reaction was started by mixing of 1 ml $CuCl_2 \times 2H_2O$, 1 ml Neocuproine (7.5 ml in methanol), 1ml 0.1 M ammonium acetate buffer; 0.1 ml of analyzed extracts and 1 ml 1 ml d. H_2O. The reaction time was 20 min at 50 °C in darkness. After cooling the absorbance was measured at 450 nm (Marchev et al., 2012). All the results from the determination of antioxidant activity were performed in triplicates and expressed as mM Trolox equivalents (mM TE) by dry weight.

Total fructans The total fructans content expressed as fructose equivalent in the obtained extracts was analysed spectrophotometrically at 480 nm by ketose specific resorcinol-thiourea reagent (Pencheva et al., 2012).

All the data were expressed as mean ± standard deviation (SD). Statistical analysis was performed with Student's t-test. A difference was considered statistically significant, when $P < 0.05$.

Results

Dry matter contents of flours obtained from *Helianthus tuberosus* L. tubers were in range 8-10 %.

The obtained extracts from Jerusalem artichoke tubers harvested during the autumn showed the highest total phenolic contents: 12-16 (in 70 % (v/v) ethanol) and up to 10 mg GAE/g DW (water extraction), respectively (Figure 1).

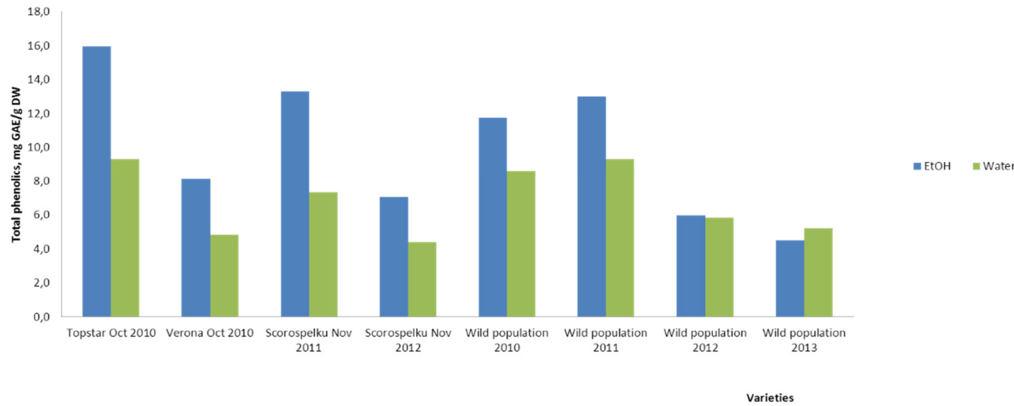

Figure 1. The total phenolic content of 70 % ethanol and water extracts from flour from tubers of *Helianthus tuberosus* L., mg/g GAE dw.

The Jerusalem artichoke varieties differed in the total concentration of phenolic content in tubers. Topstar, Scorospelcu and wild varieties from 2011 possessed the highest values. The results from our investigation were near to with reported results for total phenolic content in JA tubers 22.40 ± 0.63 GAE/g of dry extract, respectively (Yuan et al., 2013)..

To evaluate the antioxidant activities of obtained by ultrasonic extraction 70 % ethanol and water extracts from *Helianthus tuberous* tuber flours, their abilities to scavenge the synthetic ABTS radicals, as well as their power to reduce and cupric (CUPRAC) ions were investigated (Table 1).

Our study showed that the 70 % (v/v) EtOH extract from Scorospelcu and wild population 2011 showed the highest antioxidant activities (50-60 and 212-260 mM TE/g dw for methods ABTS and CUPRAC, respectively). The results for radical scavenging activities of 70% ethanol and water extracts obtained after 45 kHz ultrasonic frequency were higher than the same extracts obtained by 35 kHz ultrasonic frequency and power 200 W (Petkova et al., 2012).).

Table 1. Antioxidants activities of 70 % ethanol and water extracts from tubers of *Helianthus tuberosus* L., mM TE[+]/g dw

Varieties		70% EtOH Extract		Water Extract	
	Harvest time	ABTS	CUPRAC	ABTS	CUPRAC
Topstar	October 2010	39.6±3.3	139.1±3.1	41.36±0.9	118.1±4.2
Verona	October 2010	31.5±6.3	187.7±3.8	17.5±1.6	55.0±3.3
Scorospelcu	November 2011	46.8±3.3	212.0±1.5	25.3±1.5	94.9±3.1
	February 2012	26.2±0.4	174.1±0.3	15.7±1.4	52.9±4.2
Wild population	November 2010	51.4±1.3	170.8±3.5	35.4±3.6	121.1±0.6
	September 2011	59.2±2.6	259.7±3.8	41.8±1.7	198.1±2.1
	November 2012	19.2±0.7	169.4±2.3	23.1± 6.4	66.9±3.2
	November 2013	12.1±0.4	152.0±1.3	20.1±3.3	57.1±0.8

[+]TE: Trolox equivalents), dw: dry weight

Table 2. Fructans (inulin and FOS+) content in extracts from *Helianthus tuberosus* L. tubers after ultrasound-assited extraction, g/100 g dw

Varieties	Harvest time	70% EtOH Extract (Fru, Suc and FOS)	Water Extract (Inulin)
Topstar	October 2010	25.5 ± 5.6	45.3 ± 5.0
Verona	October 2010	24.8 ± 0.4	49.9 ± 0.9
Scorospelcu	**November 2011**	**33.6 ± 1.1**	**68.7± 0.9**
	February 2012	26.5 ± 0.1	45.3 ± 0.9
Wild population	November 2010	20.0 ± 6.3	55.2 ± 0.9
	September 2011	**21.1 ± 3.2**	**57.5 ± 1.3**
	November 2012	20.2 ± 8.4	42.5 ± 1.2
	November 2013	12.2 ± 0.9	40.5± 0.9

FOS: fructooligosacharides, Fru: fructose, Suc : sucrose, dw:dry weightThe spectrophotometric analysis of investigated flours from *Helianthus tuberosus* L. showed that in water extracts fructan fractions expresses as fructose equivalents were in the highest concentration (Table 2). All flours obtained from Jerusalem artichoke tubers contained the high molecular water soluble fraction (inulin) that was higher than the ethanol soluble low molecular fraction (fructose, sucrose and FOS). As previously reported by us results for conventional extraction of fructans from the same varieties *Helianthus tuberosus* grown on the territory of Bulgaria during a period of three years (2010-2013) (Petkova et al., 2013) and now with the application of ultrasound-assisted extraction procedure, the total fructan content in the middle and late varieties was close to 50% dw. This confirmed the data reported in literature (Praznik et al., 1998). The highest total fructan level in tuber flour was observed in Scoruspelcu variety 69 % dw., as previously reported by us (Petkova et al., 2014) and Bagaoutdinova et al. (2001) as a perspective source of inulin and FOS.

The ultrasound irradiation procedure shortened the time for extraction with 5 hours in comparison to conventional one (Petkova et al., 2013). In our previous report the application of ultrasound irradiation with frequency 35 kHz higher the extraction yield of fructans with 10 %. (Petkova et al., 2012). This may be due to the different ultrasound intensity (W/cm^2).

Discussion

The analyzed JA tuber flours consisted of many biological active substance (phenols and dietary fibers) with significant importance for human healthy nutrition. The higher antioxidant activity of tubers prevent from oxidative stress. Autumn harvest JA tubers possessed higher content of total polyphenols in comparison of spring harvest varieties. Our observation coincided with report of Seljåsen and Slimestad (2005). Meanwhile, the extracts from tubers of Jerusalem artichoke grown in Bulgarian possessed higher total phenol content (10-16 mg GAE/g dw), than the published results for Chinese Jerusalem artichoke varieties - 42.50 mg GAE/100g dw (Saikaew et al., 2010) and Polish varieties Rubik and Albik –1.7 g kg^{-1} and Albik 1.4 g kg^{-1} of total phenolics (Kapusta et al., 2013).
The results indicated that 70 % ethanol, as well and water JA tuber extracts exhibit excellent radical scavenging ability in all assays employed.

In tubers of Scorospelcu varieties has been found that the maximum amount of low- and high-molecular fructan can reach 65 %, as the greater part of tubers is a low-molecular fraction (Bagaoutdinova et al., 2001). Due to its high nutritive value and proportions of fructans, the flour of Jerusalem artichoke tubers may be fully utilized as functional food supplement (Berghofer and Reiter., 1997), because of properties of inulin to act as prebiotic (Knudsen and Hessov, 1995; Kleessen et al., 2007) and immunostimulator (Barcley et al., 2010). Therefore, the flour prepared from Scorospelcu and wild varieties H. tuberosus L. were promising source of natural antioxidants and dietary fiber.

Conclusion

The current investigation is the first comprehensive study that presented detailed information for total phenolic, total fructan and antioxidant activity of flours obtained from tubers of different varieties *Helianthus tuberosus* L. grown in Bulgaria. Application of ultrasound irradiation accelerates extraction process of biologically active substance from Jerusalem artichoke tubers, shorts extraction time and keeps active compounds unaltered. The antioxidant potential of extracts positively correlated with their phenolic contents. The flours from tubers of wild varieties 2011 and Scorospelcu were evaluated as a rich source of biological active substances. Therefore, the tuber flours of this plant present perspective source of dietary fibers and antioxidants with high importance for people and animal nutrition and for production of healthy food with well-pronounced healthy effect.

References

AOAC International 2007. Official methods of analysis, 18th edn, 2005; Current through revision 2, 2007 (On-line). AOAC International, Gaithersburg, MD, USA

Bagaoutdinova R. I., Fedoseyeva G. P., Okoneshnikova T. F., 2001. Fructose-containing carbohydrates in plants of different families localization and content. *Chemistry and Computational Simulation, Butlerov Com*., 2(5).

Barclay, T., Ginic-Markovic, M., Cooper, P., Petrovsky, N., 2010. Inulin - a versatile polysaccharide with multiple pharmaceutical and food chemical uses*. J. Excipients and Food Chem* (3).

Berghofer E., Reiter E., 1997. Production and functional properties of Jerusalem artichoke powder. Carbohydrates as organic raw materials. IV WUV Universitätserlag, 153 - 161.

Cieślik, E., Kopeć, A., Praznik, W., 2005. Healthily properties of Jerusalem artichoke flour (*Helianthus tuberosus* L.). *EJPAU* 8(2):37

Georgieva L., Mihaylova D., 2014. Screening of total phenolic content and radical scavenging capacity of Bulgarian plant species. *International Food Research Journal.* accepted (ISSN 1985-4668).

Haytova, D. 2013. Influence of foliar fertilization on the morphological characteristics and short-term storage of fruits of zucchini squash, Ecology and future – *Journal of agricultural Science and forest science,* XII(1): 33-39. BG

Ivanov I.G., Vrancheva R.Z., Marchev A.S., Petkova N.T., Aneva I.Y., Denev P.P., Georgiev V.G., Pavlov A.I. 2014л Antioxidant activities and phenolic compounds in Bulgarian Fumaria species. *Int.J.Curr.Microbiol.App.Sci* 3(2): 296-306

Kapusta, I., Krok, E., Jamro, D., Cebulak T., Kaszuba, J., Salach, R., 2013. Identification and quantification of phenolic compounds from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. *Journal of Food, Agriculture & Environment* 11 (3&4): 601 - 606.

Kara, B., Aydan, V., Akman, Z., Adiyaman, E., 2009. Determination of silage quality, herbage and hay yield of different triticale cultivars. *Asian Journal of Animal and Veterinary Advances* 4(3):167-171.

Kays, St. J., Nottingham St. F., 2008. Biology and Chemistry of Jerusalem artichoke: Helianthus tuberosus L. CRC Press.

Kleessen, B., Schwarz, S., Boehm, A., Fuhrmann, H., Richter, A., Henle, T., Krueger, M., 2007. Jerusalem artichoke and chicory inulin in bakery products affect faecal microbiota of healthy volunteers. The British Journal of Nutrition 98 (3) 540-9

Knudsen, K. E. B, I. Hessov. 1995 Recovery of inulin from Jerusalem artichoke (Helianthus tuberosus L.) in the small intestine of man. British Journal of Nutrition, 74, 101-113.

Marchev, A., A. Petrova, D. Nedelcheva, I. Lazarova, B. Trucheva, N. Kostova, V. Bankova, A. Pavlov 2011. GS/MS profiles and antioxidant activity of extract from Lavandula vera MM and Rosa damascene Mill. cell suspension cultures. Scientific works-UFT. Vol. LVIII (2). 183-188.

Mihaylova, D., Lante, A., Krastanov, A., 2013 . Total phenolic content, antioxidant and antimicrobial activity of *Haberlea rhodopensis* extracts obtained by pressurized-liquid extraction. *Acta Alimentaria*, 1-7.

Niness K., 1999. Inulin and Oligofructose: What Are They. *J. Nutr.* July 1. vol. 129 no. 7 , 1402S-1406s.

Pan, L., Sinden, M.R., Kennedy, A., Chai H., Linda E. Watson L., Graham, T., Kinghorn, D. 2009. Bioactive constituents of *Helianthus tuberosus* (Jerusalem artichoke), *Phytochemistry* 2:15–18.

Pencheva, D., Petkova, N., Denev, P., 2012. Determination of inulin in dough products, Scientific works of UFT, LIX: 339-344.

Petkova, N., Denev, P., Ivanova, M., Vlaseva, R., Todorova, M., 2013. Influence of harvest time on fructan content in the tubers of *Helianthus tuberosus* L, Nutrihort proceeding papers, Ghent, 284-289.

Petkova, N., Vrancheva, R., Ivanov, I., Denev, P., Pavlov, At., Alexieva, Y., 2012. Analysis of biologically active substances in tubers of Jerusalem artichoke (*Helianthus tuberosus* L.), Proceeding of 50 years FoodRDI "Food, Technologies & Health", 49-54.

Petrova, Iv., 2007. Dietary fibers and nutrition. Proceedings of Scietific Papers, Union of Scientists Bulgaria – Plovdiv, 44-47.

Praznik W., Cieślik E., Filipiak A., 1998. The influence of harvest time on the content of nutritional components in tubers of Jerusalem artichoke (Helianthus tuberosus L.). Proc. Seven Semin. Inulin, Belgium, 154 – 157

Saikaew, S., Tangwongchai R., Sae-Eaw A., 2010. The effect of temperature and storage time on the chemical and physical compositions changes of KaenTaWan *(Helianthus tuberosus L.) tubers after harvesting. Agricultural Science J.* 4.(3/1):249-252.

Seljåsen, R., Slimestad, R., 2005. Fructooligosaccharides and phenolics in flesh and peel of spring harvested Helianthus, *SHS Acta Horticulturae 744: I* International Symposium on Human Health Effects of Fruits and Vegetables

Shao, H.B., Chu, L.Y., Lu, Z.H., Kang, C.M., 2008. Primary antioxidant free radical. Scavenging and redox signaling pathways in higher plant cells. *Int. J. Biol. Sci.* 4, 8–14.

Stintzing, F.C., Nerbach, K.M., Mosshammer, M., Carle, R., Yi, W., Sellappan, S., Acoh, C.C., Bunch, R., Felker, P. 2005, Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. *J. Agric Food Chem*. 53 (2): 442-451.

Vlaseva, R., Ivanova, M., Petkova, N., Todorova, M., Denev, P.,2014. Analysis of fermented lactic acid dairy products enriched with inulin-type fructans // *Scientific Bulletin. Series F. Biotechnologies* XVIII: 145-149.

Yuan, X., Gao, M., Xiao, H., Tan, Ch., Dua, Y., 2012. Free radical scavenging activities and bioactive substances of Jerusalem artichoke (*Helianthus tuberosus* L.) leaves, *Food Chemistry* 133:10–14.