

Research Article

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J. Brkljača*, M. Bodroža-Solarov, J. Krulj, S. Terzić, A. Mikić
and A. Marjanović Jeromela

Quantification of Inulin Content in Selected Accessions of Jerusalem Artichoke (*Helianthus tuberosus* L.)

Abstract: Jerusalem artichoke is an excellent source of inulin. Inulin has valuable nutritional and functional attributes, and therefore it is needed to know inulin content in different accessions of Jerusalem artichoke tubers. We used rapid high-performance liquid chromatography (HPLC) method following water extraction to determine inulin content in Jerusalem artichoke tubers. HPLC conditions included Rezex RCM-monosaccharide Ca^{2+} column, deionized water as mobile phase and light scattering detection. It was found that inulin content of Jerusalem artichoke tubers ranged from 8.16 to 13.46% of fresh weight. The maximum value of inulin content in 12 accessions of Jerusalem artichoke was detected in TUB CG 32.

Keywords: inulin content, HPLC, Jerusalem artichoke

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Introduction

The Jerusalem artichoke (*Helianthus tuberosus* L.) is a native plant of North America (Kays and Nottingham, 2008) but there are around 14 collections in Europe, of which the largest one is in Serbia, and it is the third largest in the world (Terzić *et al.*, 2012).

*Corresponding author: J. Brkljača, Institute of Food Technology, University of Novi Sad, Bul. cara Lazara 1, 21000 Novi Sad, Serbia, E-mail: jovana.brkljaca@fins.uns.ac.rs

M. Bodroža-Solarov, J. Krulj, Institute of Food Technology, University of Novi Sad, Bul. cara Lazara 1, 21000 Novi Sad, Serbia

S. Terzić, A. Mikić, A. Marjanović Jeromela, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

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The Jerusalem artichoke can be used as feedstock for ethanol production, in food industry due to its very interesting nutritional, functional, health-promoting and technological properties (Luisa Beirão-da-Costa *et al.*, 2005; Lingyun *et al.*, 2007), as a substitute for lipid compounds and a supplement for sugar as high-fructose syrup (Baker *et al.*, 1993; Fleming *et al.*, 1979; Šimonová *et al.*, 2010), in animal feed as flour and dried mash, etc. (Saengthongpinit, 2005). A fresh Jerusalem artichoke tuber contains 80% water, 15% sugars and about 2% protein. Tubers contain inulin instead of starch and sucrose found in most other tubers. Inulin in various plant sources (Jerusalem artichoke and chicory) is of considerable importance in terms of its utilization. Content of inulin in both plants is >15% on a fresh weight basis according to De Leenheer *et al.* (1996) which indicates that Jerusalem artichoke is economically significant industrial crop within European cropping systems. The aim of plant breeders has been primarily to enhance tuber production and inulin content (Kays and Nottingham, 2008). Inulin, a non-digestible oligosaccharide, consists of fructose molecules (chain length from 2 to 60/70 units) linked by $\beta(2 \rightarrow 1)$ glycosidic bonds, with a terminal unit of D-glucose molecule linked to fructose by an $\alpha(1 \rightarrow 2)$ bond, as in sucrose (Suzuki, 1993; Chiavaro *et al.*, 2007). In addition to the high levels of inulin in the tubers, Jerusalem artichoke is also regarded as a good source of soluble and insoluble fiber (Terzić and Atlagić, 2009).

The inulin content of Jerusalem artichoke tubers ranges from 7 to 30% of its fresh weight (Kays and Nottingham, 2008; Saengthongpinit, 2005). Inulin content is very important because inulin has a positive effect on blood glucose attenuation, lipid homeostasis, mineral bioavailability, and it also has the ability to improve rheological characteristics, technological quality and nutritional properties of food products (Niness, 1999; Filipović *et al.*, 2013).

The aims of this study are to:

- (1) test and adopt a reliable inulin extraction and quantification procedure and
- (2) determine quantity of inulin in selected accessions of Jerusalem artichoke grown in Serbia.

Materials and methods

The trial was performed in Rimski šančevi, on the field of the Oil Crops Department of the Institute for Field and Vegetable Crops in Novi Sad, Serbia (45°19'40" N, 19°49'41" E). The material consisted of 141 accessions of Jerusalem artichoke, but the quantification of the inulin content was investigated in 12 selected accessions. The selection of these accessions was based on the results

of the total sugar content and certain phenotypic traits like tuber number and size, obtained in a wide characterization trial (Terzić, 2010).

Selected accessions were grown on dark chernozem soil in 2010 using the randomized complete block design (RCB). Each genotype was sown in four replications. Planting was done during the last decade of March. Accessions were grown in 1.5 by 3.5 m plots with 21 plants per plot (40,000 plants/ha). Plant spacing was 1 m between rows and 0.5 m between plants within rows. Tubers were planted in a nursery given standard locally recommended agronomy for cultivated sunflower. Plants were not irrigated. The nursery was fertilized with 300 kg ha⁻¹ of NPK fertilizer type 15:15:15.

Several methods for inulin extraction from Jerusalem artichoke have been developed (Kays and Nottingham, 2008). The selection of an optimal extraction method is dependent on the desired end product, equipment, volume and other factors. Considering the available resources, inulin was extracted by using a modified method from Lorenzo *et al.* (1997), using hot deionized water. The Jerusalem artichoke tubers were first washed and the cleaned tubers were ground into small pieces and steamed at atmospheric pressure for about 10 minutes to inactivate the inulin degrading enzymes. The steamed tubers were milled using a Knifetec 1095 Mill (Foss, North America). The ground tubers (20 g) were transferred into boiling water (60 ml water) for inulin extraction, where they were kept for a period of 10–15 minutes.

After that the entire mass was filtered through a muslin cloth. The hot filtered extract was collected and stored refrigerated. The samples were filtered through a 0.2 µm membrane filter before analysis by high-performance liquid chromatography (HPLC) with light scattering detection.

The HPLC system consisted of the Agilent liquid chromatography 1200 Series (Agilent Technologies, Santa Clara, CA, USA), a binary pumped system equipped with light scattering detection. The analytical column was Rezex RCM-monosaccharide Ca⁺² (300 × 7.8 mm) kept at a constant temperature of 50°C. The injection volume was 20 ml. Deionized water mobile phase was used in the isocratic gradient program at a flow rate of 0.6 ml min⁻¹. The acquisition of chromatograms lasted for 17 min.

Statistical analysis

The data were processed statistically using STATISTICA 10 Software (Statsoft Inc., 2010, Tulsa, Oklahoma). The significance of the difference between the mean values was estimated by one-way analysis of variance (ANOVA), and these were compared by the Fisher's Least Significant Difference (LSD) Test.

Results and discussion

It is assumed that there is a linear relationship between HPLC output and sample concentration. Under these conditions, the concentration of inulin in the injected sample, C_{Sample} expressed in mg/ml, and the peak area, A_{Sample} are proportional:

$$C_{\text{Sample}} = K_B \times A_{\text{Sample}}$$

The response factor K_B is defined from the concentration expressed in mg/ml and peak area of the standard solution using the average of values.

$$K_B = C_{\text{Standard}}/A_{\text{Standard}}$$

The concentration of inulin in Jerusalem artichoke sample expressed in mg/ml of Jerusalem artichoke is defined as:

$$\text{Inulin \% Jerusalem artichoke} = C_{\text{Sample}} \times 100/C_{\text{Jerusalem artichoke}}$$

where $C_{\text{Jerusalem artichoke}}$ is the effective concentration of Jerusalem artichoke expressed in mg/ml of analyzed sample solution.

Table 1: Inulin content in selected accessions of Jerusalem artichoke tubers obtained by HPLC

Accession	Inulin concentration (%)
TUB CG 32	13.46 ± 0.19 ^a
TUB CG 58	12.33 ± 0.08 ^b
CG 26	10.77 ± 0.37 ^{de}
CG 2051	11.11 ± 0.12 ^d
TUB CG 8	12.30 ± 0.05 ^b
TUB BP 5	10.40 ± 0.25 ^e
TUB CG 4	9.95 ± 0.16 ^e
TUB BP 23	8.16 ± 0.21 ^g
TUB BP 12	9.64 ± 0.64 ^f
TUB CG 61	11.75 ± 0.23 ^c
TUB BP 10	9.13 ± 0.04 ^f
TUB BP 11	11.01 ± 0.01 ^d

Note: Data are the average values of concentrations. Within column, mean values followed by different superscript letters differ significantly at $P < 0.01$ (LSD test).

Inulin content presented a maximum value in TUB CG 32, and it was 13.46%, while the lowest concentration was detected in TUB BP 23 (8.16%) (Table 1). The

differences between accessions were statistically significant. Our study is in accordance with Van Loo *et al.* (1995) who considered that typical inulin content is between 8 and 21% of fresh weight. Matias *et al.* (2011) found inulin content in Jerusalem artichoke tubers between 136.75 and 164.78 (mg/g) of fresh tubers. Our results show relatively low level of inulin in Jerusalem artichoke tubers. It could be influenced by genetic potential and different temperatures during storage (Cabezas *et al.*, 2002), while inulin content also depends on the extracting technical conditions (Lingyun *et al.*, 2007). Inulin content obtained in our samples is similar to inulin content in the chicory root which can contain up to 20% of storage carbohydrates, mainly inulin (Baert *et al.*, 1992). Douglas and Poll (1986) compared 14 chicory varieties and inulin contents varied from 10.6 to 20.5%. In spite of Jerusalem artichoke and chicory, globe artichoke has lower inulin content (2–9%), but higher degree of polymerization (Kays and Nottingham, 2008).

The comparison of the obtained chromatograms revealed that the distribution profile of peaks did not change significantly. On the other hand, the peak area varied greatly between the lowest found in accession TUB BP 23 and highest in accession TUB CG 32 (Figure 1).

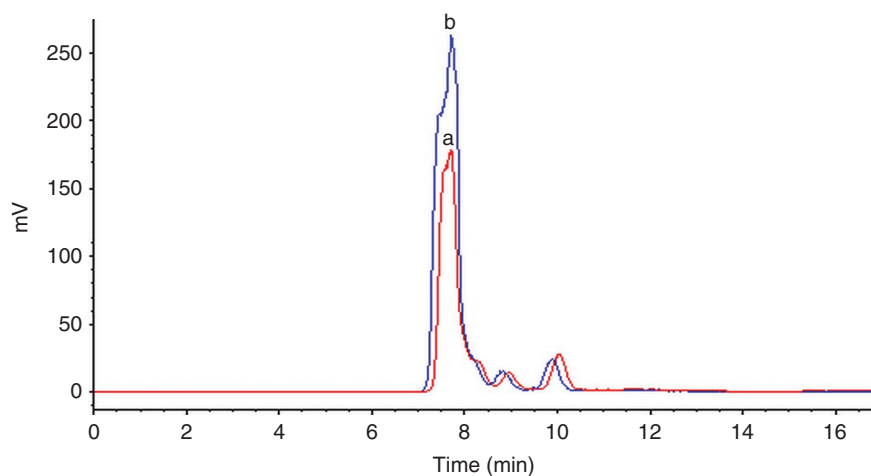


Figure 1: Chromatograms of inulin in TUB CG 32 (a) and inulin in TUB BP 23 (b)

A cluster analysis was performed to classify samples on the basis of the content of inulin in tubers. All samples are grouped in high dimensional space and thus a dendrogram was formed. In this study, an average linkage was used and

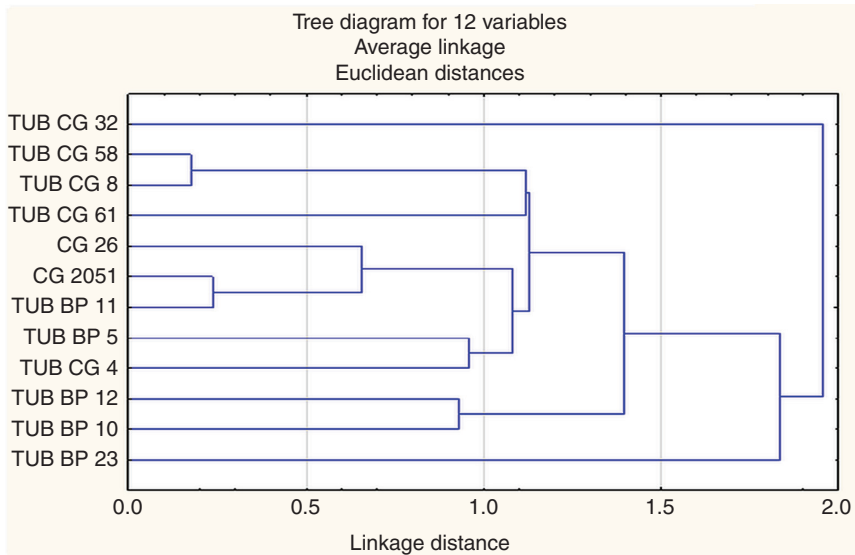


Figure 2: Diagram of cluster analysis considering the content of inulin in tubers of Jerusalem artichoke

Euclidean distances were calculated. The dendrogram (Figure 2) shows an almost clear separation of accessions originating from Montenegro (CG) which are landraces and cultivars with breeding history (BP). In general, CG accessions have higher inulin content but lower tuber yield, while BP have lower inulin content and higher tuber yield. An optimal accession for a future breeding program should comprise both high inulin and tuber yield besides positive phenotype traits, like smaller number of large tubers produced close to the plant.

Conclusion

The analyzed accessions showed relatively low inulin content that does not necessarily mean that an accession is inferior, because it may have higher total inulin yield per hectare or is more suitable for production. Interaction between yield components and genotype influenced by environment should be investigated if commercial production is expected. The quantification methods applied in this research should be improved. It is necessary to optimize the extracting conditions and in that way improve inulin extraction yields. Further research should also take into account suitable storage temperature and storage duration.

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