Characterization of inulin from dahlia tubers isolated by microwave and ultrasound-assisted extractions

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Abstract

The “green” methods as microwave (MAE) and ultrasound-assisted extraction (UAE) have been applied for simultaneous isolation of inulin from dahlia tubers. The inulin yield was 42% dw, fructose content 90-95% and reducing groups was 4.3-4.6%, respectively. The structure of dahlia inulin was confirmed by IR-FT and $^1$H and $^{13}$C NMR spectroscopy. IR-FT spectra showed bands at 817 and 873 cm$^{-1}$ assigned with β-(1→2) bond and proved the presence of 2-ketofuranose, as well as bands at 937 cm$^{-1}$ typical for α-D-Glcp residue. The shifts at 103.6 ppm in $^{13}$C NMR spectra confirmed the presence of β-(2→1)-D-Fruf residues in the linear chain of inulin. Therefore, isolated dahlia inulin consisted exclusively of (2→1)-linked β-fructofuranosyl, with terminal α-glucopyranosyl and β-fructofuranosyl units and the average degree of polymerization 19-23. MAE and UAE can be considered as appropriate approaches for simultaneous extraction of high-molecular inulin from dahlia tubers with high purity (>97%).

Introduction

Inulin is important polysaccharide from fructan family with a significant importance and role in food and pharmaceutical industry (Barclay et al., 2010). It is a biopolymer consisted mainly of D-fructose units connected by β-(2→1) linkages and terminated with a D-glucose residue linked to D-fructose by one α-(1→2) linkage (Franck, 2002). The molecular weight of inulin and its industrial application is directly dependent from the degree of polymerization (DP). Its health benefits are concerning diabetes, lipid metabolism, mineral absorption, cancer prevention, immunomodulation and prebiotic activity (Wang and Gibson, 1993; Gibson and Roberfroid, 1995; López-Molina et al., 2005; Watzl et al., 2005; Barclay et al., 2010; Cooper and Petrovsky, 2011; Miremadi and Shah, 2012; Zubaidah and Akhadiana, 2013). Inulin is low-calorie ingredient that improved rheological characteristics and nutritional properties of foods.

Dahlia, Jerusalem artichoke (Helianthus tuberosus) and chicory have been considered as sources for industrial production of inulin (inulin content >10%) (Franck, 2002). In addition, dahlia inulin found enormous application as vaccine adjuvant and drug carrier in pharmacy (Wu and Lee, 2000; Barclay et al., 2010). Therefore, isolation of inulin from dahlia tubers presents an actual and important field of work.

Several studies about inulin isolation or extraction from different plants by stirring extraction, pressure liquid extraction (PLE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) or ultrasonic/microwave assisted extraction (UMAE) have been reported (Bernal et al., 2005; Noguchi and Yamamoto, 2006; Toneli et al., 2008; Lou et al., 2009; Milani et al., 2011; Zhu et al., 2012; Petkova et al., 2014; Petkova et al., 2015; Temkov et al., 2015). The main procedures for production of inulin powder include hot water extraction, filtration, refrigeration/freezing, centrifugation, precipitation and drying (Toneli et al., 2008). Other processes as ultrafiltration, specific crystallization from aqueous solution and precipitation from solvent/water mixtures were used to enrich native chicory and dahlia inulin in the higher molecular weight fractions. Long-chain inulin could be precipitated from aqueous solutions in the presence of high concentrations of solvents especially: methanol, ethanol, and acetone (Moerman et al., 2004; Petkova et al., 2015). Moreover, acetone was evaluated as the best solvent system to increase the DP, followed by ethanol and methanol. With ethanol, the DP could be raised to 25 for chicory inulin and up to 40 for dahlia inulin, respectively (Moerman et al., 2004).

“Green” methods for extraction gain more attention because of their lower cost, reducing

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time, saving solvents and energy and increasing the efficiency of extraction and improving yields. Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) is considered as improved methods for extraction and isolation of polysaccharides, especially inulin. These two methods could accelerate the extraction process and improve bioactive compound extraction (Lou et al., 2009).

Until now numerous reports have been conducted on conventional inulin extraction from different dahlia varieties with further precipitation with aqueous ethanol (Bernal et al., 2005; Noguchi and Yamamoto, 2006; Hariono et al., 2009; Zubaidah and Akhadiana, 2013; Kosasih et al., 2015; Melanie et al., 2015).

To the best of our knowledge microwave and ultrasonic irradiation techniques have not been implemented in inulin extraction from dahlia tubers. The effect of microwave and ultrasonic power on characteristic of inulin from dahlia tubers was not investigated in details. Therefore, the aim of the current study was to isolate inulin from dahlia tubers by accelerated methods based on microwave and ultrasonic irradiation and to characterize the obtained polysaccharide.

Material and Methods

Dahlia tubers (Dahlia decorative Glory Van Noordwijk, The Netherlands) were purchased from local garden center (Plovdiv, Bulgaria) during January 2016. The plant materials were washed with tab water, sliced, air-dried and finely ground. The powder material was sieved through 0.5 mm. The moisture content of dahlia tubers was determined by drying at 105±1ºC until the constant weight (AOAC, 2000). All other chemicals were of analytical grade.

Extraction of carbohydrates from dahlia tubers

Fractional ultrasound-assisted extraction was performed to obtained soluble carbohydrates from dahlia tubers. The extraction was performed in an ultrasonic bath (VWR, Malaysia, 45 kHz and 30W) for 15 mins at 45°C with 95% ethanol in a solid to liquid ratio 1:20 (w/v). The extraction process was repeated twice and then the dahlia tuber residues were extracted twice with distilled water (1:20 w/v). The collected ethanol and water extracts were analyzed for carbohydrate content by thin-layer chromatography (TLC) and spectrophotometric methods.

TLC analysis of extracts

Ethanol and water extracts (5 μL) were performed on silica gel 60 F254 TLC plates (Merck, Germany), then the chromatogram was eluted with mobile phase n-BuOH:i-Pro:H2O:CH3COOH (7:5:4:2) (v/v/v/v) by double successive rising to a distance of 4 and 8cm. The TLC plate was dried, dipped in diphenylamine-aniline-H3PO4–acetone, heated and scanned as previously described (Petkova and Denev, 2013).

Isolation of inulin from dahlia tubers

The procedure of inulin isolation from dahlia tubers was illustrated (Figure 1). Dry dahlia powder (7g) was used as a raw material for each batch of inulin powder production. Inulin was extracted from the plant material using water as a solvent (1:10 w/v). The extraction was performed in duplicate in a microwave oven (Daewoo KOR, microwave output power 700 W and 2450 MHz frequency) for 5 mins and in an ultrasonic bath VWR USC 100 TH (Malaysia) under constant ultrasonic frequency 4 5kHz, 30 W powers at temperature of 45°C for 20 mins. The obtained extracts were precipitate with addition of four volume acetone, then cooled at -18°C for 60 mins and filtration was performed. The obtained residue was washed with 95% ethanol and acetone. Then vacuum drying was performed. The obtained inulin was characterized by different spectral and chromatographic methods.

Characterization of dahlia inulin

Melting point of isolated inulin was measured on a melting point apparatus BUCHI 510 in capillary glass tube. Water activity ($a_w$) was measured by water activity meter (AquaLab Pre, Labcell Ltd., UK).

Protein content was assessed by Bradford’s method with bovine serum albumin as a standard (Bradford, 1976). The reducing groups were determinated by PAHBAH method at 410 nm (Lever, 1972). The calibration curve was built with D-glucose as a reference in concentration range 5-100 μg/mL ($Y=0.0143x+0.0174; R^2=0.999$).

Total fructose content in dahlia extracts and isolated inulin was defined spectrophotometrically by resorcinol-thiourea methods (Petkova and Denev, 2012). In brief, dahlia extracts (100 μL) or inulin solution (2 mg/mL) were place in a glass tube of 10 mL and 100 μL resorcinol (1% in 95% ethanol solution), 100 μL thiourea (0.1% ethanol solution), 800 μL 95% ethanol and 900 μL HCl were added to them. The sample was heated for 8 mins at 80°C, cooled and filled with water until 10 mL. The absorbance of formed pink-colored complex was read at 480 nm against distilled water. The concentration of inulin in dahlia tubers expressed as fructose equivalent was
calculated using the equation: $Y=0.1174x+0.0087$, obtained from the calibration curve of fructose with $R^2=0.997$.

The total fructan content ($X$) in fructose equivalent per absolutely dry material (%) was calculated as follows:

$$X, \% = \frac{c \times r \times 0.91 \times kw}{p \times 10^6} \times 100$$

where:
- $c$ – concentration of fructose μg/mL from calibration curve linear in the range of 0.5–20 μg/mL,
- $V$ – volume of sample, mL,
- $r$ – dilution factor,
- $kw$ – moisture coefficient, calculated as $100/(100 – $moisture content$)$, here $kw=1.1452$
- $0.91$ – coefficient of hydrolysis;
- $p$ – weight of sample (inulin), g
- $10^6$ – correction coefficient from μg in g.

The results from reducing groups and total fructose content were further used to evaluate the average degree of polymerization (DP) of isolated inulin by the equation (2) (López-Molina et al., 2005). Molecular weight was calculated on the base of the obtained value of DP.

$$DP = \frac{C_{\text{fructose}}}{C_{\text{glucose}}} + 1, \text{ where } C – \text{ concentration, } \%$$

**HPLC-RID analysis of dahlia inulin**

Chromatographic separations and determination of inulin content was performed on a HPLC instrument Elite Chrome Hitachi, coupled with refractive index detector (RID) Chromaster 5450. The separation of inulin was performed on a Shodex® Sugar SP0810 (300mm × 8.0mm i.d.) with Pb$^{2+}$ and a guard column Shodex SP-G (5μm, 6 × 50mm) operating at 85°C, mobile phase distilled H$_2$O with a flow rate 1.0 mL/min and the injection volume 20 μL (Petkova et al., 2014).

**FT-IR spectroscopy**

Fourier transformation infrared spectroscopy (FT-IR) was used to elucidate and characterize the structure of isolated polysaccharides from dahlia tubers. The analysis was recorded in KBr pellets on a Nicolet FT-IR Avatar Nicolet Termo Science spectrometer in the range 4000 – 400 cm$^{-1}$ and absorption was reported in wavenumbers (cm$^{-1}$). The sample (2 mg) was pressed into pellets of KBr (200 mg).

**$^1H$ and $^{13}C$ NMR spectroscopy**

The structure of polysaccharide isolated from dahlia tubers were identified by $^1H$ and $^{13}C$ NMR spectroscopy: The $^1H$ and $^{13}C$ NMR spectra were recorded using a Bruker spectrometer operating at a frequency of 500 MHz and 125 MHz, respectively. Inulin was dissolved in D$_2$O and with a tetramethylsilane (TMS) as standard. The DP of dahlia inulin was estimated also from NMR spectrum by taking the ratio of peak integral values of carbons in fructose units ($X$) to the peak integration values of the corresponding carbons in the glucose unit, as previously described by Barclay et al. (2012) as follows: $DP_n=((X-6)/7)+1$ (3)

**Results and Discussion**

The direct extraction of inulin from fresh dahlia tubers was applied in many researches (Bernal et al., 2005; Hariono et al., 2009; Zubaidah and Akhdadina, 2013; Melanie et al., 2015), but as previously mentioned the risk from spoilage of plant material exists (Kosasih et al., 2015). The better approach is to use dry and ground dahlia tubers. This can significantly improve extraction efficiency and reduce the solvent used for precipitation.

In our study the initial moisture content in dry dahlia tubers was 12.9±0.2%. Therefore, the dry matter content in tubers for inulin extraction was significantly higher 87.3%.
The dahlia tubers were extracted with 95% ethanol and then with water to check the content of inulin and sugars in them. The first screening by TLC chromatograms of these extracts showed that a large number of carbohydrates were successively extracted by ultrasound-assisted extraction (data not shown). The presence of fructose ($R_f=0.50$), FOSs including 1-kestose ($R_f=0.37$), nystose $R_f=(0.32)$ and oligomers, equivalent with $R_f$ of used inulin standard Frutafit CLR (DP 7-9) (Sensus, Rosendaal, The Netherlands) was established in 95% (v/v) ethanol extracts, while in water extracts inulin dominated mainly. The spectrophotometric analysis of dahlia extracts revealed that tubers contained high amount of inulin 36.45 g/100g (Table 1). The low-molecular fraction extracted with 95% ethanol (fructose, sucrose and FOS) did not exceed more than 3.3 g/100g. From all known fructan sources dahlia tuber with its high inulin content can be compared with elecampane (43 g/100g dw) and dandelion roots (34 g/100g dw) as promising industrial plant material for production of this polysaccharide (Petkova and Denev, 2012).

Isolation of inulin from dahlia tubers

Inulin can be easily obtained from dahlia tubers with hot water extraction under ultrasonic and microwave irradiation (Figure 1) in good yield 41-42% on dry tuber basis. These values were higher than the reported yields of inulin from elecampane ($Inula helenium$ L) and chicory from our previous studies (Petkova et al., 2014; Petkova et al., 2015). Our results were comparable with the reports of Zubaidah and Akhadiana (2013) and Kosasih et al. (2015).

Characterization of dahlia inulin

The physicochemical characteristics of isolated dahlia inulin were summarized in Table 2. The resulting dahlia inulin presented white tasteless powder. The isolated substances contained 91-95% inulin calculated as fructose equivalent. Glucose content expressed as reducing groups were in the range of 4.6 to 4.3%. Our results were in agreement with the reported by Ananina et al. (2009) data about the standardization of inulin from dahlia $Dahlia$ single tubers which contained main substance (inulin) and bound glucose 95.72±1.05% and 4.38±0.85%, as well as data for commercial dahlia inulin (Sigma-Aldrich, Saint Louis, MO) and inulin from $Dahlia coccinea$ Cav. (Santana et al., 2016).

Melting point was significantly higher 180°C and was near to the reported in literature data for dahlia inulin (Wu and Lee, 2000; Leyva-Porras et al., 2015). This reveals high thermal stability of dahlia inulin and its further applications in different industrial areas. The water activity (0.330) coincided with data for high molecular chicory inulin Raftilene HP (DP=22) used as a reference. The amount of inulin present was 99-98% and this showed the effectiveness of the MAE and UAE methods for extraction. The percentage of inulin was high and coincided with reports of Bernal et al. (2005) for purity of inulin from $Dahlia imperialis$ tubers.

Protein content in MAE inulin fraction from dahlia tubers was 1.5%, while in inulin obtained by UAE it was less - 0.3%. Therefore, the MAE and UAE methods and purification procedures with acetone and 95% ethanol reduced significantly impurities and

<table>
<thead>
<tr>
<th>Sample</th>
<th>Low molecular fraction (Fru)</th>
<th>Water-soluble fraction (Suc and FOS)</th>
<th>Total fructans (Inulin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahlia tubers</td>
<td>3.44±0.19</td>
<td>36.45±0.04</td>
<td>39.89±0.23</td>
</tr>
</tbody>
</table>

$^1$dw – dry weight; $^2$SD – standard deviation

Figure 1. Schematic procedure for MAE and UAE of inulin from dahlia tubers
could be successfully used in production of standard dahlia inulin for food and pharmaceutical uses.

Homogeneity and molecular weight distribution

Biopolymers have a molecular weight distribution which can be characterized by the average weight molecular weight (Mw), the average number molecular weight (Mn) and polydispersity index. The data from molecular weight distribution of dahlia inulin were summarized in Table 2.

HPLC-SEC chromatograms of this polysaccharide showed single symmetric peak (data not shown), suggesting that inulin was purified completely and their molecular weights had a certain range of distribution. Praznik and Beck (1985) also reported for homogeneous mixture of polymers form native inulin from dahlia (Dahlia variabilis).

The dominant polysaccharides component, separated by HPLC-SEC displayed one symmetrical peak, corresponding to Mw=3808 Da for inulin obtained after MAE and Mw=3345 Da for UAE. It was demonstrated that ultrasonic irradiation could dramatically improve the extraction process of polysaccharides (inulin) mainly through the cavitation. The internal heating of microwave may cause hyperthermia, while ultrasonic may cause particularly degradation of inulin chain to reducing monosaccharides during extraction (Lou et al., 2009). With this statement could be explained the higher amount of reducing groups in UAE obtained inulin and lower molecular weight (Table 2).

The polydispersity index (Mw/Mn) of inulin was established to be 1.05 (Table 2). HPLC-SEC analysis showed their heterogeneity, reflecting different DP values. Our results were higher than reported by Leyva-Porras et al. (2015) for the degree of polymerization of inulin from dahlia tubers (2–12 units of fructose) and they were near to reports of Losso and Nakai (1997). It was reported that inulin from dahlia tubers showed a shorter oligomer fragmentation spectrum with major molecular weight peaks at 346.7 and 672.4g/mol (Leyva-Porras et al., 2015). The higher average molecular weight in mass was observed for the inulin extracted from dahlia tubers presented a value of 771 g/mol. In accordance with their results we also obtained high DP inulin from dahlia tubers that showed a polydispersity very close to the unit which represented a narrow molecular weight distribution. Moreover, it was reported that molecular masses of commercially available inulin from dahlia ranged from less than 1000 Da to 4000 Da with a peak value of distribution around 2500, corresponding to DP 14 (Losso and Nakai 1997). However, Moerman et al. (2004) succeeded in obtaining inulin from dahlia with DPn 43 by ultrafiltration using membrane of 5kDa.

The high molecular could be obtained also by aqueous precipitation for one week or by freeze/traw process (DPn 42). In our case we performed three different methods of evaluation of average degree of polymerization of dahlia inulin: by HPLC-SEC as Mn were divided to fructose unit 162 g/mol, by spectrophotometric analysis as content of total fructose were divided to reducing sugars content and by NMR study. The average DPn was calculated by these three methods were in range of 17-23. These results were in agreement with some reports from Praznik and Beck (1985), Frank (2006) and Hariono et al. (2009) that dahlia inulin characterized with DPn 13-20. Moreover, reported by us Mn 3345 Da by UAS dahlia inulin was near to molecular weight of native dahlia inulin (3260 Da) evaluated by gel-permeation chromatography (GPC) (Praznik and Beck, 1985). Polydisresity index (1.05) in our study was lower than previous report 1.85 and 1.35 (Praznik and Beck, 1985; Leyva-Porras et al., 2015).

The isolated inulin is in the category of standard inulin according to Franck (2002). The DP of inulin type fructans depend on the plant sources, the environmental conditions, the physiological age of the plant at harvest (Wilson et al., 1999; Monti et al., 2005). The extraction conditions (i.e., temperature, pH and time) (De Leenheer, 2008) and purification methods also can be affected the DP of inulin.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Inulin isolated from dahlia tubers by “green” extraction methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D9A Inulin</td>
</tr>
<tr>
<td>Yield, %</td>
<td>42</td>
</tr>
<tr>
<td>Purity, %</td>
<td>98</td>
</tr>
<tr>
<td>Melting point, °C</td>
<td>100-102</td>
</tr>
<tr>
<td>Water activity, g/L</td>
<td>0.330</td>
</tr>
<tr>
<td>Fructose content, %</td>
<td>91.2</td>
</tr>
<tr>
<td>Reducing groups, %</td>
<td>4.5</td>
</tr>
<tr>
<td>Protein, %</td>
<td>1.5</td>
</tr>
<tr>
<td>Appearance</td>
<td>White powder</td>
</tr>
<tr>
<td>Taste</td>
<td>Neutral</td>
</tr>
<tr>
<td>Sweetness</td>
<td>none</td>
</tr>
<tr>
<td>pH</td>
<td>6.7</td>
</tr>
<tr>
<td>Weight molecular weight (Mw), Da</td>
<td>3808 Da</td>
</tr>
<tr>
<td>Number molecular weight (Mn), Da</td>
<td>3000 Da</td>
</tr>
<tr>
<td>Polydispersity index</td>
<td>3.05</td>
</tr>
<tr>
<td>DPn by HPLC-SEC analysis</td>
<td>23</td>
</tr>
<tr>
<td>DPn from spectrophotometric analysis</td>
<td>21</td>
</tr>
<tr>
<td>DPn by NMR spectra</td>
<td>22</td>
</tr>
</tbody>
</table>
FT-IR spectra

FT-IR spectra of dahlia inulin was performed to elucidate the main functional groups and their general assignments were summarized in Table 3. Typical bands for inulin were found in FT-IR spectra. The FT-IR spectrum can be divided into four spectral regions: region I (from 3500 to 2500 cm\(^{-1}\)), region II (from 2500 to 1500 cm\(^{-1}\)), region III (from 1500 to 900 cm\(^{-1}\)) and region IV so-called fingerprint region below 900 cm\(^{-1}\).

In the first region the broad band at 3319 cm\(^{-1}\) was appeared that could be assigned with stretching vibrations of OH groups. A sharp band at 2933 cm\(^{-1}\) and a shoulder at 2880 cm\(^{-1}\) were also observed. These bands were due to asymmetric C-H stretching vibration and C-H symmetric stretching vibration of CH\(_2\) groups, respectively. In the second region only one bands at 1650 cm\(^{-1}\) was appeared that was not specific for inulin and was assigned with absorption of water, because of hygroscopic properties of this homopolysaccharide. In the third spectral region between 1500-900 cm\(^{-1}\) bands typical for C-C stretching in pyranose ring, C-O and C-O-C deformation modes were appeared. The bands at 1130 cm\(^{-1}\) was characteristic for C-O-C ring stretching vibrations and bands at 1031 cm\(^{-1}\) was assigned with C-O stretching vibrations. In last region bands useful for conformational studies of inulin was found. The band at 937 cm\(^{-1}\) was assigned with α-D-Glcp residue in carbohydrate chain. Our assignments were in accordance with previous reports of FT-IR spectra of isolated inulin from different dahlia varieties (Bernal et al., 2005; Melanie et al., 2015).

In addition bands at 873 and 817 cm\(^{-1}\) in inulin dahlia spectra confirmed CH\(_2\) ring vibration of β-anomer and the presence of 2-ketofuranose. Similar bands were reported for inulin from elecampane, as previously described by Petkova et al. (2015). A distinctive absorption bands at 669 and 598 cm\(^{-1}\) in our spectrum were similar to reports of Melanie et al. (2015) and showed the presence of pyranose rings in polymer chain. No significant differences in FT-IR spectra of dahlia inulin obtained after MAE and UAE were observed.

NMR studies of inulin isolated from dahlia tubers

In \(^1\)H NMR spectra of inulin was found typical chemical shifts for glucose and fructose units: \(^1\)H NMR (500 MHz, D\(_2\)O) δ 5.35, 5.01, 4.93, 4.80, 4.49, 4.40, 4.16, 4.01, 3.82, 3.76, 3.68, 3.63, 3.47 ppm. The \(^1\)H-NMR spectrum of dahlia MAE inulin contained isolated resonance for the single anomic α-glucose proton was observed at 5.35 ppm (Figure 2 A). Anomeric glucose signal H-1 showed low intensity in comparison with the high intensity of fructose units.

All protons from inulin were with chemical shifts in the range from 3.47 to 4.93 ppm. The obtained \(^1\)H
NMR spectra showed the presence of fructose unit (~4.20 ppm) and polysaccharide chain terminates with fructofuranosyl residue (4.16 ppm). In addition, the integration of the H-1 signal of the glucose moiety at δ 5.4 ppm and the H-3 and/or H-4 signals of the preponderant fructosyl units between δ 3.30 and 4.40 ppm gave mean DP₅. Under these extraction conditions, the DP₅ distribution of inulin obtained for spectrophotometry analysis ranged from 15 to 19.

In ¹³C NMR spectra of dahlia inulin chemical shifts typical only for fructose units were observed (Figure 2 B): ¹³C NMR (126 MHz, D₂O) δ 103.19, 81.03, 76.93, 74.23, 62.09, 60.84 ppm. The spectra contained prominent shifts for C₁–C₆ carbons (C₁ 60.8 ppm, C₂ 103.2 ppm, C₃ 76.9 ppm, C₄ ~74 ppm, C₅ ~81 ppm and C₆ ~62.1 ppm) of fructosyl residue due to fructose repeated units. The ¹³C shifts from glucose were not observed (Figure 2 B) due to the low quantity in sample. The superposition of glucose shifts were observed in other studies and was reported for inulin from echinacea, dahlia, and stevia (Wack and Blaschek, 2006; Fontana et al., 2011; Lopez et al., 2015). In the ¹³C NMR spectra were observed only one shift at 103.19 ppm corresponding to the C-2 carbon involved in β-(2→1)-D-fructofuranosyl-fructose bonds. The data from ¹³C NMR spectra confirmed chemical structure of inulin from dahlia tubers composed mainly of fructose units linked with β-(2→1) bonds and of only one terminal glucose unit linked α-(1→2). This structure is characteristic for inulin-type fructan obtained from Composite family plants (Wack & Blaschek, 2005; Barclay et al., 2012).

**Conclusion**

The current research demonstrated the efficiency of two “green” methods for isolation of inulin from dahlia tubers. Based on HPLC, FT-IR and NMR analyses, inulin was evaluated as the major component of dahlia tubers in high yields 42% and purity more than 97%. Dahlia tubers were evaluated as a rich source of high molecular inulin (DP 19-26), that is important characteristics for its functional properties, related to prebiotics, dietary fibre, role lipid metabolism diabetes control and immunomodulation.

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