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ISOLATION AND CHARATERIZATION OF INULIN FROM TAPROOTS OF COMMON CHICORY (CICHORIUM INTYBUS L.)

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ABSTRACT

Polysaccharide inulin has been isolated from the dry taproots of common chicory *Cichorium intybus* L. by hot water extraction procedure under high-pressure conditions and further precipitation with acetone at low temperature. The obtained inulin was characterized by the measurement of melting point and angle of optical rotation. The total fructose content was determined by resorcinol spectrophotometric method. The purity of isolated polysaccharide was defined by HPLC-RID method for analysis of inulin. The reducing groups were evaluated by PAHBAH. The protein content was analyzed by Bradford protein assay. IR-FT spectrum was recorded in KBr pellets and the absorption was reported in wavenumbers (cm^{-1}).

The isolated substance from chicory roots presented a white powder, without taste. It characterized with the melting point 165°C, the angle of optical rotation $[\alpha]_D^{25} = -22$(c = 0.5; H_2O) and gave positive Selivanoff reaction, an indication for the presence of ketose groups. This carbohydrate was

characterized with the fructose content 96%, purity 97%, reducing groups 3.6%, degree of polymerization (DP) 27 and the molecular weigh 4.3 kDa. The protein content in the polysaccharide fraction was below 0.2%. The IR-FT spectrum were characterized by absorption brands at 3330 cm^{-1} ν(OH); strong complex absorption at 1170 cm^{-1}, 1087 cm^{-1}, 1030 cm^{-1} related to valent stretching vibrations of (C – C), (C – O), (C – O –C) groups and ring vibrational modes in furanoside structure. The bands at 827, 860 and 938 cm^{-1} belonged to β 2→1 glycosidic bond. These data suggest that the isolated polysaccharide substance is inulin-type fructan.

Key words: *chicory, inulin, HPLC analysis, IR-FT spectrum*

INTRODUCTION

Chicory (*Cichorium intybus* L.) is a biennial plant belongs to the *Asteraceae* family with many applications in the food industry. Its roots are rich source of inulin [13]. It is a reserve polydisperse plant polysaccharide, member of the fructan family, consisting mainly of β-(2→1) fructofuranosyl units (Fm), and a terminal α-glycopyranose unit (1→2) (GFn). The degree of polymerization (DP) of inulin varies from 2 to 70 [17].

Because of its healthy effect inulin are recommended for treating and curing diabetes mellitus, alimentary corpulence, atherosclerosis, and dysbacteriosis. It also improves mineral absorption, possessed prebiotic and immunomodulating properties [2, 3]. Inulin is used as soluble dietary fiber and texture modifier in food production and as a substrate for the production of high fructose content syrups (HFCS) used in beverage industry [9, 22].

Due to their wide distribution in nature and significant role in industry, the extraction, isolation and characterization of inulin-type fructans are still gaining attention in recent years [24]. The main sources for inulin industrial production are chicory witloof, Jerusalem artichoke and dahlia [9].

Many investigations were developed to set optimum extraction conditions in order to improve inulin extraction from plants, temperature, extraction time, and solvent/solid ratio were identified as the most important factors influencing the yield [1, 23]. The main procedures for production of inulin powder include extraction, filtration, refrigeration/freezing, centrifugation, precipitation and drying [23]. Because of its water solubility, all inulin extraction methods described in the literature used hot water as a solvent, with only small differences in temperature and extraction time. For

industrial production of inulin hot water diffusion process has been applied followed by refining with ion exchanger, concentration and spray draying [9]. Inulin isolation from chicory roots could be done also by batch extraction at 70°C with continuous stirring and clarification by filtration trough silica-chitosan bed [8]. Laboratory investigations include extraction procedures from dry chicory roots by hot water diffusion at an average temperature of 80 ± 2°C for 1 h with continuous stirring [23], from globe artichoke by distilled water (80°C) at pH 6.8 (by NaOH) to avoid inulin hydrolysis at pH < 6 [22] and from *H. tuberosus* L by hot deionized water at 85°C for 2 – 3 min [7].

Clearing procedure by active carbon pretreatment of the extract was reported for inulin from Jerusalem artichoke tubers [1, 7]. Other widespread technique for clarification is carbonation. For many years this method is applied for refining inulin, because of its advantages as effectiveness and low-cost price [16]. For precipitation of inulin solvents as ethanol, acetone or 2-propanol were applied [7, 12].

The aim of the current study was isolation and physicochemical characterization of inulin obtained from taproots of medicinal plant *Cichorium intybus* L.

MATERIALS AND METHOD

Inulin has been isolated from common chicory (*Chicorium intubus* L.) grown on the territory of Bulgaria (Chehlare village). The roots were collected in October 2010, and then they were washed with tap water and dried at 40°C in the vacuum oven. The ground taproots were extracted with hot water (~90°C) under pressure 1.5 MPa for 2 – 3 min. The extraction was repeated twice, the filtrates were mixed and pH was adjusted to 8.0 by adding $Ca(OH)_2$. The mixture was left at room temperature for 1 h. After that, the sludge was filtrated. The filtrate was neutralized to pH 7 at temperature 60 – 65ºC by oxalic acid. An activated carbon was added and then filtration procedure was done again. The concentrated filtrate was precipitated by addition of acetone and left to settle down at 2 – 5°C. The resulting amorphous substance was filtrated, washed twice with ethanol, once with acetone and finally dried at 40°C (Figure 1)

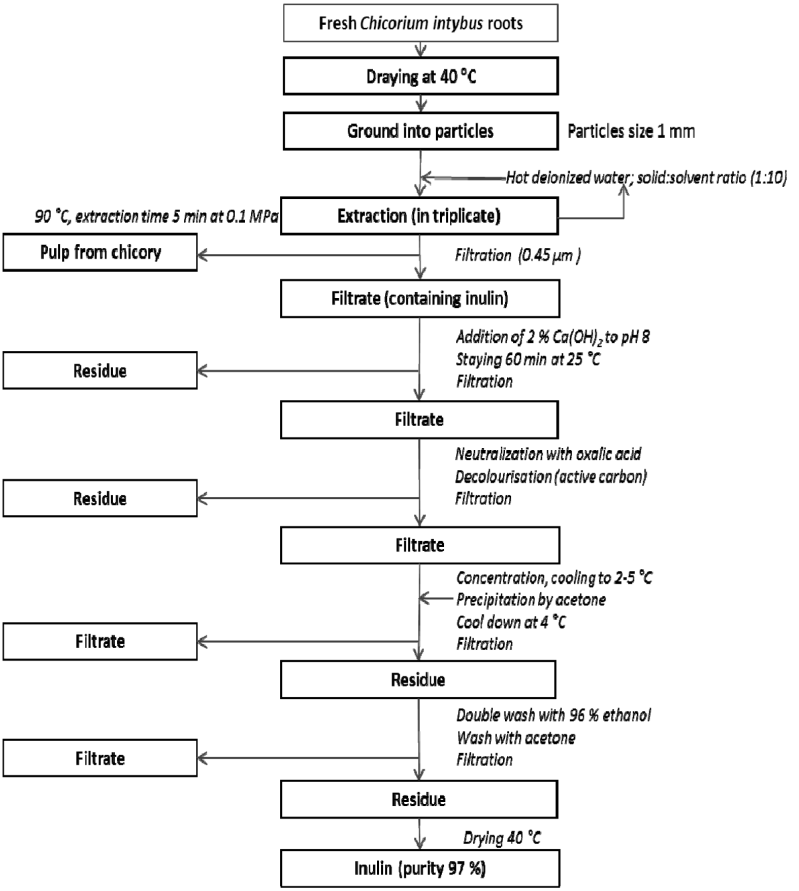

Figure 1. *Schematic procedure of inulin extraction from common chicory*

Melting point of inulin was measured on a melting point apparatus BŰCHI 510 in capillary glass tube. Optical rotation of 5% water solutions of inulin samples was determined on automated polarimeter Polamat A (Carl Zeiss, Jena, Germany) in a tube 1 dm long with volume 10 ml using sodium lamp as light source.

Protein content was assessed by Bradford's method with bovine serum albumin as a standard [6].The reducing groups were assayed by PAHBAH,

as measuring the absorbance at 410 nm [14]. The calibration curve was built by use of D-fructose (Mw = 180.16, Fluka) as a reference.

The fructose content was analyzed by resorcinol assay with measuring absorbance of the color complex at 480 nm [20].

Inulin content in the isolated substance from roots of common chicory was analyzed by HPLC-RID method. The inulin solution was filtered through a 0.45 μm filter prior to analysis. Chromatographic separations were performed on HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector Shimadzu RID-10A. The control of the system, data acquisition, and data analysis were under the control of the software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). The chromatographic separations were performed on a Shodex® Sugar SP0810 with Pb^{2+} a guard column (50 × 9.2 mm i.d.) and an analytical column (300 mm × 8.0 mm i.d.). The mobile phase used for separation was distilled water with flow rate 1.0 ml/min. The concentration of the sample was 8 mg/ml and the injection volume 20 μL.

To evaluate the average degree of polymerization (DP) of isolated inulin the equation (1) were applied [15]. Molecular weight was calculated on the base of the obtained value of DP.

$$DP = C_{fructose}/C_{glucose} + 1, \text{ where C – concentration,\%} \quad (1)$$

Fourier transformation infrared spectroscopy (FT-IR) was used to elucidate and characterize the structure of isolated substance from common chicory. The analysis was recorded in KBr pellets on a Nicolet FT-IR Avatar Nicolet Termo Science spectrometer in the range 4.000 – 400 cm^{-1} and absorption was reported in wavenumbers (cm^{-1}). The sample (2 mg) was pressed into pellets of KBr (200 mg).

For the characterization and identification of isolated inulin, comparative study were made with commercially available inulin Frutafit® TEX (DP = 22) from *Cichorium intybus* (Sensus*,* Rosendaal, the Netherlands).

Statistical analysis was performed using MS Excel 2010.

RESULTS AND DISCUSSION

The procedure for extraction and purification of inulin form medicinal plant common chicory were easy to perform. The following data were characteristics for isolated inulin: yield 12%; odorless and tasteless white powder; soluble in water, with melting point 164 – 165°C and $[\alpha]_D^{25} = -22$

(c = 0.5; H_2O). The negative optical rotation could be explained with the presence of β-configuration of the glycoside bonds between the fructofuranosyl units. The negative values of optical rotation of inulin isolated from dahlia, topinambour and burdock were also reported in the literature [5, 7, 10, 18].

The reducing groups in inulin isolated from roots of common chicory were established to be 3.6% dw. Similar values to our results were reported for the purified samples of inulin obtained from different plant sources: elecampane, dahlia, Jerusalem artichoke and chicory have glucose content of 2.2 – 2.6% [19], 4.3% [2], 4.9 – 5.2% [1] and 6% [4], respectively.

The studied powder material contained average 96.1 ± 0.7% fructose defined by ketose specific method with resorcinol. The calculated degree of polymerization of isolated inulin was 27. This value is close to the DP 23 reported by Praznik & Beck for chicory inulin [21] and 25 for high performance inulin isolated from chicory witloof [9]. The molecular weight of isolated inulin on the base of its DP was established to be 4.3 kDa.

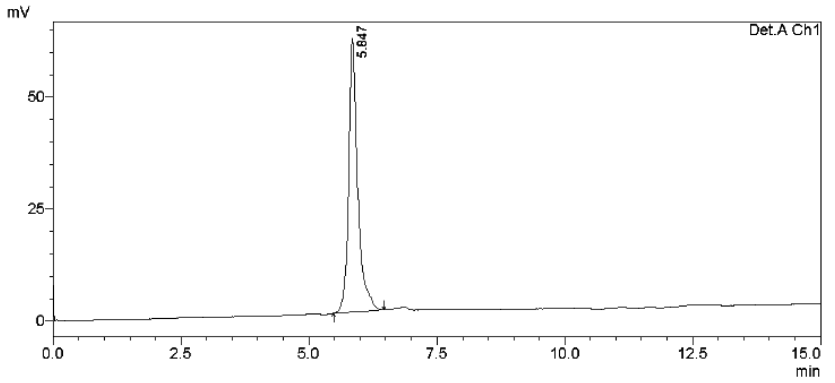

Figure 2. *HPLC chromatogram of isolated inulin*

The purity of inulin was tested by HPLC-RID. The obtained chromatogram showed the presence of only one peak without any interfering compounds (Figure 2). The retention time of the investigated substance (t_R = 5.85 min) is the same with that of pure inulin (DP = 22) used as a reference. The amount of inulin present in the isolated substance from common chicory was 97%. The high purity of inulin showed the effectiveness of the applied method for extraction and purification. Above

95 – 96% purity of inulin from *Helianthus tuberosus* L. applying near procedure for isolated was reported by Denev et al. and Zhang et al. [7, 25]. The protein content in the isolated inulin was 0,3%.

The IR-FT spectra of the investigated substance contained band typical for inulin-type fructans (Figure 3).

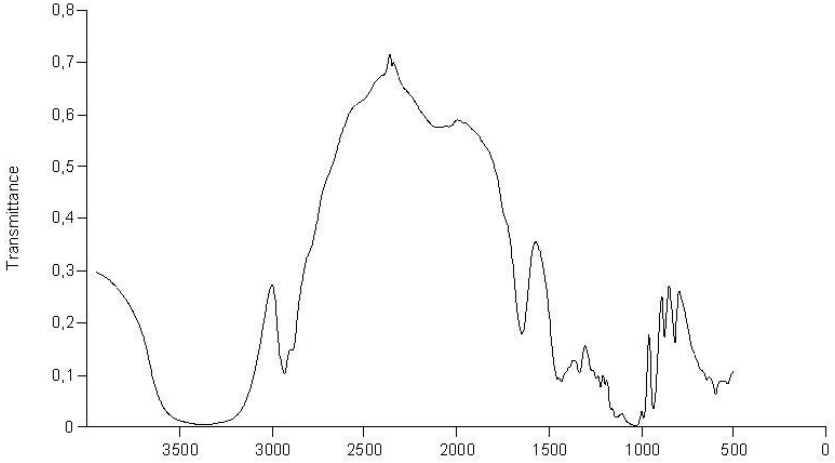

Figure 3. *IR-FT spectra of isolated inulin from chicory*

The detail information about the IR-FT spectra of inulin isolated from common chicory was presented in Table 1. The bands at 1170 cm^{-1}, 1087 cm^{-1}, 1030 cm^{-1} are characteristic for (C – C), (C – O), (C – O – C) stretching vibrations in furanose ring. 2-ketose in furanose form contain band at 874 cm^{-1} и 817cm^{-1}, which are evidence for presence of β-(2→1) glycosidic bonds.

Table 1. *General assignment of IR-FT spectra of inulin*

Wavenumber, cm^{-1}	Experimental IR bands, cm^{-1}	Assignment
3200 – 3400	3361	ν_{O-H} (OH); intermolecular H-bonds
2933 – 2981	2931	$\nu_{C-H}^{as}(CH_2)$
2850 – 2904	2880	$\nu_{C-H}^{s}(CH_2)$
1664 – 1634	1635	Absorption of water

1416 – 1430	1430	$\delta_{C-H}{}^{s}(CH_2)$
1335 –1336	1335	$\beta_{O-H}(OH)$
1225 – 1235	1220	$\beta_{O-H}(OH)$
1125 –1162	1135	$\nu_{C-O-C}{}^{as}$ (C – O – C)
1015 – 1060	1031	ν_{C-O} (C-O)
985 – 996	987	ν_{C-O} (C-O)
930	935	α-D-Glc*p* residue in chain
892 – 895 874	873	Anomeric bendings δ(C1 – H), ring vibration (2-ketofuranose)
817	817	2-ketose

Our observation is similar to the reported bands for inulin by Grube & Olennikov [11, 18]. The obtained results from IR spectrum prove that the isolated substance from roots of common chicory is inulin-type fructan.

CONCLUSION

The proposed procedure for extraction and purification of inulin from common chicory gave final product with high yield and purity. Inulin was characterized with DP 27, which will define its functional properties and its future application in healthy nutrition as a potential prebiotic and as texture modifier in food. The roots of medicinal plant common chicory as well as inulin isolated from them are a potential source of soluble dietary fibers, which could be used in preparation of functional food and nutritional formula with well-pronounced healthy effect.

The proposed analytical methods for inulin analysis could be applied for determination of its purity, content in food products and additives, for food labeling purpose or for revealing adulteration.

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