OBTAINING AND IDENTIFICATION OF INULIN FROM JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*) TUBERS

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Abstract: The growing demand of the Russian population for healthy food dictates the need in functional ingredients production increase. Inulin, the polysaccharide of natural origin, has a wide range of functional activity. This article grounds the expedience of inulin obtaining from Jerusalem artichoke tubers and considers effective technological methods of ensuring high yield and quality of the target product. It was demonstrated that application of vibration with frequency 24 Hz for 60 min at temperature 30–35°C intensifies the extraction process, and fractionation of the extract on membranes with pore diameter 2, 3 and 5 kDa allows to obtain inulin with certain physicochemical properties. The membrane separation results in three inulin fractions: low molecular (DP = 2–10), medium molecular (DP = 11–18) and high molecular (DP = 19–35) fraction. The medium molecular fraction of inulin, which is used as prebiotic and fat substitute in food technology, was studied using FTIR spectroscopy and 1H-13C NMR spectrometry. The obtained spectral characteristics have led to a conclusion that the investigated sample of inulin is highly competitive with the best world analogues. The authors thoroughly describe the method of determining the degree of polymerization and average molecular weight of the investigated polysaccharide using ^{1}H-^{13}C NMR spectroscopy. It has been established that inulin obtained by improved technology has the degree of polymerization DP = 13–14 and molecular weight 2124–2286 Da. The results of this work have practical value for production of inulin from Jerusalem artichoke tubers and theoretical value for the chemistry of natural compounds.

Keywords: Functional ingredients, Jerusalem artichoke, inulin, degree of polymerization, FTIR, NMR spectroscopy

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INTRODUCTION

According to analytical agencies worldwide, the most promising area of food industry development is the production of functional food. Total consumption of these product in Europe is about 100 000 tons per year and in Russia – 1 400 tons per year.

Relevance of healthy nutrition is confirmed by studies indicating direct correlation between human immune status and consumed food. Manufacturers are expanding the range of prophylactic products by means of various functional ingredients: dietary fiber, vitamins, antioxidants, polyunsaturated fatty acids, pro- and prebiotics [1, 2].

Inulin, polysaccharide of natural origin, is an effective prebiotic. Inulin is a polymer consisting of several fructose residues (from 10 to 36) in furanose form (β, D-fructofuranose) and one glucose residue in pyranose form (α, D-glucopyranose), connected through β-2,1 glycoside bonds. Molecular weight of inulin is 5000–6000. Inulin produces D-fructose and a small amount of glucose during acid or enzymatic hydrolysis. Inulin and the intermediate products of its lysis (inulids) do not have reducing abilities [1, 3].

According to several studies, inulin belongs to reserve carbohydrates [4]. It is formed in leaves of plants during photosynthesis and accumulates in stems and roots of composite plants (mainly). Inulin is stored in vacuoles in form of spherocrystals.

Inulin is considered a soluble dietary fiber and a functional ingredient. Because inulin undergoes fermentation by microflora of the large intestine and is not absorbed in the stomach or small intestine, regular consumption of inulin with food provides the following health effects:

– Creating of optimal conditions for growth and development of normal intestinal microflora; prevention of goiter; increased resistance to bacterial and viral infections of the digestive system, as well as to the introduction of various parasites;

– Regulation of carbohydrate metabolism: the acidic gastric juice environment ensures the hydrolysis of inulin yielding fructose, which is absorbed by the body without insulin, reducing the hunger;

– Normalization of at metabolism: decrease of cholesterol and triglycerides level in blood, which prevents the development of blood vessel atherosclerosis;
– Reduction of body weight (overweight problems) through activation of fat disposal process associated with the processes of glucose digestion;
– Normalization of sugar level in blood: the molecules of inulin, unsplitted by hydrochloric acid in stomach, adsorb a large amount of alimentary glucose and hinder its absorption into the bloodstream, thereby reducing the sugar level in blood after meal.
– Stable glucose level decrease leading to the normalization of insulin production by pancreatic cells;
– Promotion of energy production: the major part of energy required for normal human life is obtained by glycolysis. Since fructose is much more easily absorbed by the body, the cells do not develop energy hunger. Moreover, short fragments of inulin molecules incorporated into cell membrane facilitate the passage of glucose inside the cells. Inulin promotes glycogen synthesis by improving the glucose utilization. This provides a higher level of energy metabolism;
– Normalization of metabolism: unlike non-utilized glucose, which turns into products of fat metabolism, fructose is used by the body entirely, preventing the development of obesity, vessel atherosclerosis, coronary heart disease, arterial hypertension;
– Integrated effect on the functional activity of the liver. Inulin stimulates the synthesis of protein, cholesterol, bile acids by improving the glycose utilization. Due to disposal of toxic substances in the intestines and blood, inulin significantly relieves the liver and keeps its fighting potential against various diseases and environmental factors [1].

Inulin possesses both physiological and technological functionality. It forms a creamy gel with a short fat-like texture together with water and thus simulates the presence of fat in the diet products, ensuring the full flavor and texture. In addition, inulin improves stability of air-concentrated product, such as ice cream and food emulsions (spreads, sauces) [5].

Dietary norm of inulin intake is 5–8 g per day. One portion produced for nutrition purpose contains 10–50% of recommended daily intake. If addition of inulin has technological purpose, the dosage may be higher, because inulin starts to work as a texture and flavor enhancer at concentrations more than 2% [6–8].

The application of inulin in the food industry is constantly growing. There is information about usage of inulin in production of various kinds of bread made from wheat and rye-wheat flour, shortbread products and cakes, waffles and gingerbread. It has been proved that adding of inulin makes the bread healthier and improves a number of technological effects – shape stability, porosity, oven and moisture losses, yield. Moreover, inulin increases the consumer qualities of bread: improves its external appearance and flavor, slows down hardening. Recommended dosage is 2.5–3.0% to weight of flour [9].

Thanks to its versatility inulin has found application in dairy industry for production of milk, milk products, butter, cheese, ice cream. The most famous inulin-containing products: kefir "Biomax effektivnyj" by Vimm-Bill-Dann Company and yogurt line "Ermigurt prebiotics" by Ermann Company. The Group of companies "Galaktika" designed a new product – pasteurized inulin-enriched milk. This is a healthy functional product, ideal for dietic nutrition – the milk with inulin contains only 1% of fat. One glass or 200 g of such milk provides 20% of recommended daily need in inulin for the organism [10].

Inulin is widely distributed in plants, but its content and degree of polymerization can be different. The data on quantitative and qualitative inulin content in different crop plants are presented in Table 1.

The main current source of inulin in the European countries is root chicory. Chicory (*Cichórium íntybus*) is a genus of biennial or perennial herbs of Asteraceae or Compositae family. The genus includes two cultivated species and from four to six wild species. Chicory contains up to 20% of inulin. The cultivation of chicory in Russia is limited [11].

Another representative of inulin-containing root vegetables is yacon (*Smallanthus sonchifolius*). Yacon tubers vary in shape and size. One tuber can weigh up to 850-900 g and contain up to 19% of inulin on a wet weight basis. Yacon application perspectiveness for inulin production is differently estimated by the experts [12].

Jerusalem artichoke is one of the most promising natural sources of inulin.

Jerusalem artichoke (*Helianthus tubersrosus L*) belongs to the Asteraceae family and is an annual plant [13]. According to biological characterization it is a plant with direct folious stem. The stem can grow up to 5 m depending on growing conditions. Stooling of

Table 1. Weight fraction and degree of polymerization of inulin from different sources [1, 9, 10]

Plant	Weight fraction of inulin, g/100 g of raw material	Mean degree of polymerization
Banana	0.3–0.7	≤ 5
Onion	1–1.75	≤ 12
Leek	3–10	≤ 12
Garlic	16	≥ 5
Yacon	15–19	≥ 30
Chicory	15–20	≥ 40
Jerusalem artichoke	17–22	≤ 40

Jerusalem artichoke varies between 1 and 5 stems. Inflorescence is a multi-flowered head with bright yellow flowers. Head diameter varies from 7 to 11 cm with consideration to ray flowers [13].

Tuber coloring is white, purple-red, light brown. The dominant shape is pear, but it can also be oblong, oval and fusiform. The tubers of some cultivars have uneven surface caused by the presence of growths. The average weight of tubers varies from 10 to 100 g (usually 30–80 g) depending on the cultivar and growing region. The high farming level allows to obtain 500 g tubers [13]. Jerusalem artichoke tubers contain about 22% of inulin on a wet weight basis [14, 15].

Thanks to its agrobiological properties including high cold- and drought-resistance, low demand towards soil and fertilizers, Jerusalem artichoke is widely cultivated in Russia. The Russian breeders have bred inulin-containing cultivars of Jerusalem artichoke with regular geometric shape that are suitable for industrial processing.

The State Register of breeding achievements currently include 4 cultivars of Jerusalem artichoke: "Vyl′gortskij" (Institute of Biology of Komi Science Center, Ural Division, Russian Academy of Sciences), "Interes" (Maykop station of VIR, KFH "Topinambur" LLC, Rodnik Zdorov'ya "IKSI" LLC), "Nakhodka" (Maykop station of VIR) and "Skorospelka" (Rodnik Zdorov'ya "IKSI" LLC and KFH "Topinambur" LLC) and one cultivar of topinsolnechnik (Maykop station of VIR) [16–18].

Analysis of literature sources revealed that Jerusalem artichoke is a multifunctional agricultural crop and can serve as raw material for production of many different common, functional and dietary products, biologically active and food supplements, medicines. Jerusalem artichoke also has significant biotechnology and bioenergy potential. Tubers and overground parts are used for ethanol production and microbiological synthesis of protein, glycerin, organic acids. At the same time, chemical composition, biochemical and technological properties of Jerusalem artichoke are affected by various environmental factors and are characterized by considerable variability. For example, the content of inulin, degree of its polymerization, qualitative and quantitative composition of oligofructose and free sugars depend on cultivar, growing conditions, time of harvesting, post-harvest ripening and storage conditions [1].

The comprehensive processing of tubers with sorting of raw materials according to technological indicators and further effective usage of sorted parties should be considered the most rational, taking into account the physiological and biochemical features of artichoke .

The Department of food of animal origin technology of Kuban State Technological University developed the concept of deep and complex Jerusalem artichoke processing. It allows to organize a flexible and stable production of food and ingredients from Jerusalem artichoke tubers, quick and cost-effective adjustment of product range depending on the quality of incoming raw materials.

Jerusalem artichoke can become the basis for creation of a large-scale industrial production of inulin in Russia. However, deep processing of Jerusalem artichoke and obtaining of high-quality target products according to the world standards require to resolve a number of technological problems.

The aim of this work is to improve the identification methods and technology of production of inulin from Jerusalem artichoke tubers.

OBJECTS AND METHODS OF STUDY

Jerusalem artichoke tubers of "Interes" cultivar were used for this study. They were washed, cleaned, blanched and crushed into particles with size approx. 1–2 mm. Then the water extraction using vibration with following technological parameters was conducted: water duty 1 : 2, frequency of vibration 24 Hz, process time 60 min, extragent temperature 30–35°C.

The extract obtained from Jerusalem artichoke tubers was fractionated using membranes with selectivity 5, 3, 2 kDa. It yielded four fractions, two of them contained high molecular and medium molecular inulin. After that, the concentrate was evaporated under vacuum and dried. Commercial inulin powder was obtained as a result.

Identification of medium molecular fractions of inulin was performed by IR spectroscopy on IR spectrometer Spectrum Two with ATR (United States). ^{1}H-^{13}C NMR spectra of inulin samples were recorded on Agilent/54 400 spectrometer (United States) at operating frequency 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei.

RESULTS AND DISCUSSION

Existing technologies of inulin production from Jerusalem artichoke tubers can be represented as the process flow diagram shown on Fig. 1. Extraction is the primary process determining yield and quality of the final product. Hot water (approx. 80°C) is currently used as extragent in most cases. It is added to the previously crushed tubers in various amounts (water duty from 1 : 2 to 1 : 10). The process is usually conducted in tanks with stirrer, continuous extractors are also used: screw, rotary pulsed and others. We have proposed and grounded an effective method of extraction using vibration with the following parameters: vibration frequency 24 Hz, process duration 60 min, extragent temperature 30–35°C. These parameters allow to obtain inulin yield about 90–96% from the theoretically possible [2].

The increasing of target product yield with application of vibration occurs because of the fact that vibration causes increasing particle motion relative to each other, as well as to their center of mass. As a result, interaction surface of the components involved in these processes increases; heat and mass transfer become more intensive. Besides, the classic extraction applied previously heated water. To increase the temperature of extragent additional energy costs are required, which increases the cost of the final product. There is no need in preheating when conducting mass transfer using vibration. This positively affects both quality and cost of the target product [18–21].

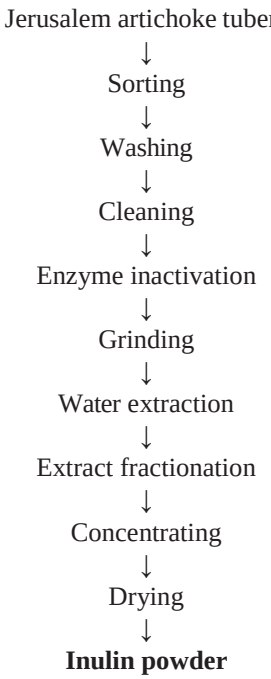

Fig. 1. The process flow diagram of production of inulin from Jerusalem artichoke tubers.

The extract obtained from tubers of Jerusalem artichoke contains inulin with various molecular weights, fructooligosaccharides, proteins, amino acids and other water-soluble substances. The ratio between different carbohydrate factions can vary depending on the raw material, growing and storage conditions and other factors. Therefore, we offer to fractionate the Jerusalem artichoke extract obtained using vibration into separate fractions. Conducted scientific and technical search revealed that the fractionation using ultra and nanofiltration is the most appropriate method. The usage of membranes with selective 5, 3, 2 kDa yielded three fractions for various purposes, including high molecular inulin for medicine and medium molecular inulin for food industry.

We applied FTIR spectrometry and ^{1}H-^{13}C NMR spectroscopy for identification and researching the quality of inulin powder (medium molecular fraction) obtained using improved technology.

The Raftiline ST inulin from Belgian company Beneo Orafti derived from chicory was taken as the reference model.

The IR spectra of reference and experimental samples (Fig. 2 and 3) contain broad absorption bands in the region 3 150–2 800 cm^{-1} that indicates the stretch vibrations of hydroxyl groups of inulin.

Bands in the region 1 636–1 628 and 1 427–1 403 cm^{-1} indicate the presence of esterified carboxyl groups, peaks 1 629 and 1 404 cm^{-1} signalize about the hydroxyl groups. The presence of glycoside bonds typical for inulin in reference and experimental samples is confirmed by the presence of peaks in the region 1 245–1 115 cm^{-1}. Absorption band in the region 1 170–950 cm^{-1} with peak at 1 028 cm^{-1} reveals the presence of OH groups of glucose in inulin. Absorption bands with maxima at 933, 870, 819, 937 and 818 cm^{-1} are typical for 2–1 bonds of fructofuranose residues that serve as links between α,D-glycopyranose and β, D- fructofuranose in the inulin molecule [22, 23].

IR spectra of experimental and reference samples are mostly identical. However, the experimental sample (Fig. 3) revealed a stronger absorption band in the range 1 245–1 404 cm^{-1}. It can be explained by high content of esterified carboxyl and hydroxyl groups that indicate the presence of pectin.

The comparison between spectrometry graphs of experimental and reference samples suggests that the experimental sample of inulin powder obtained using improved technology is identical to the reference, but has a slightly higher content of pectin.

We used ^{1}H-^{13}C NMR spectroscopy to determine the chemical structure of inulin. Data from the ^{1}H and ^{13}C NMR spectra allow to determine monomeric composition, presence and localization of functional groups, configuration of glycoside bonds, degree of polymerization and mean molecular weight of polysaccharide.

It is known that inulin is a polymer consisting of a single α,D-glycopyranose residue and several β,D- fructofuranose residues connected by glycoside bond [24].

The head chain of inulin molecule has a fragment of saccharose residue, where the ratio of α,D-glycopyranose and β,D-fructofuranose is 1 : 1, as shown in Fig. 4. The fundamental difference between the structure of saccharose and inulin lies in the number of fructofuranose residues per one glycopyranose residue. It is possible to determine the ratio of α,D-glycopyranose and β,D-fructofuranose residues using ^{1}H NMR method: each H atom in polysaccharide has characteristic integral peak indicators.

Hence, we researched in detail the spectra of saccharose using NMR 1H (Fig. 5), ^{13}C NMR (Fig. 6) first.

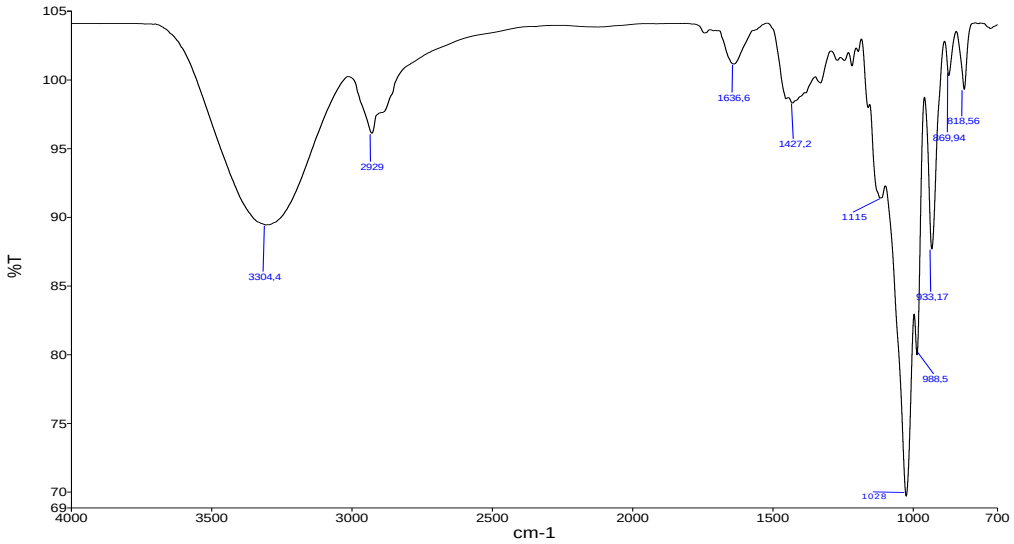

Fig. 2. IR spectrum of commercial inulin from chicory (reference model).

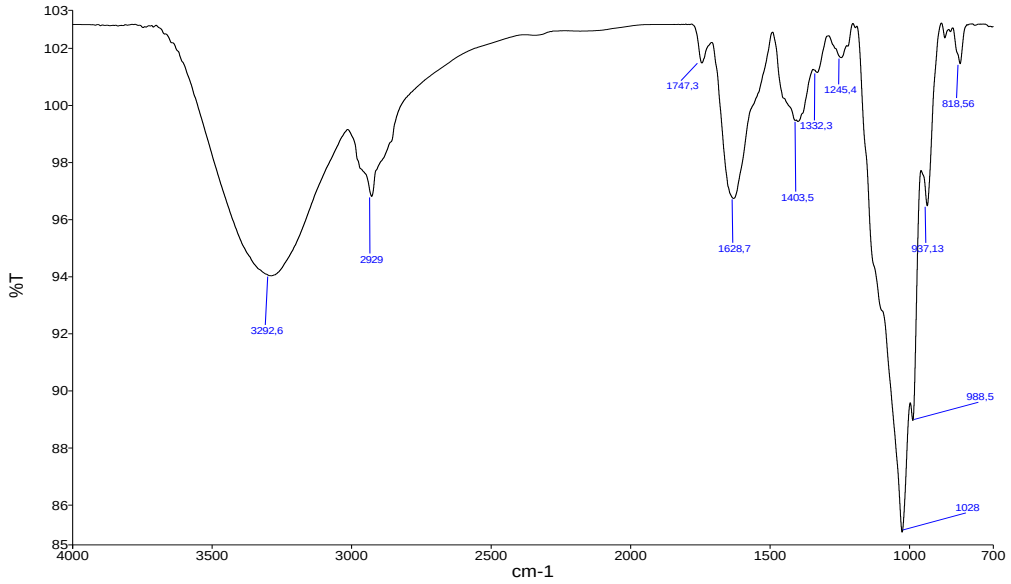

Fig. 3. IR spectrum of inulin from Jerusalem artichoke (experimental sample).

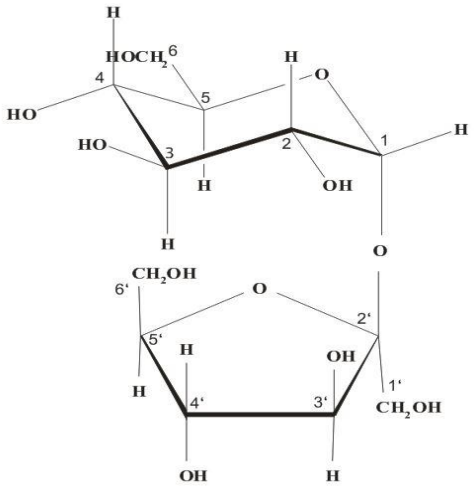

Fig. 4. Chemical structure of saccharose.

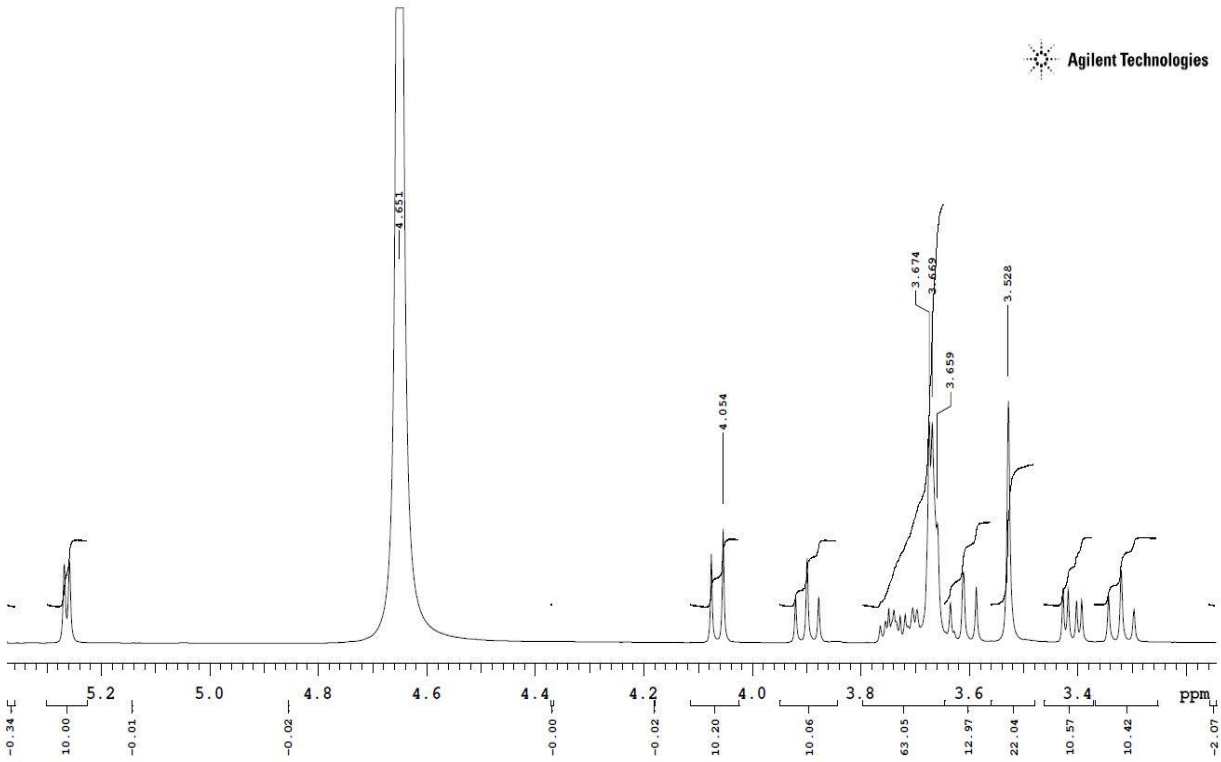

Fig. 5. [1]H NMR spectrum of saccharose.

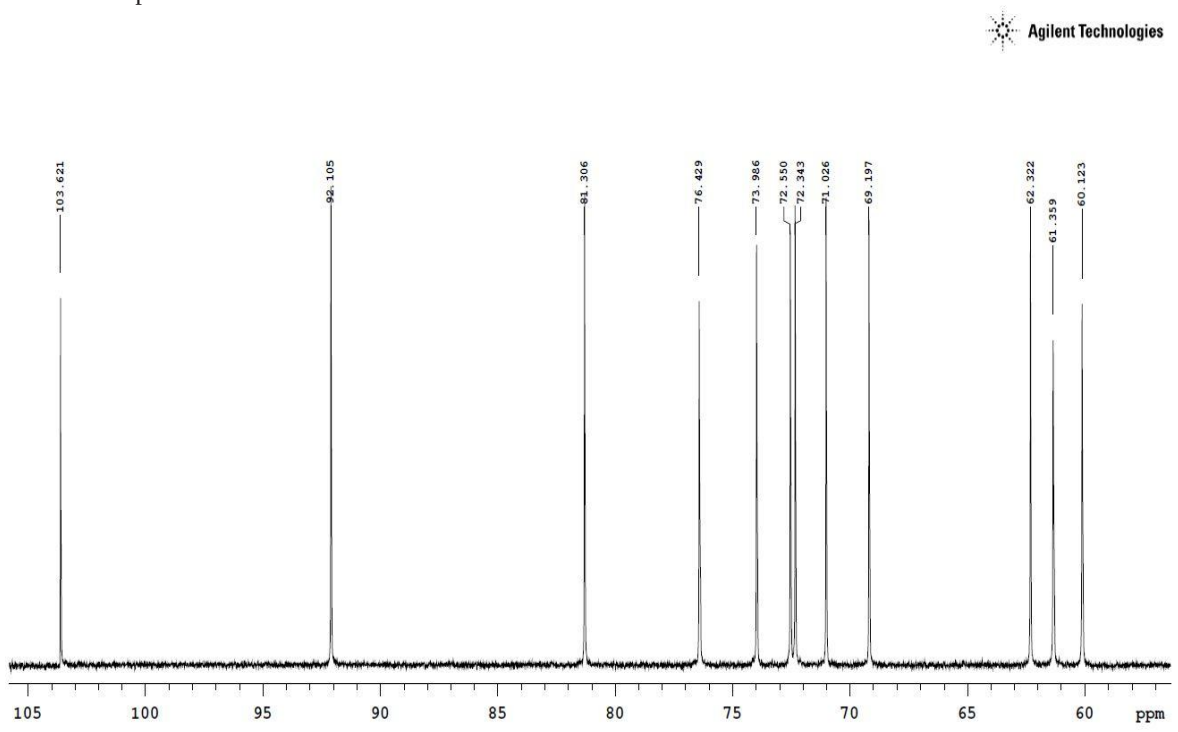

Fig. 6. [13]C NMR spectrum of saccharose.

Combination of these methods allows clear identification of all signals of hydrogen and carbon atoms in the saccharose molecule. Description of signals derived from [1]H and [13]C NMR spectroscopy is presented in Table 2.

One can see in [1]H NMR spectrum the signals of equatorial 1-H glycopyranose residue in the region 5.3 part per million (ppm) and 3′-H proton of the fructofuranose residue in the region 4.1 ppm that are suitable for further analysis. They are isolated and do not mix with the other signals. Their surface is easily interpretable, which allows to use these signals for further research of inulin samples.

The next step was examination of spectra of commercial inulin from Beneo Company (reference) and inulin obtained using improved technology. Results of [1]H and [13]C NMR spectrometry are presented in Fig. 7–10.

Table 2. Chemical shifts of hydrogen and carbon atoms in ^{1}H and ^{13}C NMR spectrum of saccharose (chemical shifts relative to the internal standard TMS)

Number of hydrogen atom	1H, δ, ppm	13C, δ, ppm
1-H	5.27	92.1
3'-H	4.06	76.4
4'-H	3.90	74.0
5'-H	3.73	81.3
5-H	3.69	72.5
6-CH2	3.68	62.3
6'-CH2	3.67	60.1
4-H	3.62	72.5
1-CH2	3.53	61.4
2-H	3.41	71.0
3-H	3.32	69.2

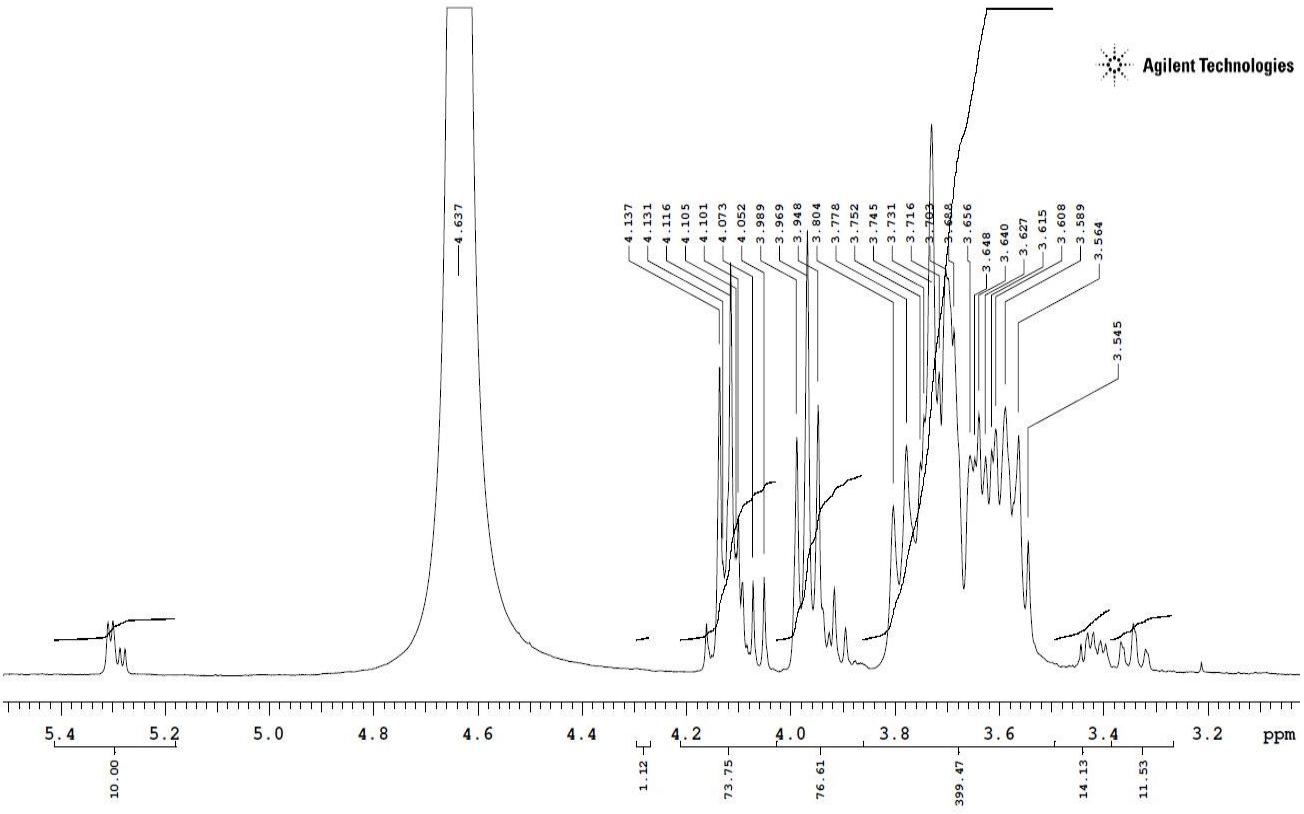

Fig. 7. ^{1}H NMR spectrum of commercial inulin from Beneo Company.

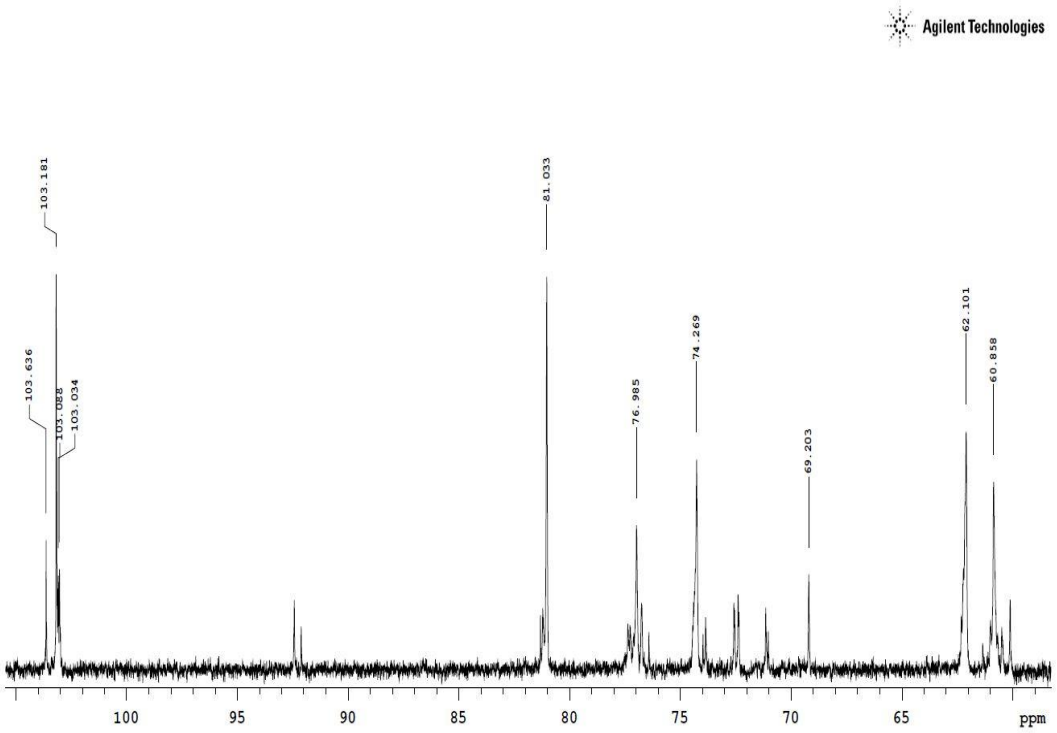

Fig. 8. ^{13}C NMR spectrum of commercial inulin from Beneo Company.

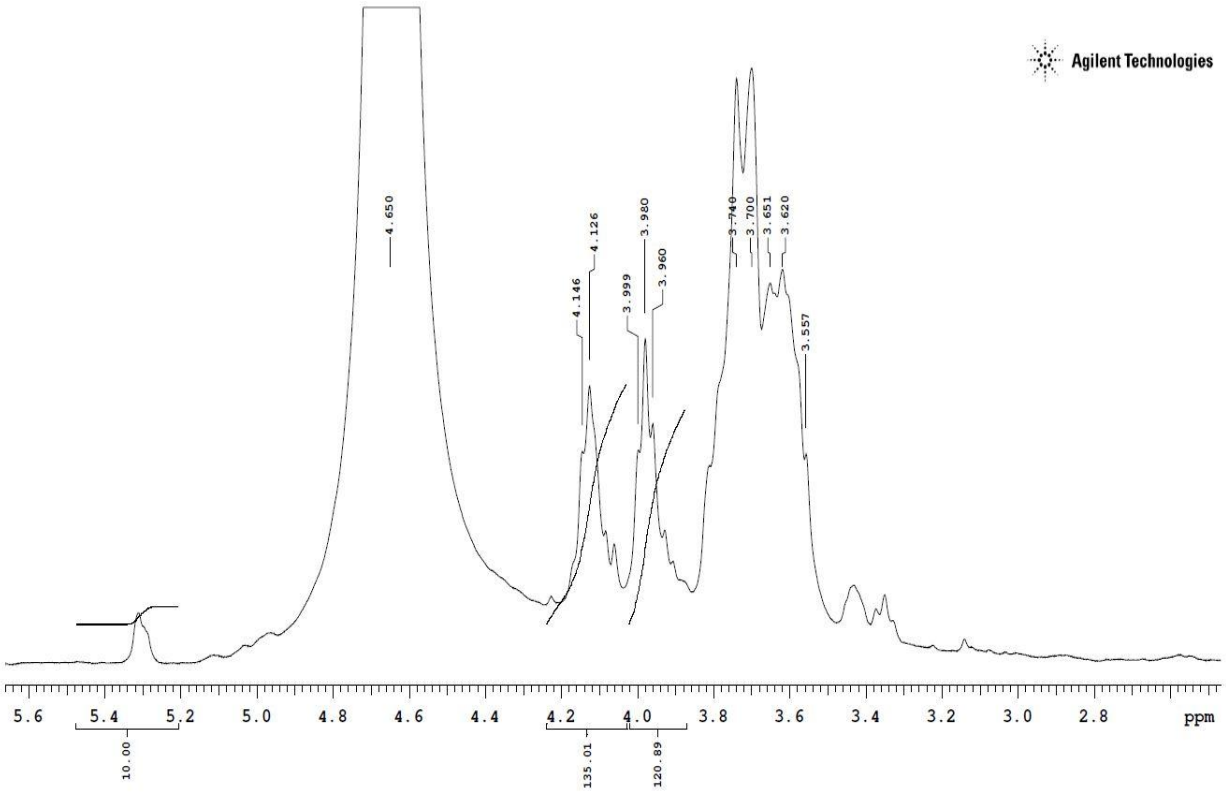

Fig. 9. ^{1}H NMR spectrum of inulin obtained using improved technology.

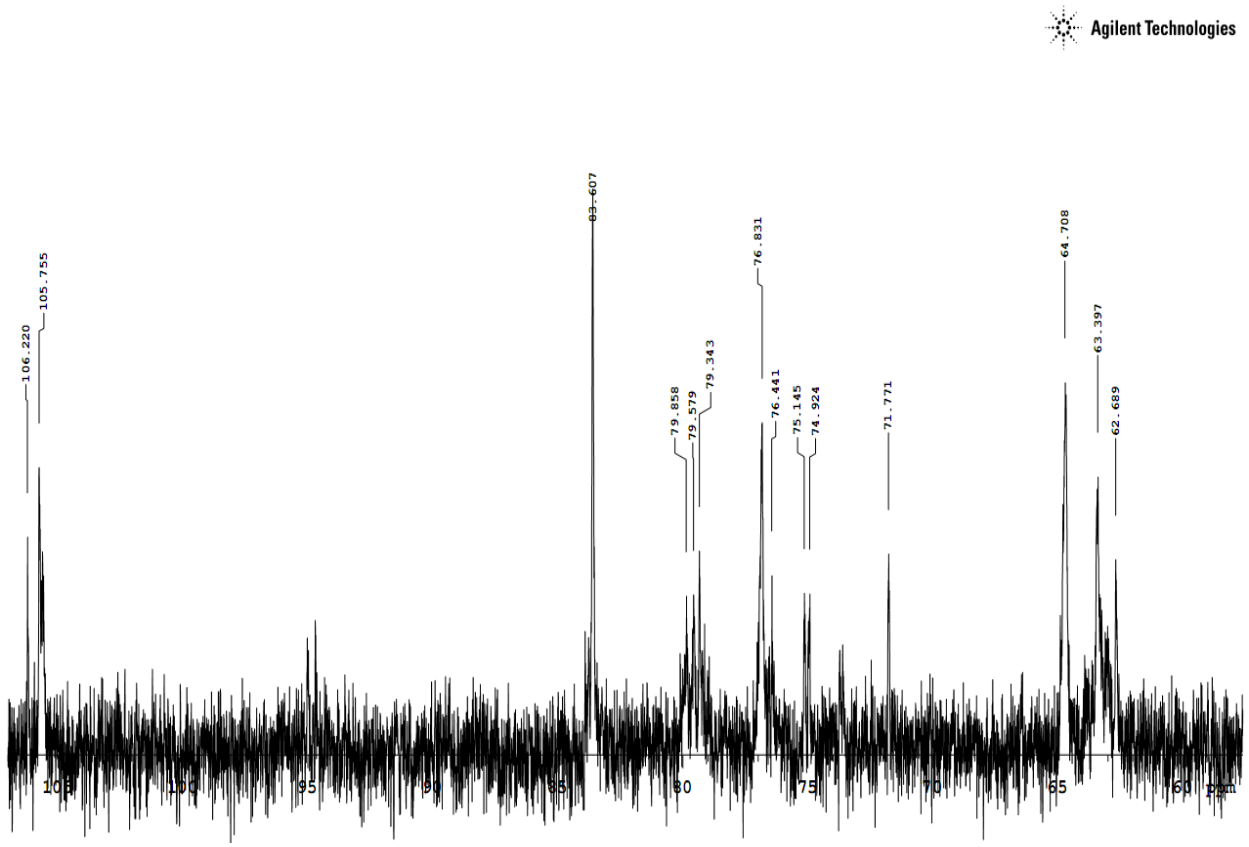

Fig. 10. ^{13}C NMR spectrum of inulin obtained using improved technology.

One can see that presented spectra of commercial inulin from Beneo Company and inulin obtained by improved technology differ by the integral intensity of signals, which belongs to α,D-glycopyranose and β,D-fructofuranose residues.

For the sample of commercial inulin from Beneo Company the ratio of integrated signal intensity of hydrogen atoms in 1-H glycopyranose form and 3′-H fructofuranose form is 1 : (7–8), i.e. one glycopyranose fragment in polysaccharide has 7–8 fructofuranose residues. Molecular weight of this compound lies between 1 314 and 1 476 Da.

For the inulin obtained using improved technology, the ratio of the integrated intensity of the same signals is 1 : (12–13), i.e. molecular weight is 2 124–2 286 Da. The higher molecular weight of experimental sample is indicated by integral surface increase in 1H NMR spectrum. This is associated with increased viscosity of the sample solution in comparison to the sample of commercial inulin from Beneo Company at the same solution concentration.

CONCLUSION

Experimental data indicate that the inulin derived from Jerusalem artichoke using improved technology has a fairly high degree of polymerization of DP = 13–14 and molecular weight 2 124–2 286 Da and therefore possesses good technological properties and physiological activity.

This allows to draw a conclusion about the prospect of its application in the food production as gelation agent, fat substitute and sustained action prebiotic.

Thus, obtaining of inulin from Jerusalem artichoke tubers using vibratory extraction and fractionation with ultra- and nanofiltration methods provides high yield of the target product and excellent quality that is highly competitive with the best world analogues.

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