UDC 615.322
https://www.doi.org/10.34907/JPQAI.2021.47.11.003

DEVELOPMENT OF AN EXPRESS TECHNIQUE FOR THE ISOLATION OF INULIN FROM THE ROOTS OF THE ELECAMPANE (*INULA HELENIUM* L.)

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Inulin is a natural plant polyphructosan, the importance of which in modern medicine and pharmacy as well as the food industry is difficult to overestimate. The available patented processes for the production of inulin are characterized by a low product yield and a significant duration. The aim of the study was to develop an express technique for the isolation and quantitative determination of inulin from the roots of elecampane (Inula helenium L.). To speed up the process of extracting the biologically active substances from the elecampane roots, as well as to increase the yield of inulin, it was decided to use an ultrasonic bath. Varying the parameters of the process, it was possible to choose the optimal conditions for extracting inulin from the elecampane roots under ultrasound treatment: raw material grinding – 0.5–1.0 mm, temperature – 80°C, extraction multiplicity – 3, extraction duration – 15 min., ultrasound frequency – 35 kHz, the ratio of raw materials and extraction solvent – 1 g per 15 ml. A further increase in the extraction time under the conditions of an ultrasonic bath leads, obviously, to the destruction of water-soluble polysaccharides. The optimal conditions for the purification of the polysaccharide complex of the elecampane roots with the production of pure inulin were also selected. The proposed method makes it possible to intensify the process of obtaining inulin from the elecampane roots and reduce the duration of the process to 6–7 hours, as well as increase the product yield up to 20.63±0.36% in terms of absolutely dry raw materials. The technique can be used for express analysis of the quality of the elecampane roots and for industrial production of inulin from this type of raw material.

Keywords: inulin, water-soluble polysaccharides, ultrasound, elecampane (*Inula helenium* L.)

Inulin is a natural polypructosan, which is partially broken down in the gastrointestinal tract to fructose, the importance of which in modern medicine and pharmacy, as well as the food industry, is difficult to overestimate. The non-split part of inulin, being an active sorbent, removes a lot of toxins from the body starting from heavy metals, radionuclides up to excess low-density lipoproteins. Inulin, being a prebiotic, contributes to the normal functioning of the gastrointestinal tract, which is especially important, since, according to Roszdravnadzor, up to 90% of Russians suffer from some degree of dysbacteriosis. In addition, inulin exhibits pro-kinetic activity, stimulating the contractility of the intestinal wall and providing normal stool. On the basis of inulin, a lot of medicines and biologically active additives, including domestic ones, are produced. Due to its moisturizing and prebiotic action, inulin is used in cosmetology, in the production of creams, shower gels, shampoos and conditioners, antiperspirants, masks and serums, cosmetics for children. Inulin is a popular sweetener for diabetic patients. In addition, inulin plays the role of a fat substitute and is used for the production of low-calorie confectionery and dairy products [1–3].

Inulin is produced only from plants by extraction with water followed by purification. The available patented processes for production of inulin are characterized by a low product yield and a significant duration, the extraction of raw materials takes up to 3–5 days [4, 5]. The main industrial sources of inulin today are specially grown raw materials: jerusalem artichoke tubers (up to 18% inulin), chicory roots (up to 40% inulin). At the same time,"chicory" inulin has contraindications for people with varicose veins and chronic respiratory diseases. Other sources of inulin are also widely known, in particular, such available plants that have significant raw materials reserves on the territory of the Russian Federation as burdock, dandelion, and elecampane [6].

Elecampane (*Ínula helenium* L. – syn.: *Aster helenium* (L.) Scop., *Aster officinalis* All., *Corvisartia helenium* (L.) Mérat, *Helenium grandiflorum* Gilib.) – perennial plant species of Elecampane (*Ínula helenium* L.) genus, sunflower family *(Asteraceae)*, grows everywhere in Europe, Asia and Africa [7–9]. There is a method for production of inulin from the elecampane roots, including preparation of inulin-containing raw materials, its mechanical cleaning, washing of roots, rhizomes and stems, their crushing and mixing. The mixed and crushed pieces of raw material are extracted twice with hot water at a temperature of 75°C for 2–3 days with constant stirring. The resulting extract of inulin as a whole is treated with 96% ethyl alcohol in a ratio of 1: 1 by volume, followed by precipitation of inulin at temperature of minus 16°C. The disadvantage of this method is the duration of the process, low inulin yield and a large amount of impurities in the finished product [10].

One of the promising physical methods of influencing on substances in order to intensify technological processes is a method based on the use of mechanical vibrations in the ultrasonic range. It was found, for example, that using ultrasound with frequency of 19–44 kHz, it is possible to extract flavonoids, tannins, phenolic glycosides, coumarins, and anthocyanins from plants with the extraction process duration reduction by 1–2 orders of magnitude [11]. This not only significantly accelerates the process of extracting useful substances from plants, but also increases the yield of the main product in comparison with other extraction methods [12,13].

The purpose of the study is to develop an express technique for obtaining inulin from the elecampane roots using an ultrasonic bath.

MATERIALS AND METHODS OF STUDY

To intensify the process of extracting water-soluble polysaccharides (WSP), ultrasonic bath "Grad 40–35" was used, weighing was carried out using analytical balance "A&D GH – 202", drying to a constant mass was provided in a hot-air oven "Vityaz GP-40". Purified water was used as an extraction solvent, and the remaining process parameters were selected experimentally.

When developing the method, the elecampane roots were used, purchased in one of the pharmacies in the city of Voronezh (manufacturer – Fitofarm LLC, series 170617).

RESULTS AND DISCUSSIONS

Initially, the optimal conditions for the extraction of water-soluble polysaccharides (WSP) from the elecampane roots using an ultrasonic bath were determined. The raw material fineness, the extraction temperature, the extraction multiplicity and duration, the ratio of raw materials to extraction solvent, and the ultrasound frequency were varied. All the determinations were carried out in three repetitions. The results of the experiment are shown in Tables 1, 2, and 3.

Thus, the optimal conditions for the extraction of water-soluble polysaccharides (WSP) from the elecampane roots were selected: the raw material fineness – 0.5–1.0 mm, the temperature is 80°C, the extraction multiplicity is 3, the extraction duration is 15 minutes, the ultrasound frequency is 35 kHz, the ratio of raw materials to extraction solvent is 1 g per 15 ml. A further increase in the extraction time under the conditions of an ultrasonic bath leads, obviously, to the destruction of water-soluble polysaccharides. The use of ultrasound with frequency above 40 kHz also leads to the destruction of biologically active substances and is not used in the production of herbal medicinal products [11].

Further studies were directed to the development of a method for the purification of the obtained water-soluble polysaccharides from the elecampane roots. The precipitate obtained after precipitation of water-soluble polysaccharides with ethanol contains impurities of pectin, some pigments, and some organic acids. To remove pectins, it was decided to provide interaction with calcium salt after dissolving the resulting WSP precipitate in water, and to purify it from pigments the interaction shall be provided with finely dispersed aluminum oxide [14]. After filtering the resulting impurity precipitate under vacuum, it was decided to remove the remaining impurities by passing the solution through the cationite and anionite columns, for which ion

Table 1

THE RESULTS OF THE QUANTITATIVE DETERMINATION OF WATER-SOLUBLE POLYSACCHARIDES (WSP) (% IN TERMS OF ABSOLUTELY DRY RAW MATERIALS) IN THE ELECAMPANE ROOTS WITH VARYING RAW MATERIAL FINENESS AND ULTRASONIC BATH TEMPERATURE (WITH THREE-TIME EXTRACTION FOR 15 MINUTES EACH USING AN ULTRASOUND FREQUENCY OF 35 KHZ, THE RATIO OF RAW MATERIALS AND EXTRACTION SOLVENT – 1 G PER 15 ML)

Temperature, °C	Raw material fineness, mm		
	0.2–0.5	0.5–1.0	1.0–2.0
60	15.32±0.40	15.19±0.34	12.25±0.50
70	22.42±0.26	24.90±0.37	18.28±0.42
80	26.12±0.25	31.57±0.44	25.99±0.39

Table 2

THE RESULTS OF THE QUANTITATIVE DETERMINATION OF WATER-SOLUBLE POLYSACCHARIDES (WSP) (% IN TERMS OF ABSOLUTELY DRY RAW MATERIALS) IN THE ELECAMPANE ROOTS WITH VARYING MULTIPLICITY AND DURATION OF EXTRACTION (WITH RAW MATERIAL FINENESS OF 0,5–1,0 MM, THE ULTRASONIC BATH TEMPERATURE OF 80°C, USING AN ULTRASOUND FREQUENCY OF 35 KHZ, THE RATIO OF RAW MATERIALS AND EXTRACTION SOLVENT – 1 G PER 15 ML)

Duration of extraction, min.	Multiplicity of extractions		
	1	2	3
10	11.34±0.40	18.47±0.40	24.68±0.34
15	15.78±0.51	20.80±0.52	31.57±0.42
20	16.80±0.32	22.97±0.60	28.96±0.40

Table 3

THE RESULTS OF THE QUANTITATIVE DETERMINATION OF WATER-SOLUBLE POLYSACCHARIDES (WSP) (% IN TERMS OF ABSOLUTELY DRY RAW MATERIALS) IN THE ELECAMPANE ROOTS WITH VARYING THE RATIO OF RAW MATERIAL TO EXTRACTION SOLVENT AND ULTRASOUND FREQUENCY (WITH THREE-TIME EXTRACTION FOR 15 MINUTES EACH, RAW MATERIAL FINENESS OF 0,5–1,0 MM, THE ULTRASONIC BATH TEMPERATURE OF 80°C)

Ratio of raw material and extraction solvent (g:ml)	Ultrasound frequency, kHz		
	15	25	35
1:10	14.68±0.30	21.86±0.43	23.49±0.30
1:15	15.86±0.45	26.37±0.30	31.57±0.44
1:20	16.90±0.37	24.30±0.52	27.96±0.46

exchange columns with anionite in the hydroxyl form AV-17-8 and cationite in the hydrogen form KU-2-8 were selected.

The degree of purification of the finished product was provided by thin-layer chromatography (TLC), comparing it with a standard inulin sample (plates – Silufol, system – 55% ethanol, developer – solutions of resorcinol and sulfuric acid diluted with subsequent heating, Rf~0.81) [15,16].

The complex of the experimental works performed makes it possible to propose the following method of isolation and subsequent quantitative gravimetric determination of inulin in the elecampane roots. To obtain inulin, the analytical sample of the raw material is crushed to particles of 0.5–1.0 mm in size. About 1 g (exact weight) of the crushed raw material is placed in a flask with a capacity of 50 ml, 15 ml of purified water heated to the boiling point is added, and placed into an ultrasonic bath with frequency of 35 kHz at temperature of 80°C, then extracted for 15 minutes. The extraction is repeated 2 more times with adding 15 ml of water. The water extracts are combined and filtered through 3 layers of gauze with a cotton swab placed in a glass funnel with a diameter of 5 cm. Precipitation is carried out with a triple amount of 95% ethyl alcohol, mixed, cooled in a freezer at a temperature of –18°C for 1 hour. The content of the flask is then filtered through a pre-dried and weighed ashless paper filter laid in a glass filter POR 16 with a diameter of 40 mm, under vacuum at residual pressure of 0.4–0.8 atm.

The resulting precipitate is dissolved in 10 ml of purified water heated to 80°C, 5 drops of a 50% solution of calcium chloride and 0.5 g of fine aluminum oxide powder are added, hold for 20 minutes, then filtered under vacuum at residual pressure of 0.4–0.8 atm. The resulting filtrate is successively passed through ion-exchange columns with anionite in the hydroxyl form AV-17–8 and cationite in the hydrogen form KU-2–8, taking into account the capacity of the ion-exchange resins to the eluate pH of 6.5–7.5 and the degree of inulin purity equal to 97%. To precipitate inulin, a three-fold amount of 95% ethanol is added to the eluate again with stirring, cooling in the freezer at –18°C for 1 hour, filtration of the precipitate is carried out through a pre-dried ashless paper filter under vacuum at residual pressure of 0.4–0.8 atm. The filtration residue is washed sequentially with 15 ml of a solution of 95% ethyl alcohol in purified water (3:1), 10 ml of mixture of ethyl acetate and 95% ethyl alcohol (1:1). The filter with the residue is dried first in air, then at temperature of 100–105°C to a constant mass.

Table 4

METROLOGICAL CHARACTERISTICS OF THE METHOD OF QUANTITATIVE DETERMINATION OF INULIN IN THE ELECAMPANE ROOTS

N	f	X	S^2	S	S_x	P, %	t(P, f)	Δx	ε, %
10	9	20.63	0.02552	0.15975	0.05052	95	2.2622	0.36	1.75

The inulin content in terms of completely dry raw materials is calculated using the standard formula:

$$X = \frac{(m_2 - m_1) \times 100 \times 100}{m \times (100 - W)},$$

where m_1 – the mass of the dried filter, g; m_2 – the mass of the dried filter with residue, g; m – the weight of the raw material, g; W – the loss in the mass of the raw material during drying, %.

The proposed method makes it possible to intensify the process of obtaining inulin from the elecampane roots and reduce the time spent on it to 6–7 hours, as well as increase the product yield to 20.63±0.36% in terms of completely dry raw materials.

The metrological characteristics are given in Table 4, where N is the number of repetitions, f is the number of degrees of freedom, X is the average value to be determined, S^2 is the dispersion, S is the standard deviation, S_x is the standard deviation of the average value, P is the confidence probability, t(P, f) is the Student t-test, Δx is the half – width of the confidence interval of the value, ε is the relative error of the average result. Thus, the relative error of the proposed method with a confidence probability of 95% is 1.75%.

CONCLUSIONS

An express technique of isolation and quantitative determination of inulin from the elecampane roots has been developed, which can be used for quality control of this type of raw material and industrial production of inulin. The optimal conditions for the extraction of water-soluble polysaccharides (WSP) from the elecampane roots were selected: the raw material fineness – 0.5–1.0 mm, the temperature – 80°C, the extraction multiplicity – 3, the extraction duration – 15 minutes, the ultrasound frequency – 35 kHz, the ratio of raw materials to extraction solvent – 1 g per 15 ml. In addition, the optimal conditions for cleaning the polysaccharide complex of the elecampane roots were selected, which are reduced to the precipitation of pectins by calcium salts, the absorption of aluminum pigments by oxide, followed by passing the extract through ion-exchange columns. The proposed technique allows you to intensify the process of obtaining inulin from the elecampane roots and reduce the process duration to 6–7 hours, as well as increase the product yield to 20.75% in terms of completely dry raw materials.

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