Elaboration of an express technique for inulin extraction from the roots of elecampane (Inula helenium L.)

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Abstract. Inulin is a natural plant polyfructosan which has a high significance for modern medicine and pharmacy as well as food industry. Existing patented technologies for inulin production are characterized by a low product output and long extraction time. The aim of the research was to develop an express method for the extraction and quantification of inulin from the roots of elecampane (Inula helenium L.). An ultrasonic bath was used in order to speed up the process of extracting biologically active substances, as well as increasing the output of inulin from the roots of elecampane. By varying process conditions, it was possible to determine optimal conditions for the extraction of inulin from elecampane roots during ultrasonic treatment: grinding raw material to 0.5 - 1.0 mm, a temperature of 80 ºC, extraction rate 3, extraction time 15 min, ultrasound frequency 35 kHz and ratio of raw materials to extractant of 1 g per 15 ml. This method reduced extraction time to 6-7 hours, as well as increased product output by 20.63 ± 0.36 % in terms of completely dry raw material. A further increase in extraction time under ultrasonic bath conditions leads to the destruction of water-soluble polysaccharides. Also optimal conditions for purification of the polysaccharide complex of elecampane roots for obtaining pure inulin were determined. The technique can be used for express analysis of the quality of elecampane roots, as well as for the industrial production of inulin from this type of raw material.

1. Introduction

Inulin, being a prebiotic, contributes to the development of the normal functioning of the gastrointestinal tract, which is especially important, since according to Roszdravnadzor, up to 90% of Russians suffer from varying degrees of dysbiosis. In addition, inulin exhibits prokinetic activity, stimulating the contractility of the intestinal wall and providing normal stool [1,2].

Inulin is used to produce a mass of drugs and biologically active additives, including domestic ones. Due to its moisturizing and prebiotic effect, inulin is also used in cosmetology in the production of creams, shower gels, shampoos and conditioners, antiperspirants, masks and serums, and cosmetics for children. Inulin is also a popular sweetener for patients with diabetes. Inulin plays the role of a fat substitute and is used for the production of low-calorie confectionery and dairy products [3,4].

Inulin is obtained exclusively from plant objects by extraction with water, followed by purification. Existing patented technologies for producing inulin result in low product yields and considerable duration, with the extraction of raw materials taking up to 3-5 days [5,6]. The main industrial sources
of inulin today are specially grown raw materials: Jerusalem artichoke tubers (up to 18% inulin) and chicory roots (up to 40% inulin). At the same time, “cyclic” inulin has contraindications for people with varicose veins and chronic respiratory diseases. Other sources of inulin are widely known, in particular such accessible plant objects with significant raw material reserves in the Russian Federation as common burdock, medicinal dandelion and elecampane. Additionally, a species of perennial plants of the genus Elecampane (Inula) of the family Asteraceae grows ubiquitously in Europe, Asia and Africa [7].

There is a method of producing inulin from the roots of elecampane which includes the preparation of inulin-containing raw materials, their mechanical cleaning, washing roots, rhizomes and stems, then grinding and mixing them. The mixed and crushed pieces of raw materials are extracted twice with hot water at a temperature of 75 °C for 2-3 days with constant stirring. The resulting extract of inulin in bulk is treated with 96% ethanol in a ratio of 1:1 by volume, followed by precipitation of inulin at a temperature of minus 16 °C. The disadvantages of this method are the long duration of the process, the low yield of inulin and a large number of impurities in the finished product [8].

The aim of this study was to develop an express technique for producing inulin from elecampane roots using an ultrasonic bath.

2. Materials and methods

To intensify the process of extracting water-soluble polysaccharides (WSPS), a Grad 40-35 ultrasonic bath was used [9,10], weighing was performed on an A&D GH-202 analytical balance, and drying to constant weight was carried out in a Vityaz GP-40 dry heat oven. Purified water was used as an extractant; the remaining process parameters were selected experimentally.

When developing the methodology, roots of elecampane purchased in one of the pharmacies in the city of Voronezh were used (manufacturer "Krasnogorskleksredstva", a series of 070919).

3. The discussion of the results

Initially, the optimal conditions were determined for extracting a high amount of WSPS from the roots of elecampane using an ultrasonic bath. They varied by the fineness of the raw materials, the temperature regime of extraction, the multiplicity and duration of extraction, the ratio of raw materials and extractant, as well as the frequency of ultrasound. All determinations were performed in triplicate. The experimental results are shown in tables 1, 2 and 3.

Thus, the optimal conditions for extracting WSPS from the roots of elecampane were identified: the fineness of the raw material was 0.5-1.0 mm, the temperature was 80 °C, the extraction multiplicity was 3, the extraction duration was 15 minutes, the ultrasound frequency was 35 kHz and the ratio of raw material to extractant was 1 g per 15 ml. A further increase in the extraction time under the conditions of an ultrasonic bath clearly leads to the destruction of water-soluble polysaccharides.

Table 1. The results of the quantitative definition of WSPS (% in terms of completely dry raw materials in the roots of the ninety high during varying grinding of raw materials and the temperature of an ultrasonic bath (three times extraction of 15 minutes with a frequency of ultrasound of 35 kHz, ratio of raw materials and extragent of 1 g per 15 ml))

| Comminution of raw material, mm | 0.2-0.5 | 0.5-1.0 | 1.0-2.0 |
|-------------------------------|---------|---------|---------|
| Temperature, °C               |         |         |         |
| 60                            | 15.34±0.31 | 14.09±0.41 | 13.06±0.42 |
| 70                            | 21.43±0.42 | 24.61±0.43 | 19.19±0.29 |
| 80                            | 27.11±0.36 | 33.92±0.31 | 26.01±0.28 |
Table 2. The results of the quantitative definition of WSPS (% in terms of completely dry raw materials) in the roots of the ninety high with varying multiplicity and duration of extraction (at the grinding of raw materials of 0.5-1.0 mm, the temperature of an ultrasonic bath of 80 °C, 35 kHz is ultrasound frequency, raw and extragent ratio of 1 g per 15 ml)

| Extraction multiplicity | 1          | 2          | 3          |
|-------------------------|------------|------------|------------|
| Duration of extraction, min. |            |            |            |
| 10                      | 10.90±0.31 | 17.82±0.31 | 23.73±0.43 |
| 15                      | 14.98±0.42 | 21.77±0.44 | 33.92±0.34 |
| 20                      | 17.51±0.43 | 23.25±0.42 | 27.80±0.31 |

Table 3. The results of quantitative definitions of WSPS (% in terms of completely dry raw materials) in the roots of the ninety, high at a varying ratio of raw materials and extragent and frequency of ultrasound (three times extraction of 15 minutes, shredding of raw materials of 0.5-1.0 mm, Ultrasonic bath temperature of 80 °C)

| Frequency of ultrasound, kHz | 15     | 25     | 35     |
|------------------------------|--------|--------|--------|
| Ratio of raw materials and extractant (g:ml) |        |        |        |
| 1:10                         |        |        |        |
| 15.81±0.41                   |        |        |        |
| 20.60±0.32                   |        |        |        |
| 26.52±0.41                   |        |        |        |
| 1:15                         |        |        |        |
| 16.50±0.37                   |        |        |        |
| 27.41±0.41                   |        |        |        |
| 33.92±0.33                   |        |        |        |
| 1:20                         |        |        |        |
| 17.88±0.42                   |        |        |        |
| 25.02±0.41                   |        |        |        |
| 29.84±0.31                   |        |        |        |

Further studies were aimed at developing a method for purifying the obtained water-soluble polysaccharides from the roots of elecampane. The precipitate is obtained from water-soluble polysaccharides with ethanol contained impurities of pectin, some pigments, and some organic acids. Pectins were removed by dissolving the obtained WSPS precipitate in water, then introducing a calcium salt. Pigments were removed with finely dispersed aluminum oxide [11]. The degree of purification of the finished product was determined by thin-layer chromatography, comparing it with a standard inulin sample (Silufol plates, 55% ethanol system, developer-solutions of resorcinol and sulfuric acid diluted with subsequent heating, Rf ~ 0.81).

The set of experimental findings makes it possible to propose the following method of selection and subsequent quantitative determination of inulin in the roots of elecampane. To obtain inulin, the analytical sample of raw materials is crushed to particles of 0.5-1.0 mm. 1 g of crushed raw materials is placed in a flask with a capacity of 50 ml, to which 15 ml of purified water are added, heated to boiling point, placed in an ultrasonic bath with a frequency of 35 KHz at 80 °C for 15 minutes. Extraction is repeated 2 times more. Deposition-precipitation is carried out three times using 95% ethanol, stirred and cooled in the freezer at -18 °C for 1 hour. The contents of the flask are then filtered through a pre-dried and weighted, ashless paper filter, laid in a 40 mm POR glass filter with a diameter of 40 mm, under a vacuum at a residual pressure of 0.3-0.7 atm. The resulting sediment is dissolved in 10 ml water heated to 80 °C. Then 5 drops of 50% calcium chloride solution and 0.5 g fine powdered...
aluminium oxide are added, let stand for 20 minutes, before filtering under a vacuum at residual pressure 0.4-0.8 atm. The resulting filtrate is passed through ion exchange columns with anionitis in a hydroxyl form AV-17-8 and cationite in hydrogen form KU-2-8, taking into account the capacity of ion exchange resins up to pH eluate 6.5-7.5 and the purity of inulin equal to 97%. For deposition of inulin to the eluate again add triple amount of 95% ethanol when stirring, then cooling in a freezer at -18 degrees Celsius for 1 hour, then filtering the sediment through a pre-dried groundless paper filter under vacuum at residual pressure of 0.3-0.7 atm. The filter with precipitation is dried first in the air, then at a temperature of 100-105 °C until getting permanent mass.

The metrological characteristics are shown in the table. 4 (where N - number of repeats, f - number of degrees of freedom, X - average defined value, S2 - variance, S - standard deviation, Sx - standard deviation of average, P - confidence probability, t (P,f) - Student criterion, zx - the half-width of the confidence interval of the magnitude, the relative error of the average result). Thus, the relative error of the proposed methodology with a 95% confidence level is 1.75 %.

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

4. Conclusion
An express method of extracting and quantifying inulin from elecampane roots has been developed, which can be used in the quality control of elecampane roots and the industrial production of inulin from this type of raw material. Optimal conditions of extraction of WSPS from elecampane roots are: grinding of raw materials to 0.5 - 1.0 mm, temperature - 80 °C, extraction multiplicity - 3, duration of extraction - 15 minutes, ultrasound frequency of 35 kHz and ratio of raw materials and extragent of 1 g to 15 ml. The optimal conditions for purification of the polysaccharide complex of elecampane roots were also determined: reducing the deposition of pectins with calcium salts; absorption of pigments with aluminium oxide and the subsequent flow of the extract through ion exchange columns. The proposed method also reduces extraction time to 6-7 hours, as well as increases product output by 20.75 percent in terms of completely dry raw materials.

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