Fructose biopolymers contained in roots of *Heliánthus annuus*

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**Abstract.** The article investigates fructose biopolymers contained in the roots of *Heliánthus annuus*. The raw material has been analyzed in terms of the content of inulin and other accompanying components, which are both advantageous for developing new functional food products and challenging for inulin extracting. Because in the composition of preparations from the sunflower root, this may be an advantage for the future food product, but when extracting inulin, this can create certain difficulties. These components are polyphenolic substances, namely tannins. Literature sources describe the presence of polyphenols in conflicting amounts, which justifies our research objective. The article presents a study of the yield of inulin in the extract at different extraction temperatures. We have determined the moisture content of the crushed sunflower root, the content of extractives in percent in absolutely dry raw materials, the content of tannins in percent in absolutely dry raw materials, as well as the content of inulin. The research aims to apply the results obtained for the further development of a functional food product.

**1. Introduction**

Despite the improvement in living conditions, many people suffer from diabetes mellitus, dysbiosis, obesity, which significantly reduce the working capacity of population. A balanced diet, which includes food components with a positive effect on human health, plays an important role in the prevention of these diseases. Such food components are fructans, including inulin, positively affecting metabolism, stabilizing blood glucose levels, and also having a prebiotic effect [1].

Fructans are polymers of fructose containing inulin, levan, and oligofructose. Oligofructose belongs to the fructan subgroup s that contains less than 10 fructose monomers. Inulin and levan are fructans, but in inulin fructose residues are linked by β-2,1 bonds (figure 1), in levan fructose residues are linked by β-2,6 bonds [2].
Inulin was first isolated by the German chemist and pharmacist Rose Valentine the Younger in 1804 from the root of the flowering plant _Inula helenum_, which belongs to the _Asteraceae_ family [3]. Inulin is a reserve polysaccharide in plants. In 2018, the U.S. Food and Drug Administration confirmed that the addition of inulin increases the nutritional value of foods [4]. In 1992, the U.S. Food and Drug Administration also approved inulin as a safe and effective nutritional supplement for infants.

Inulin is a prebiotic and this property attracts the main interest of the scientific and industrial communities and is used in food technology. Due to the β-bond of fructose residues, which is not hydrolyzed by the enzymes of the human digestive system, inulin is not digested or absorbed in most parts of the human digestive system. Inulin travels through the gastrointestinal tract to the left descending colon, where it is fermented by the beneficial bacteria; this beneficially affects the microbiome of the gastrointestinal tract [5].

1.1. Inulin in the food industry
Inulin, due to its ability not to be absorbed by the human body, is referred to as dietary fiber. As a dietary fiber, it is used to reduce the calorie content of food. Inulin has many applications in food production, such as lactic acid products, soft drinks, and confectionery. The most frequent is that it can be used as a substitute for sugars or fats, depending on the biopolymer chain length.

Compared with long-chain inulin, short-chain, low molecular weight inulin is more readily degraded by enzymes as a result of the formation of sweet oligosaccharides and monosaccharides. Short-chain inulin can be used to replace sugar, since the mixture of hydrolysis residues of such an inulin provides enough sweet taste [6]. Inulin itself has a neutral taste and adds no aftertaste [7]. Short-chain inulin retains water better than long-chain inulin and has rheological properties suitable for its application in chocolate making [8].

Inulin can also be added to a product as a supplement to replace fat. Compared with short-chain inulin solutions, long-chain high molecular weight inulin exhibits higher solution viscosity and gel structure even when used at low concentrations [9; 10]. In addition, inulin is poorly water-soluble. Kaur and Gupta [11] showed that in aqueous solutions, it forms microcrystals. In the mouth these microcrystals create a creamy texture that makes the feeling of a greasy product. As a fat substitute, inulin can be used in foods that contain liquids. Due to this, inulin is used in cream fillings, dairy products, and frozen desserts [12].

However, there is a limitation on the use of inulin in food. For example, inulin cannot be used in soft drinks or fruit jams, as it is unstable in acidic media and can be hydrolyzed to oligofructose and monosaccharides. This property of inulin must be considered when creating a product with an acidic medium [13].

1.2. Beneficial properties of inulin for human health
Inulin was tested for other beneficial properties to use it as efficiently as possible in the food and other industries. Inulin shows many potential therapeutic benefits, such as reducing intestinal inflammation,
reducing the risk of various types of cancer, improving intestinal motility, increasing calcium absorption, antioxidant activity, etc. [14].

Inulin possesses anti-cancer and anti-inflammatory properties. Hijová et al. [15] demonstrated that consumption of foods containing inulin prevents precancerous changes and inflammation that contribute to the development of colon cancer.

Consuming food containing inulin helps prevent colorectal cancer [16]. Inulin-based drug delivery systems are designed to assist the fight against other types of cancer, for instance breast cancer [17]. It should also be noted that inulin has antidiabetic effects. An inulin based product lowers blood sugar levels and normalizes metabolism. The most part of inulin is not cleaved in the acidic environment of the stomach and is not hydrolyzed by enzymes of the human body. Inulin absorbs a significant amount of glucose and toxic products of impaired metabolism. Thus, excess glucose and toxic metabolic products are excreted from the body preventing its absorption into the bloodstream, which helps to lower blood sugar after meals.

Chicory is known for a high inulin content and is economically profitable. However, raw material has been insufficient for the Russian processing industry. Russia imports dried chicory root from other countries, such as Ukraine and India. Chicory is not grown in the required volumes due to the laboriousness of its cultivation in comparison with other crops, as it requires a large share of manual labour primarily in harvesting its long roots. Scientists try to create new chicory varieties with roots more convenient and suitable for the requirements of modern industrial technologies [18].

The use of Jerusalem artichoke to obtain inulin is also not common. Currently, there is no raw material on an industrial scale or a complex of machines for the mechanized technology of Jerusalem artichoke cultivation. For these reasons, the development of a technology for growing Jerusalem artichoke and obtaining inulin from it requires large capital investments [19].

The possibility to use other raw materials for inulin production is interesting. For the purpose of economic efficiency of inulin production, it is possible to use secondary raw materials such as plant waste. For example, the use of waste from sunflower growing. Sunflower belongs to the family Asteraceae, the same as chicory and Jerusalem artichoke. Ten years ago, the area of sunflower cultivation has increased by 20% and in 2019 reached 8.6 million hectares. [20]. After harvesting sunflower, about 7 t/ha of dry organic matter of plant residues remains in the field [21]. Among these residues are inulin containing sunflower roots, although there are very few studies on the inulin content in various parts of sunflower.

Thus, our research aims to investigate biopolymers based on fructose and tannin contained in the roots of *Heliánthus annuus*. We have also set an objective to study the yield of inulin in the extract at different temperatures, which is important for the development of a functional drink.

2. Materials and methods

In the present work, the method of sunflower root extraction was selected based on the literature data.

2.1. Materials

Dried crushed root *Heliánthus annuus* was used for our research.

2.2. Methods

The studies were performed in the laboratory of ITMO University. The following methods were applied:

2.2.1. The method for determining the moisture content. The mass fraction of moisture was measured using a moisture analyzer MOC63u (Shimadzu, Japan).

2.2.2. The method for determining the extractives content. The determination done according to the normative document for the study of medicinal plant materials [22]. The arithmetic mean of the results of two parallel determinations is taken as the final test result. The determination was performed three
times on two parallels. Initial measurements showed that the content of extractives in dried sunflower root is low. To solve this problem, the sample weight was five times increased.

An analytical sample of sunflower root is crushed and sieved through a sieve with holes 1 mm in diameter, after which a sample weight is taken with a mass of 1 g. The sample weight is placed in a conical flask, 50 ml of distilled water is poured, the flask is closed with a stopper, weighed with an error of not more than 0.01 g and left for 1 hour. Then the flask is connected to a reflux condenser, heated to boiling and the liquid is kept at a slight boiling for 2 hours. After cooling, the flask with the content is closed again with the same stopper, weighed and the mass loss is supplemented with distilled water. The content is thoroughly shaken and filtered through a dry paper filter into a dry flask with a capacity of 150–200 ml. After 25 ml of the filtrate is pipetted into a porcelain dish 7–9 cm in diameter, previously dried at 100–105 °C to constant weight and weighed on an analytical balance. The filtrate is evaporated in a water bath to dryness, dried at a temperature of 100–105 °C for 3 hours. Then the dry residue of the filtrate is cooled for 30 minutes in a desiccator with anhydrous calcium chloride on the bottom, and then weighed.

The content of extractives ($X_1$) in percent in absolutely dry raw materials is calculated by the formula:

$$X_1 = \frac{m \cdot 200 \cdot 100}{m_1 \cdot (100 - W)},$$

where $m$ – the mass of the dry residue in the cup, g;
$m_1$ – the mass of raw materials, g;
$W$ – loss in mass during drying of raw materials, g.

2.2.3. The method for determining the tannins content. Determination of the content of tannins in medicinal plant raw materials was conducted by the titrimetric method. Determination according to the normative document for the study of medicinal plant materials [22]. The arithmetic mean of the results of two parallel determinations is taken as the final test result. The determination performed three times on two parallels. Initial measurements showed that the content of extractives in dried sunflower root is low. To solve this problem, the sample weight was increased five times.

An analytical sample of sunflower root is crushed and sieved through a sieve with holes of 3 mm in diameter. Take a sample weight 2 g with an error of not more than 0.001 g.

The sample weight is placed in a conical flask, poured in 250 ml of boiling water and kept in a boiling water bath with a reflux condenser for 30 min. The content of the flask is cooled to room temperature and decanted about 100 ml into a conical flask through cotton wool. Then take with a pipette 25 ml of the resulting liquid into another conical flask, add 500 ml of distilled water, 25 ml of indigosulfonic acid solution. The resulting solution is titrated with 0.1 N solution of KMnO$_4$ until golden yellow coloration.

Control test. 525 ml of distilled water is poured into a conical flask, add 25 ml of indigosulfonic acid solution. The resulting solution is titrated with 0.1 N solution of KMnO$_4$ until golden yellow coloration.

The content of tannins ($X_2$) in percentage in absolutely dry raw materials is calculated by the formula:

$$X_2 = \frac{(V - V_1) \cdot 0.004157 \cdot 250 \cdot 100 \cdot 100}{m \cdot 25 \cdot (100 - W)},$$

where $V$ – the volume of exactly 0.1 N potassium permanganate solution consumed for extraction titration, ml;
$V_1$ – volume is exactly 0.1 N solution of KMnO$_4$ consumed for titration in the control analysis, ml;
0.004157 – the amount of tannins corresponding to 1 cm exactly 0.1 N solution of KMnO$_4$ solution (in terms of tannin), g;
$m$ – the mass of raw materials, g;
2.2.4. The resorcinol method for determining the inulin content in the extract. Sunflower root contains fructosides and fructans (most of them are inulin). The method is based on the fact that inulin is soluble in water, but not in 95% alcohol. The rest of the fructosides are soluble in both solvents. Therefore, the alcoholic extract contains no inulin, while the aqueous extract contains fructans and fructosides. The content of fructosides and fructans in terms of inulin and absolutely dry raw materials as a percentage is calculated by a specific indicator of light absorption. The amount of inulin is found as the difference between the sum of fructosides and fructans and separately the sum of frutosides. The photocolorimeter KFK-3.01 was used in this study.

An analytical sample of sunflower root is crushed and sieved through a sieve with a hole diameter of 2 mm. A sample weighing 1.0 g is placed in a conical flask, in the case of determining the sum of fructosides and fructans, 60 ml of water is added to the flask and kept in a boiling water bath for 30 minutes. In the case of determining fructosides, 60 ml of 95% rectified ethanol are added to the flask and kept in a boiling water bath for 30 minutes using a reflux condenser. The extract is cooled to room temperature, filtered through a cotton swab into a 200 ml volumetric flask. The flask is washed with 10 ml of water and filtered into the same volumetric flask. The extraction is repeated two times. 3 ml of a 10% solution of lead acetate is added to the resulting extract and left for 10 minutes. Then 3 ml of 5% sodium hydrogen phosphate solution is added and left for 5 minutes. The volume of the solution is brought up to the mark with distilled water. The extract is filtered with a paper filter. 4 ml of the filtrate is placed in a 100 ml volumetric flask. The volume of the solution is brought up to the mark with water (solution A). 5 ml of 0.1% resorcinol solution is added to two volumetric flasks with a capacity of 25 ml. In the first flask, 5 ml of distilled water (reference solution) is placed, in the second flask 5 ml of solution A is added (analyzed samples). The volume of the solutions in both flasks is brought up to the mark with hydrochloric acid HCl. The content of the flasks is kept in a water bath at 80 °C for 20 minutes. Then, the content of the flasks is cooled and brought to the mark with water.

The optical density of the analyzed sample is measured on a spectrophotometer at a wavelength of (480 ± 2) nm in a cuvette with a layer thickness of 10 mm relative to the reference solution.

The content of the sum of fructosides and fructans in terms of inulin and absolutely dry raw materials in percent \(X_3\) was calculated by the formula:

\[
X_3 = \frac{D \cdot 200 \cdot 100 \cdot 25 \cdot 100}{498 \cdot m \cdot (100 - W)},
\]  

(3)

The content of the sum of fructosides in terms of inulin and absolutely dry raw materials in percent \(X_4\) is calculated by the formula:

\[
X_4 = \frac{D \cdot 200 \cdot 50 \cdot 25 \cdot 100}{498 \cdot m \cdot (100 - W)},
\]  

(4)

The content of the sum of fructans in terms of inulin and absolutely dry raw materials in percent \(X_5\) is calculated by the formula:

\[
X_5 = X_3 - X_4,
\]  

(5)

where \(D\) – optical density of the analyzed sample;

498 – specific absorption rate of the products of interaction of inulin with resorcinol in an acidic medium;

\(m\) – mass of raw materials, g;

\(W\) – humidity, %.
3. Results and discussion
Among the information currently available, the sunflower root was investigated for the presence of inulin by the authors [23; 24]. Table 1 presents the results of studying sunflower root in literary sources. There are articles where only the total content of polysaccharides was determined. Some researchers suggest that sunflower accumulates significantly less fructans in contrast to related species, but the reason for this was not established [25]. However, no more literary sources on the content of fructans in one-year-old sunflower were found, therefore we have investigated sunflower root available on the market to accumulate data on the content of inulin.

Table 1. The research results.

| Quantitative characteristics                                    | Value          |
|----------------------------------------------------------------|----------------|
| The moisture content, %                                         | 2.58 ± 0.35    |
| The extractives content, % in absolutely dry raw materials     | 30.55 ± 0.41   |
| The tannins content, % in absolutely dry raw materials         | 11.19 ± 0.22   |
| The content of the sum of fructosans in terms of inulin and absolutely dry raw materials, % | 5.99 ± 0.13 |

The research results are presented in the table (table 2).

Table 2. The research results.

| Quantitative characteristics                                    | Value          |
|----------------------------------------------------------------|----------------|
| The moisture content, %                                         | 13.52 ± 0.87   |
| The extractives content, % in absolutely dry raw materials     | 22.49 ± 0.17   |
| The tannins content, % in absolutely dry raw materials         | 4.54 ± 0.05    |
| The content of the sum of fructosans in terms of inulin and absolutely dry raw materials, % | 4.12 ± 0.01 |

The content of inulin in the investigated sunflower root is slightly less than in the literature. Besides, the content of extractives in the test sample is less than in the literature. We assume that this is due to different raw materials used, but the results can be considered close to those in the literature. The tannin content is significantly less compared with the stated in the literature. This fact may be regarded as beneficial as the astringent flavor of tannin can affect the taste of the beverage being developed. Moreover, tannin can make bonds with polysaccharides decreasing their water solubility.

To improve the technology of inulin extraction from sunflower root, it is necessary to study how the yield of inulin into the extract depends on the extraction parameters. An important parameter is the water temperature. It is necessary to identify the optimum temperature, at which the maximum yield of inulin is observed. For this, the yield of inulin was measured at 6 different temperatures (figure 2).
The study revealed that the extraction temperature affects the inulin content in the extract. A mathematical relationship was found between the temperature of inulin extraction and its concentration in solution. The optimum extraction temperature is about 60 °C for 30 minutes. At lower temperatures, inulin does not completely pass into the extract and is lost during filtration from the sediment, and at temperatures above 60 °C the amount of inulin decreases. When exposed to high temperatures inulin is destroyed with the release of oligofructose and monosaccharides.

4. Conclusions
We have studied the sunflower root. The investigated dried root contains 22.49% extractives, 4.54% tannins, and 4.12% inulin. We have also investigated the yield of inulin into the extract at different extraction temperatures, which is important for the development of a functional drink. The optimum extraction temperature is 60 °C, which allows the maximum yield of inulin into the extract.

Thus, we can see a promising biopotential of the sunflower root for inulin extraction. The subsequent development of a functional food product containing inulin can contribute to solving the problem of modern society – poor-quality unbalanced nutrition. The data obtained in the study enables to implement a project for the development of a functional drink. The development of methods for the isolation and determination of inulin from sunflower root is necessary, since this raw material as a source of fructose biopolymers has been little studied in the international and Russian literature.

Let us note the resource-saving orientation of the project for the use of sunflower root, which is a massive waste of sunflower growing industry in Russia. The presence of sunflower root on an industrial scale in Russia distinguishes this raw material from others, such as chicory and Jerusalem artichoke.

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