Isolation and Separation of Inulin from *Phalaris arundinacea* Roots

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The inulins are a group of oligosaccharides that contain fructose. They belong to the class of carbohydrates known as fructans. The aim of this paper is to determine the quantity of proteins, lipids, and glucides by known standard methods, from roots of Phalaris arundinacea. The inulin was determined by a fast and simple way, based on the fact that in the presence of concentrated sulphuric acid, vanillin and inulin form a red complex which has a characteristic in the absorption spectrum at 520 nm. The qualitative evidentiation of inulin was done in agreement with the USP 23 criteria: the solubility test, specific rotation, percentage of iron, reducing sugars, microbial load and from the physico–chemical point of view, melting point, Molish test, with lugol and resorcinol. Inulin was characterized with the help of UV–Vis absorption spectrophotometric methods. The use of undigestable polyglucides (inulins) may improve taste, texture and humidity of many foods.

Keywords: inulin, Phalaris arundinacea, undigestable polyglucides

Inulins are found in the roots of *Phalaris arundinacea*. They are composed of fructose units with a terminal glucose molecule, being a nonreducing polyglucide [1].

Inulins are used by plants for storing energy, being found at the level of roots and rhizomes. Many plants that synthesize and accumulate inulin do not accumulate other glucides (such as starch). Inulin has begun to be used in foods, due to its nutritional and functional characteristics [2]. Inulin increases the absorption of calcium and magnesium, being a promoter for intestinal bacteria growth [3]. From the nutritional point of view it is a soluble fiber. Inulin can be digested by the enzymes from the human body (amylases), adapted to the digestion of starch. Inulin can pass undigested a big part of the digestive tract; bun in colon will be metabolized by bacteria [4]. Following the action of bacteria on inulin it is released carbon dioxide and/or methane [5]. Digestion does not decompose the inulins in monosaccharides and these do not have effect on the blood sugar level [6]. The polysaccharide has a minimum impact on the blood sugar level and can be consumed by diabetics or persons with issues regarding the blood sugar level. It can be used as sweetener (replacing sugar) in fatty and flour foods. Inulin is found in almost 36000 plant species. Inulin is a polydispersable GFn molecule. Its structure is a linear chain of linked fructose molecules (2-1), with a molecule of terminal glucose. Inulins are polymers formed of fructose units (96%), having a terminal glycosidic group (4%) (fig. 1).

Between the fructose units there are beta-(2-1) glycosidic bonds. Inulins from plants have between 2 and 140 fructose units [7]. The simplest type of inulin is

HOCH2 O H Fructose H HOCH2OH OH H Fructose H HOCH2OH H H H HOCH2OH H H H HOCH2OH H H H HOCH2OH H H H H HOCH2OH H H H H HOCH2OH H H H H H H H H

Fig. 1. Structure of the inulins

1-kestose, which includes 2 molecules of fructose and one of glucose. Those that have terminal glucose are known as alpha–D–glucopyranosyl–[beta–D–fructopyranosyl] (n–1)–D–fructofuranoside (GpyFn). The others without glucose are beta–D–fructopyranosyl–[D– fructofuranosyl] (n–1)–D–fructofuranoside (FpyFn), where n is the number of fructose residues and py is the abbreviation for the pyranosyl group.

Through hydrolysis there can be obtained oligofructose, with different degrees of polymerization (DP) ≤ 10 [8]. Inulin is hygroscopic; it dissolves easily in hot water and hard in cold water. The inulin quantity in plants depends on species, natural conditions and plant maintenance. It is easily decomposed to fructose. Inulin is a unique natural polysaccharide that contains fructose in proportion of 95% and represents a reserve hydrocarbonate. There are known 3 forms of inulin; α inulin (amorphous white powder), β -inulin (colourless crystals) and γ -inulin [9]. These are distinct by the

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molecular mass, polymerization degree, dissolving temperature, method of obtaining [10].

Form α is formed by freezing the inulin solution; form β by alcohol precipitation; form γ is obtained after applying all the processes related to the action of different temperatures. All forms can transform from one to the other.

The aim of the paper was the development of a simple and cheap extraction method for inulin (efficient purification for the removal of coloured compounds, tannins and other contaminants).

It was also aimed the characterization of the extracted inulin and the oligofructosaccharides and also the isolation and separation of inulin molecules from the roots of *P. arundinacea* and emphasize this substance through spectrometric method comparing with standard inulin.

Experimental part

Fresh roots (Phalaris arundinacea)

The roots of *P. arundinacea* were harvested from Banat, the west area of Romania.

The roots were washed with water and disinfected with solution of sodium hypochlorite, and then rinsed with water.

The fresh material was cut in water, with a blender. The obtained mix of roots of *P. arundinacea*, was kept at 65–70°C for 15–20 min. The literature indicates hot water (95°C) with good results for the isolation of inulin from roots of *P. arundinacea*.

The resulted extract of brute inulin was then concentrated under reduced pressure and crystallized in form of pasta, at low temperatures (4°C) for 30 h. The product was recuperated through pulverization and in the end dried in order to obtain a white powder.

The process is divided in 2 phases. The first phase was done after the harvesting of roots and involved the transport and slicing of *P. arundinacea* roots in order to produce

"slices" from which inulin is extracted with hot water in a special recipient.

The effluent from the recipient that circulates in countercurrent was then treated in a primary purification stage, resulting impure syrup. The primary purification stage implies a gas process of the effluent, at a high pH. CaCO₃ is added, the residue precipitates along with peptides, some anions, degraded proteins and colloids and is harvested in special recipients.

During this initial purification stage, a foam type product is obtained, rich in calcium and organic matter that can be used at improving soil structure.

The second stage of the process comprises of the refining of the *P. arundinacea* effluent, using resins that exchange anionic and cationic ions for demineralization. After the demineralization, the *P. arundinacea* effluent is sterilized by passing through a filter. The sediment is removed and the supernatant was kept for further use.

This was clarified through filtration in vacuum using diatom filter. To the liquid, ethanol 96% was added step by step, for the crystallization of inulin.

Sterilization is then followed by evaporation and drying by pulverization and the final product is collected as dry powder.

The crystallized inulin is recuperated by filtration, and then dried by lyophilization (fig. 2).

The yield of the process was calculated with the formula: Inulin extraction yield (%) = (inulin content × volume of extraction liquid / mass of P. arundinacea roots) × 100.

Determination of dry matter, ash, protein, fat and antioxidant activity of the P. arundinacea roots preparations

Dry matter, ash, protein and fat contents in the extracts from P. arundinacea roots were determined according to the official methods of AOAC.

Carbohydrate determinations were performed using Official Methods of Analysis of AOAC International [11– 14]. Phenolic determinations were performed according

↓ Harvested in October, Obtained from Banat's aria (Ron	nania)	
Pretreatment system		
↓ Ground into particles]	
Multi-stage countercurrent Extraction (hot water)	90°C extraction time 20	
↓ Filtration	min; solvent:solid ratio 12:1	
Filtrate(containing inulin)]	Fig. 2. Schematic procedure of <i>P. arundinacea</i>
↓ Centrifugation	-	inulin production
Supernatant		
↓ ↓	-	
Carbonation		
↓ Filtration		
Filtrated		
Decolourisation (active carbon):	certain pH value, and then	
	introducing CO ₂ to an	
Deionization (anion and cation resin exchangers); ↓	Refining other pH value	
Concentration and drying		
Inulin (purity > 95%)		
REV. CHIM. (Bucharest) ♦ 66 ♦ No. 4 ♦ 2015 http://	- /www.revistadechimie.ro	473

Constituents (%)	P. arundinacea roots	
Moisture	6.17 ± 0.01	
Dry matter	97.3 ± 0.1	
ash	4.8 ± 0.03	
protein	6.2 ± 0.1	
fat	1.52 ± 0.06	
*Total carbohydrate	71.01 ± 0.02	
Mono- and disaccharides	24.3 ± 0.015	
glucose	3.6 ± 0.002	
fructose	5.8 ± 0.003	
sucrose	14.9 ± 0.01	
Inulin fraction	62.4 ± 0.03	
DP=3-10	47.2 ± 0.02	
DP>10	15.2 ± 0.01	
Phenolic fraction	6.54 ± 0.02	
Antioxidant activity/(nmol/g of fresh mass)	317.2 ± 3.1	
*Calculated by different. Values are mean \pm standard deviation (SD).		

 Table 1

 CHEMICAL COMPOSITION OF P. ARUNDINACEA ROOTS

 (g/100g on dry weight basis %)

to the AOAC procedure. Additionally, the total phenolic content was also determined using Folin–Ciocalteu reagent. The antioxidant activity was performed using 2,2–diphenyl–1–picrylhydrazyl (DPPH) radical. The extracts were dissolved in 10 mL of 80 % ethanol, then 0.05 mL of the solution were added to 1.95 mL solution of DPPH radical (60.8 μ mol/L), mixed and incubated for 30 min in the dark. The absorbance was measured at λ =515 nm and the results were expressed as mmol of Trolox equivalent per g of sample [15].

Determination of inulin content

For the determination of inulin content from the extract of *P. arundinacea* a simple and fast method was used, based on the fact that in the presence of concentrated sulfuric acid vanillin and inulin form a red complex which has a characteristic in the absorption spectrum at 520 nm. The colored complex follow the Lambert–Beer law in the concentration field of 0.2–1.2mg/L inulin. Readings are done with UV–Vis spectrophotometer (T60U, PG Instruments Limited, WIN UV ® version 5.05) and quartz cuvettes of 1 cm thickness were used. Inulin analysis was done in agreement with the USP 23 criteria: the solubility test, specific rotation, percentage of iron, reducing sugars, microbial load. Inulin was characterized from the physico– chemical point of view, melting point, Molish test, with lugol and resorcinol [16, 17].

Results and discussions

Determination of quality of inulin is absolutely necessary for the approval, introduction in therapeutics or as raw material in the pharmaceutical or food industry. Solid–liquid extraction is a separation technique, which involves the extraction of some components from a solid sample (*P. arundinacea*) in an adequate solvent [18, 19]. The chemical composition varies depending on the cultivar, pedoclimatic conditions and the applied agro technique. In table 1 it is presented the chemical composition of roots of *P. arundinacea*.

The analytes can be absorbed on to the specific surface of the sample particles, when the interaction can be overcome by the use of a solvent with a high affinity (solubility) for analyte [20–22], or the analyte molecules can be physically embedded in the sample matrix, and in

this case for their extraction it is necessary the physical destruction of the texture of the sample [23]. These methods are applied on the basis of some empirical parameters that are related to the solubility of the analytes in the chosen solvent and on the applied methods of work [24]. The nature of the analyte–matrix interactions depends widely on the origin of the sample. The humidity degree of a plant product must be between some values that allow the plant product conservation. If the humidity percentage is too high, a part of the enzymatic processes that happen in the interior of the cell are going on, leading to the degrading of the active principles [25]. Because the roots of *P. arundinacea* contain a high quantity of sugar at the determination of proteins, the mix foams strongly at the beginning, provoking the spillage of the mix and potential losses. In order to avoid this phenomenon there was used the adding of some drops of octilic alcohol (can be introduced a few balls of glass, with the help of which the foam is breaking mechanically). Foaming can be avoided if the sample is left with acid overnight, before warming. When there are big quantities of elements of the group of alkaline-ground metals, their sulphates precipitate provoking losses by co precipitation.

The operation lasts long, depending on the quantity and nature of the oxidized substance (especially for the samples rich in fats). For the determination of total nitrogen it has to be highlighted the fact that in the process of mineralization it is not realized the passing to ammonia salts of the oxidized nitrogen combinations (nitrates, nitrites, or some organic nitro combinations, notrozo, nitril) [26]. Lipids are characterized, from the physical and chemical point of view, by a series of constants (density, solidification point, etc.) and indices (acidity, saponification and iodine). The acidity index represents the quantity of KOH, in mg, necessary for the neutralization of free fatty acids in a gram of fat. Natural fats are neutral.

Their acidity appears during preservation or handling, with a follow-up of the action of oxygen from air and of water vapours, process that can be generally represented by the reaction: The acidity index set at different moments gives indication on the intensity of the process of fat degradation. The method of determination of the acidity index is based on the reaction of neutralization of free fatty acids with KOH of known titre, in the presence of an



Fig. 3. UV–Vis absorption spectrum of the inulin from *Phalaris* arundinacea roots

indicator (phenolphthalein) [27, 28]. Ash represents the inorganic substance resulted after the burning of a plant product, and the ratio between the organic and inorganic products represents a value that characterizes the plant product.

Determination of insoluble ash in diluted HCl had as aim the setting of the content of chlorides insoluble in water of some heavy metals (Pb, Hg, Cd).

Our results have shown an antioxidant activity of the extracts of roots of *P. arundinacea*. This shows that the non inulinic components have the capacity to increase the protection against lipid peroxidation in the organism. Inulin purification method can be compromised by removing the impurities, when it can be degraded to the inulin chain and by color reactions, Maillard reaction (reaction of reducing sugars and proteins or amino acids that can produce brown-color molecules, this thing is possible also in natural conditions when some foods are stored or heated), but also by contamination and incorrect removal of taste and smell. Inulin was characterized with the help of UV-Vis absorption spectrophotometric methods. Inulin presents a strong absorption in the visible region of the spectrum, having the ε molecular absorption coefficients of ~ 105 M^{-1} x cm⁻¹ at a wavelength of 520 nm (fig. 3). The electronic absorption spectra in the UV-VIS domain of the polysaccharide molecules present 2 distinct bands of absorption: B band (Soret) in UV region and Q band in the visible region.

The presence of Q band at wavelengths and intensities higher than in case of the homogenous polysaccharides represents one of main advantages of use of nonhomogenous polysaccharides as detection markers. Q band presents a specific split, in Qx band (at a higher wavelength) and Qy band (at a lower wavelength), respectively, called also the Davidov split band. This is a specificity of different types of polysaccharidic compounds, in liquid form, solid or as components of some natural complex polymeric matrices. In vicinity of the Q bands there can be detected vibration bands due to vibrational transitions [29, 30]. Extraction and isolation of inulin can be done from roots of *P. arundinacea*, obtaining a very good yield.

The extraction yield of inulin calculated with the formula R(%) = (content of inulin x extraction volume liquid/mass of roots of *P. arundinacea*) x 100, indicates that the substance is a polyfructan that is very soluble in water.

The polyhydroxi groups increase the polar character. All reactions for qualitative analysis of inulin were done in agreement with the USP 23 criteria: the solubility test,

specific rotation, percentage of iron, reducing sugars, microbial load and from the physico-chemical point of view, melting point, Molish test, with lugol and resorcinol were positive. Melting point: 176–181°C; Density: 1.35 g/ cm³; Sensitive: Hygroscopic; Stability: Stable; Incompatible with strong oxidizing agents. Inulin from roots *of P. arundinacea* had the same physical aspect, solubility, melting point and Rf value, as those in the literature [31, 32].

Hydroxyl groups (–OH) represent the most important functional group of the glucide. Inulin has many beneficial characteristics for the functional foods. Leaves and roots have a slightly bitter taste (because of sesquiterpene lactone). The gelification property may make from this substance a component of cheeses with low fat content, sauces, soups etc. Its properties allow the easy processing of the frozen wastes, in cereal bars. In addition, as a glucide, inulin can substitute sugar, when a low content of sugar is wanted.

Conclusions

The results obtained from the analyses run on roots of P. arundinacea lead to the extraction and identification of inulin. The inulin extraction from roots of P. arundinacea was done using water as solvent. The yield of inulin was 14% w/w. Identification of inulin by spectrophotometers of absorption UV-Vis from roots of *P. arundinacea* gave results that were compared to the sample of inulin used as reference. The chemical composition of the roots of P. arundinacea presented in this study indicates the fact that P. arundinacea may be a good source of functional compounds (inulin, chicoric acid, quercetin glucuronid, chlorogenic acid, and other cafeoilquinic acids). The value of the antioxidant capacity increases the protection against lipid peroxidation, underlines that the non-inulinic fractions from the extract of *P. arundinacea* rich in phenols, may be a supplementary factor in improving the physiologic activity of inulin. We consider that P. arundinacea should be researched with more care due to the possible nutritional and therapeutic use of its extracts. Inulin has more beneficial properties for health; this alimentary fiber can be used as probiotic agent in functional foods, to stimulate the development of intestinal bacteria. Being soluble in hot water, the substance could be easy to incorporate in drinks, dairy products and pastry products.

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