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Procedia Chemistry 16 (2015) 78 – 84

Procedia Chemistry

International Symposium on Applied Chemistry 2015 (ISAC 2015)

Characterization of Inulin from Local Red Dahlia (*Dahlia* sp. L) Tubers by Infrared Spectroscopy

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Abstract

Inulin consists of soluble dietary fiber and insoluble dietary fiber, which is undigested carbohydrate produced by inulin extraction from local red dahlia tuber (Dahlia sp. L). Extraction of inulin was carried out through gelatinization by heating at a temperature of 90 ºC for 30 minutes at pH 5 (control) and 10, respectively, then precipitated with ethanol to obtain inulin and continued with characterization by infrared spectroscopy (FT-IR). Based on FT-IR spectral analysis, the results showed characteristic absorption bands of inulin structure, OH stretch (3350 cm-1) and carbonyl (1645 cm-1). In addition, four characteristic regions can be distinguished from 3600 to 2500 cm-1, 2500 to 1550 cm-1, 1500 to 900 cm-1 and under 900 cm-1. The difference of infrared spectra obtained from each sample of inulin could be caused by different structure and influence of glucose, sucrose and mannan existed in inulin and the purification stage during treatment in alkaline condition.

Peer-review under responsibility of Research Center for Chemistry, Indonesian Institute of Sciences

Keywords: inulin; local red dahlia; extraction; soluble dietary fiber; infrared spectral

1. Introduction

Inulin is a linear biopolymer consisting of D-fructose units linked by β (2→1) glycosidic linkages. Typically, a glucose molecule resides at the end of each fructose chain and is connected through an α -1,2 bond, as in sucrose. The glucose unit exists in the inulin macromolecule in pyranose form and fructose exists in furanose form[1]. The

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Peer-review under responsibility of Research Center for Chemistry, Indonesian Institute of Sciences
doi:10.1016/j.proche.2015.12.027

particular characteristic of inulin structure is its β-2,1 bonds. These linkages prevent inulin from being digested like a typical carbohydrate and are responsible for its reduced caloric value and dietary fiber effects[2].

Inulin is a mixture of polysaccharides with different degrees of polymerization[3]. Inulin is obtained from different plants differ in their degree of polymerization (DP). The differences in DP between different inulin account for their distinctly different functional attributes. Long chain length inulin has low solubility properties and the ability to develop inulin microcrystals when sheared in water and milk[2].

Nutritionally, inulin is plant-derived carbohydrate with the benefits of soluble dietary fiber and is categorized as a prebiotic. It is not digested or absorbed in the small intestine, but is fermented in the colon by beneficial bacteria. Functioning as a prebiotic, inulin has been associated with enhancing the gastrointestinal system and immune system. In addition, it has been shown to increase the absorption of calcium and magnesium[4,5]. The absorption of these bio-elements from the food is implemented through active transportation in the upper part of the small intestine. Usually, with proper nutrition, about 30% from calcium taken with the food is absorbed. In the large intestine, most of the calcium is found as insoluble complexes. Due to the influence of short-chain fatty acids, which are formed during inulin fermentation from the bacteria, the solubility of calcium increases and its absorption through passive diffusion also increases. In phytotherapy, inulin is considered as natural nutritional component, which has beneficial impact on humans health by lowering blood sugar and cholesterol, and is having application for the treatment of patients with diabetes and cardiovascular diseases[5].

Due to its beneficial impact, there is an increasingly interest to use inulin as a desirable ingredients in processed foods since it has unusually adaptable characteristics. Furthermore, it can be used to replace sugar, fat and flour. The inulin has less increasing impact on blood sugar and is not insulemic. Therefore, it is suitable for diabetics and potentially beneficial for managing blood sugar related illness[6,7]. Recently, inulin is widely used in foodstuffs (table spreads, baked goods, fillings, dairy products, frozen desserts and dressings) and medical formulations[8,9].

Commercially available inulins are obtained mainly from chicory, artichoke and dahlia. Red dahlia (*Dahlia* sp. L) tuber from Sukabumi (West Java) is the best local dahlia variety when compared with other varieties of dahlia tuber in producing inulin. However, inulin characterization from local red dahlia has not been established. Therefore, the objective of the present study is the characterization of inulin fiber from local red dahlia using infrared spectroscopy since this method is one of the most often used spectroscopic tools for characterization, physical properties and interaction of carbohydrates.

2. Materials and Methods

2.1. Materials

Local red dahlia (Dahlia spp. L.) tubers grown in Sukabumi (West Java) and obtained from local farmer, were used for the extraction of inulin. The commercial inulin as reference was obtained from Orafti, Belgium. All the chemicals and solvents used were of analytical grade and purchased from local distributors.

2.2. Extraction of inulin

Local red dahlia tubers were sorted and added with water ratio of 1 : 2, blended and homogenized at 4000 rpm for 15 minutes, then adjusted to pH 5 (control) and 10. Extraction process was carried out in a water bath shaker with a rotational speed of 150 rpm at 80 ºC for 30 minutes. Suspension was obtained as inulin hydrolysate. Then, inulin hydrolysate was filtered through 80 mesh sieve. Filtrate was added with ethanol 50% and allowed to stand for 18 hours at -10 ºC and then filtered. The precipitate was obtained as inulin.

2.3. Chemical Characterization of Inulin

The moisture content, insoluble and soluble dietary fibers were measured using gravimetric method. Reducing sugars were determined according to Somogyi-Nelson. Inulin in samples was analyzed using cysteine-carbazole according to Kierstan (1980)[10].

2.4. Infrared spectroscopy (IR) analysis

The infrared (IR) spectral analysis was performed on Shimadzu FTIR IRPrestige-21 with ATR (Attenuated Total Reflectant) Attachment 8000. Five milligrams of dried inulin was homogenized with KBr, the mixture was pressed to form tablet and subject to analysis in the range of 4,000 – 400 cm^{-1}.

3. Results and Discussion

3.1. Chemical characterization of inulin fibers from red dahlia tubers

Preliminary analysis of the chemical properties of inulin from local red dahlia tubers were performed, and the obtained results are presented in Table 1.

Table 1. Chemical characteristics of inulin from local red dahlia tubers

Composition	Inulin from local red dahlia tubers		Inulin Orafti
	pH 5	pH 10	
Moisture (%)	93,4385	93,5188	2,5702
Total dry matters (%)	6,5615	6,4812	97,4298
Reducing sugar (mg/mL)	13,7400	5,4800	3,900
Inulin (%)	29,3501	43,7782	92,00
Insoluble dietary fiber (%)	2,0420	1,1430	55,0177
Soluble dietary fiber (%)	6,1027	9,7300	15,7386

From table 1, it can be observed that chemical composition of inulin in the condition of increased alkalinity were generally distinct, in which total sugars, inulin content and soluble dietary fiber were increased and reducing sugar and insoluble dietary fiber were decreased.

Extracting inulin by heating treatment resulted in extracts contain a complex mixture of various sized chain lengths of fructose linked β(2→1) with, occasionally, an α-D-glucopyranosyl residue at the reducing end of the chain, along with fructose, glucose, sucrose, salts, fats, proteins and amino acids[11]. Then proteins and larger molecules were removed by filtration.. Thermal treatments in inulin extraction can alter the ratio between insoluble and soluble fibers, total dietary fiber content, and their physicochemical properties. However, these modifications depend on the type of plant material and on the nature of the treatment[12].

There is an increment of inulin content and soluble dietary fiber by increasing alkalinity in inulin extraction. Inulin content and soluble dietary fiber of inulin in pH 5 and pH 10 are 29,3501%, 43,7782% and 6,1027% and 9,7300%, respectively. These increases could be caused by heating treatment with alkaline condition and precipitation with ethanol. Previous research investigated the influence of extraction conditions on the solubility of dietary fibers and observed that high temperature extraction resulted in the highest levels of soluble fibers while extraction in acidic buffer yielded the lowest[13]. Moreover, alkaline condition could increase yield by hydrolyzing insoluble constituents into soluble which increases the extraction yield[14]. Besides, precipitation with ethanol separates the soluble dietary fiber polysaccharides from low-molecular-weight sugars and starch hydrolysis products[15,16]. This method was applied to reduce the content of mono- and disaccharides, salts or amino acids at the low molecular weight range, and to reduce the content of proteins, cellulose fibers and other debris at the high molecular weight end. Yet, if the low degree polymerization of fructooligosaccharides is the expected product, normally enzymatic hydrolysis is used to break down the high molecular weight fractions to achieve a common quality composition[11].

3.2. Infrared spectral of inulin fiber from local red dahlia tubers

The infrared spectroscopy is a rapid and convenient method for the investigation of functional groups of polysaccharides[17]. IR spectra of the investigated inulins were taken in KBr to obtain information for their structural and functional groups. This method was selected for its fast analysis and qualitative information for certain functional groups. In recent years, infrared spectroscopy can characterize the molecules as containing or lacking certain functional groups which representing as their finger prints. Through comparison with known spectrums, identification of compounds based on this functional group could be easily achieved[18]. The specificity of carbohydrates originates from the geometry of the many O–H groups and the configuration of the C–O, C–C, and C–H bonds in the skeletal base configuration[19].

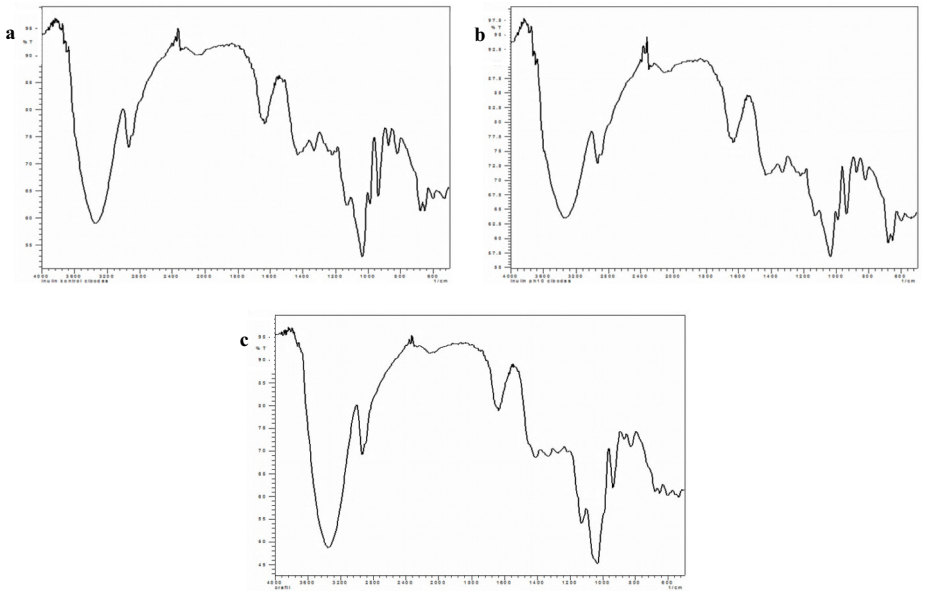

Figure 1. Infrared spectrum of inulin pH 5 (a), inulin pH 10 (b) from local red dahlia and commercial inulin (c)

The IR spectrum of inulin in Figure 1 can be divided into four characteristic spectral regions, from 3,600 to 2,500, 2,500 to 1,550 and 1,500 to 900 cm-1, under 900 cm-1 (the so-called finger-print region, which is characteristic for every compound). Also, from the overall spectrum of inulin from red dahlia tubers, a similar trend was obtained that FT-IR spectrum for red dahlia inulin was essentially identical to chicory inulin and artichoke inulin showing OH stretch (3300 cm^{-1}) bands as the characteristics of inulin[20,21].

In the first region, around 3,300 cm-1, was showed a broad asymmetric band due to the valence vibrations of hydroxyl groups (OH stretching). In 300-2700 cm^{-1} region appears a sharp band with middle intensity around 2930 cm^{-1} assigned to the valence vibration of C-H asymmetric stretching of CH_2 and a shoulder at 2890 cm^{-1} attributed to C–H symmetric stretching of CH_3.

In the second region were observed the absorption bands of the carbonyl group due to the valence vibrations of C=O bonds, which are very characteristic for the carbohydrates[1]. Low intensity band at 1629 cm^{-1} represented C=C bond, but can't be used as specific for inulin in biological mixture, as usually in this region is a strong absorption of amides[22].

The spectral region between 1500 and 900 cm^{-1} is mostly dominated by a complex sequence of intensive peaks due mainly to strongly coupled C–C, C–O stretching and C–O–H, C–O–C deformation modes of various oligo- and polysaccharides[23]. Two bands at 1,330 and 1,400 cm-1 found in the spectra of inulin, and they were associated to in-plane bending vibrations and internal deformations of CH, CH_2 and OH groups from the fructose ring, whereas the first one for symmetric and the second one for asymmetric deformations[1]. In this region, it can be seen that the most intensive absorption bands are strong complex absorption at 1130 and 1030 cm^{-1} which assigned to valence stretching vibrations of C-O and C-O-C groups and ring vibrational modes in the composition of cyclic structures[24].

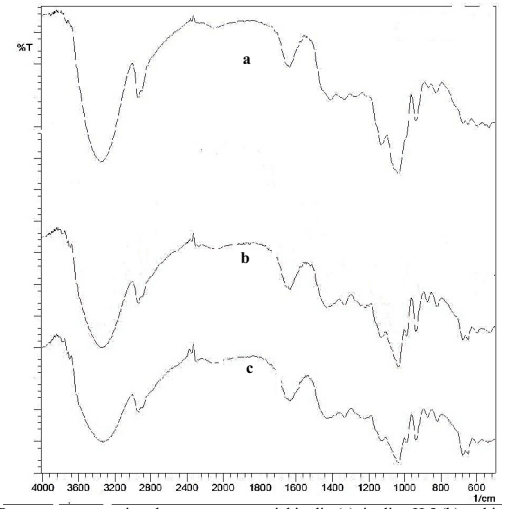

Figure 2. FT-IR spectrum comparison between commercial inulin (a), inulin pH 5 (b) and inulin pH 10 (c).

In the last region under 900 cm^{-1} or so-called fingerprint region features a variety of characteristic absorption bands and are useful for conformational studies of carbohydrates[22]. The distinctive absorption bands at 670, 650 and 590 cm^{-1} represent pyranose rings and also characteristics of bond stretching vibration of the bio molecule[25]. The difference in commercial inulin and inulin from local red dahlia appeared in 800-600 cm^{-1} region which indicates the presence of other carbohydrates are possibly caused by the different structure and influence of glucose, sucrose and mannan[22, 26] and the purification during treatment in alkaline condition.

Table 2. Wavelength numbers (cm^{-1}) and %Transmittance of infrared spectrum of inulin from local red dahlia and commercial inulin

Inulin pH 5		Inulin pH 10		Commercial Inulin	
Wave numbers (cm^{-1})	% Transmittance	Wave numbers (cm^{-1})	% Transmittance	Wave numbers (cm^{-1})	% Transmittance
597,93	63,654	596,0	63,051	596,0	60,261
650,01	61,403	650,01	59,691	650,01	60,709
678,94	61,328	678,94	59,158	677,01	61,115
819,75	71,975	817,82	70,121	827,46	70,939
867,97	73,955	935,48	64,211	867,97	72,608
935,48	64,091	987,55	63,215	935,48	62,015
987,55	62,674	1033,85	56,855	1031,92	45,217
1033,85	52,850	1126,43	63,949	1128,36	54,101

1130,29	62,358	1242,16	71,346	1213,22	69,763
1242,16	72,377	1330,85	56,855	1271,09	69,601
1330,88	72,489	1406,11	71,184	1330,88	68,914
1429,25	71,689	1429,25	70,893	1408,04	68,587
1629,85	77,553	1629,85	76,554	1629,85	79,138
1645,28	78,072	1645,28	77,144	1645,28	79,496
2891,29	75,170	2889,37	74,360	1745,58	91,973
2931,8	73,063	2933,73	72,919	2897,08	71,539
3327,21	59,136	3296,35	63,769	2933,73	69,344
3350,35	59,022	3323,35	63,479	3350,35	48,787

4. Conclusions

Extraction process by heating treatment to obtain inulin from local red dahlia tubers (Dahlia spp L.) at 80 ºC for 30 minutes by adjusting pH to 5 (control) and 10, affects the alteration of molecular structure of inulin. Based on infrared spectral analysis, the results showed characteristic absorption bands of inulin structure, OH stretch (3350 cm-1) and carbonyl (1645 cm-1). In addition, four characteristic regions could be distinguished from 3600 to 2500 cm-1, 2500 to 1550 cm-1, 1500 to 900 cm-1 and under 900 cm-1. The difference of infrared spectra obtained from each sample of inulin could be caused by different structure and influence of glucose, sucrose and mannan existed in inulin and the purification during treatment in alkaline condition.

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