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Extraction and Identification of Inulin-Type Fructo-Oligosaccharides from *Dahlia pinnata* L.

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A method for obtaining high yields of high-quality inulin from dahlia tubers was developed in the present study. The steps involved were the extraction of inulin, determination of inulin concentration and elucidation of inulin structure. The highest yield of inulin was obtained by ultrasonic- and microwave-assisted extraction (UMAE) (5.77 %), followed by stirring extraction (4.97 %) and indirect sonication (5.09 %). The concentrations of inulin extracted from dahlia tubers using UMAE were determined to be 41.94 % (w/w) and 33.52 % (w/w) by 3,5-dinitrosalicylic acid and high-performance liquid chromatography, respectively. Analysis of inulin extracted from dahlia tubers by Fourier-transform infrared spectroscopy confirmed the presence of an inulin-type fructan that is the same as commercial inulin. ^{1}H NMR and ^{13}C NMR spectroscopy indicated that it has a degree of polymerization of 19-20 and a molecular weight of 3.173-3.316 Da.

Keywords: Inulin, Dahlia tubers, Ultrasonic- and microwave-assisted extraction, 3,5-Dinitrosalicylic acid, HPLC, NMR spectroscopy.

INTRODUCTION

Inulin is a linear polymer of D-fructose units joined by β(2→1) linkages with a terminal D-glucose molecule linked to fructose by an α(1→2) bond similar to that in sucrose [1]. Classified as a reserve carbohydrate by several studies [2], inulin forms in the leaves of composite plants during photosynthesis and mainly accumulates in the stems and roots. It is stored in vacuoles in the form of spherocrystals. In United States of America and United Kingdom, inulin is commercially produced from chicory (*Cichorium intybus*) roots and Jerusalem artichoke (*Helianthus tuberosus*). However, in Indonesia, tropical plants such as dahlia (*Dahlia pinnata* L.) are potential sources of inulin; inulin extraction from dahlia tubers would reduce Indonesia's dependence on imported inulin and increase the economic value of local foods.

Inulin extracted from dahlia tubers is of high quality because it contains soluble and insoluble fibres [3]. Several methods have been developed to improve inulin extraction yields: stirring extraction, indirect sonication and ultrasonic- and microwave-assisted extraction (UMAE). Stirring extraction is often used for comparing inulin extracted from several different samples, even though it gives the lowest yields compared with other extraction methods [4,5]. Stirring extraction of inulin with water as a solvent generates yields of 65.60 % from chicory [6] and 4.40 % [3] and 14.00 % [7] from dahlia tubers. Inulin extraction by indirect sonication of Jerusalem artichokes generates a yield of 83.60 % [4], while extraction by UMAE generates 99.03 % of inulin from burdock root [5].

Reports on inulin extraction from dahlia tubers have been limited to mentioning extraction yield and have not addressed the inulin concentration in the extract. The concentration of inulin in Jerusalem artichoke extract was 52.5-65.7 % (w/w) as determined by 3,5-dinitrosalicylic acid (DNS) method [4]. Furthermore, high-performance liquid chromatography (HPLC) was conducted on gembili extract (*Dioscorea esculenta*) [7], but the concentration of inulin obtained using HPLC has not yet been reported. Studies on inulin extracted from dahlia tubers were conducted by Hariono *et al*. [8] who acetylated inulin to produce an inulin ester and determined its structure. However, the concentration and chemical structure of inulin extracted from dahlia tubers have not yet been revealed.

Therefore, the present study aimed to evaluate different extraction methods to obtain high yields of inulin from fresh dahlia tubers, to determine the concentration of inulin by 3,5-dinitrosalicylic acid (DNS) method and HPLC and to elucidate the structure of inulin by spectroscopic methods.

EXPERIMENTAL

Dahlia tubers (*D. pinnata* L.) from plants with red pompom flowers, red crown strands and a flower diameter of 6-8 cm were obtained from Batu Malang, Indonesia, while commercial

inulin was obtained from Sigma-Aldrich and Beneo-Orafti. All chemicals used were of analytical grade and were used as received without any further purification.

Inulin extraction: Inulin extraction was performed by stirring extraction [3], modified indirect sonication [4] and UMAE [5] with a slight modification.

Determination of inulin concentration: Inulin concentrations were determined using HPLC [9] and DNS according to Lingyun *et al*. [4]. The inulin concentration using HPLC was calculated using eqn. 1:

$$\text{Inulin concentration (\%)} = \frac{\text{Inulin extract content}}{\text{Inulin standard content}} \times 100 \quad (1)$$

The determination of inulin concentration by DNS method is based on the reduction of 3,5-dinitrosalicylic acid to the colorant 3-amino-5-nitro-salicylic acid and the oxidation of the aldehyde group of reducing sugars to carboxylic acid [10]. Total carbohydrate concentration was determined by the phenol-sulphuric acid method [11] using glucose (Sigma-Aldrich) as a standard. Reducing sugar concentration was determined by DNS method using D-fructose (Sigma-Aldrich) as a standard [10]. The inulin concentration was measured from the difference between total carbohydrate and reducing sugar concentrations [4]. The inulin concentration using DNS method was calculated using eqn .2:

$$\text{Inulin conc. (\%)} = \frac{\text{Inulin content} \times \text{Volume of extraction liquid}}{\text{Mass of dahlia powder}} \times 100 \quad (2)$$

Structure determination of inulin: The structure of inulin was elucidated by Fourier-transform infrared (FTIR) spectroscopy [12], ^{1}H and ^{13}C nuclear magnetic resonance (NMR) [13] and distortionless enhancement by polarization transfer-135 (DEPT-135) [14].

RESULTS AND DISCUSSION

Inulin extraction: The inulin extracted from fresh dahlia tubers by the three different methods gives different results (Table-1). UMAE was found to give the highest extract yield of 5.77 %. The low extraction yields for all methods are possibly due to the filtering of fresh tuber extracts, which selects only the white precipitate at the end of the process. Susdiana [15] reported that inulin extraction with water results in a white precipitate at the end of extraction, while Widowati *et al*. [3] stated that the content of inulin in fresh tuber extracts is low because they contain 83.21 % water. This result is similar to that obtained by Kays and Nottingham [16], who reported that inulin extracted from tubers was approximately 2-9 % of the tuber's weight.

TABLE-1
INULIN EXTRACTION YIELD FROM DAHLIA TUBER USING VARIOUS METHODS

Methods	Inulin yield (%)	Inulin content by DNS (%)
Stirring extraction	4.97 ± 0.04[a]	11.42 ± 0.24[a]
Indirect sonication	5.09 ± 0.03[a]	33.84 ± 0.53[b]
UMAE	5.77 ± 0.01[b]	41.69 ± 0.05[c]

[a,b,c]Mean values with different superscript letters in the same column differ significantly (P < 0.05).

The results of inulin extraction by stirring and indirect sonication were not significantly different (P > 0.05). This was likely due to the use of water, which reduced the release of inulin. The yield using UMAE was higher because it combines ultrasonic and microwave treatments. According to Vilkhu *et al*. [17], ultrasonic treatment increases the contents of phenolic and aromatic compounds, anthocyanin and polysaccharides. Furthermore, microwave treatment provides internal heat, which causes increased internal pressure that improves the amount of inulin extracted from fresh tuber extracts [5].

Physico-chemical analysis of inulin extracted from dahlia tubers: The different methods for extracting inulin from fresh tubers resulted in extracts with different physicochemical properties (Table-2). Extraction using UMAE resulted in the highest contents of total sugar, total dietary fibre and soluble dietary fibre. Total sugar content is an indicator of the amount of inulin because total sugar is the sum of all monosaccharides, oligosaccharides, polysaccharides and their derivatives, as reported by Dubois *et al*. [11]. Therefore, the highest amount of total sugar results in the highest amount of inulin. Thus, the highest content of total dietary fibre and soluble dietary fibre obtained using UMAE is complimentary to the inulin content. Total dietary fibres are food contents that cannot be hydrolyzed by digestion enzymes [18]. Inulin is a soluble dietary fibre that also cannot be digested by enzymes, but it can be fermented by colon microflora [19].

TABLE-2
PHYSICO-CHEMICAL ANALYSIS OF INULIN EXTRACT DAHLIA TUBERS WITH VARIOUS METHODS

Physico-chemical analysis (%)	Inulin extraction		
	Stirring extraction	Indirect sonication	UMAE
Total sugar	23.91±0.06[a]	67.04±1.33[b]	73.21±0.36[c]
Total dietary fiber	35.61±0.59[a]	40.34±0.67[b]	51.02±0.30[c]
Soluble dietary fiber	32.51±0.69[a]	36.82±0.78[b]	47.72±0.32[c]

[a,b,c]Mean values with different superscript letters in the same row differ significantly (P < 0.05).

Determination of inulin concentration: The concentrations of inulin extracted from fresh tubers obtained using the different methods were determined by DNS method and UMAE was found to yield the highest inulin concentration of 41.69 % (Table-1). Variant analysis showed that the extraction methods significantly affected the concentration of inulin (P < 0.05). This was due to the differences in the extract weight, total sugar amount and sugar reduction in the different methods [4]. The results showed that the inulin content from this experiment was higher than that reported by Matias *et al.* [20] who showed that the inulin content in Jerusalem artichokes was 13.67 %.

The highest content of inulin obtained using UMAE was confirmed by HPLC and the result showed that the commercial inulin and inulin extracted from fresh tubers provided only a single peak (Fig. 1). The retention time of both samples were the same at 2.833 min, with the commercial inulin showing a higher peak area. Therefore, it can be concluded that inulin extracted from fresh tubers was similar with the standard

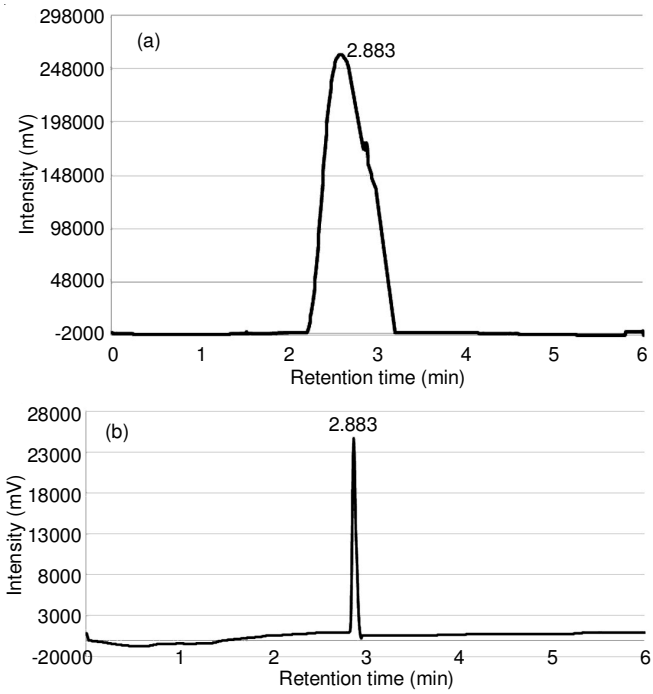

Fig. 1. Chromatograms of (a) inulin (Sigma) and (b) inulin from dahlia tubers

compound (Sigma-Aldrich). This similarity was confirmed by observing spectra using UV-visible spectroscopy, which showed a peak at 286 nm. This result is the same as that reported by Hariono *et al.* [8]. HPLC showed that inulin extracted from fresh tubers using UMAE had a purity of 33.52 % compared to that of standard inulin. Overall, compared to HPLC, DNS revealed an 8.42 % higher concentration of inulin.

Structural determination of inulin: FTIR was used for the identification of inulin extracted from fresh tubers using standard inulin. The FTIR spectra of the commercial inulin and inulin extracted from dahlia tubers had a similar pattern (Fig. 2). The FTIR spectrum of inulin extracted from dahlia tubers had absorption bands at 3417, 2924, 1635, 1427, 1334, 1272, 1219, 1126, 1033, 987, 933 and 594 cm^{-1}. The peaks at 987 and 1126 cm^{-1} indicated the presence of inulin [12].

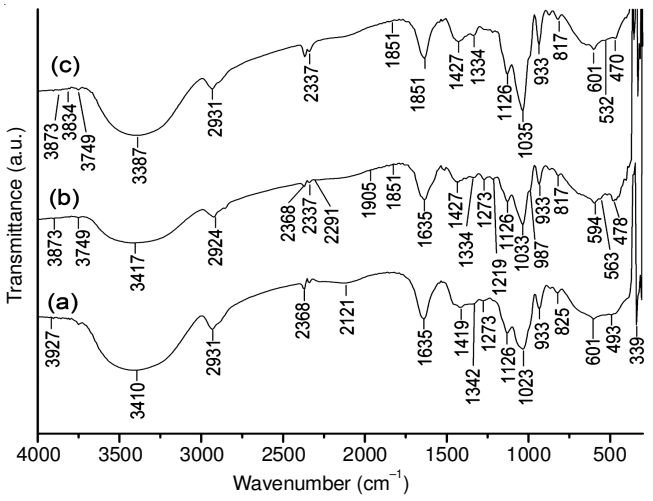

Fig. 2. FTIR spectra of inulin (a) inulin (Beneo), (b) inulin from dahlia tubers and (c) inulin (Sigma)

The hydroxyl groups that are abundant in the molecular structure of inulin were indicated by the absorption band at 3417-2924 cm^{-1} [21]. The band at 1635-1427 cm^{-1} indicated the presence of esterified carboxyl groups. Absorption at 1033 cm^{-1} was related to the ketal group, while the presence of fructose with β-(2→1) glycosidic bonds in the commercial inulin and inulin extracted from dahlia tubers was indicated by the peak at 933 cm^{-1} [22]. The FTIR spectra of the commercial inulin and inulin extracted from dahlia tubers were largely identical. However, the experimental sample had an absorption band at 1334-1219 cm^{-1}, showing high contents of esterified carboxyl and hydroxyl groups, which indicate the presence of pectin. The same result was reported by Barkhatova *et al.* [23] who concluded that inulin extracted from Jerusalem artichokes using improved technology has a relatively higher pectin content.

^{1}H and ^{13}C NMR allows the clear identification of all hydrogen atoms in the inulin molecule. The ^{1}H NMR spectrum is presented in Fig. 3 and the interpretations of the ^{1}H and ^{13}C NMR spectra are presented in Table-3. The ^{1}H NMR spectrum showed signals at 5.27 ppm, indicating equatorial 1-H glycopyranose residues, which is characteristic for a fructose unit and the 32-H proton of the fructofuranosyl residue that terminates the polysaccharide chain presented a signal at 4.04-4.12 ppm.

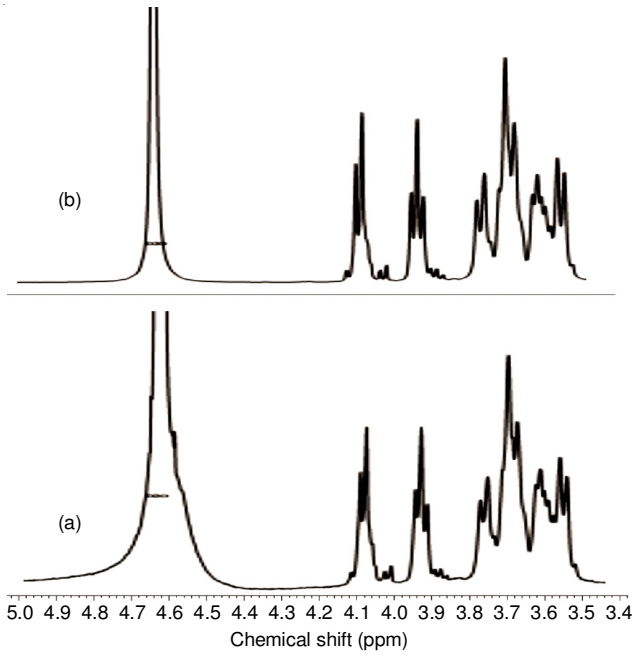

Fig. 3. ^{1}H NMR spectrum of (a) inulin from dahlia tubers and (b) inulin (Sigma)

The spectra of inulin extracted from dahlia tubers and of the commercial inulin indicated α,D-glycopyranose and β,D-fructofuranose residues. For the commercial inulin, the ratio of integrated signal intensities for the hydrogen atoms in the 1-H glycopyranose and 32-H fructofuranose forms was 1:20, *i.e.* one glycopyranose fragment in the polysaccharide has 20 fructofuranose residues. The molecular weight of this compound is 3,316 Da. For inulin extracted from dahlia tubers, the ratio of the integrated intensities for the hydrogen atoms in the 1-H glycopyranose and 32-H fructofuranose forms was 1:(19-20), *i.e.* the molecular weight is 3.173-3.316 Da. The

TABLE-3
^{1}H AND ^{13}C NMR CHEMICAL SHIFTS OF INULIN AND INULIN EXTRACT FROM DAHLIA TUBERS

Residue	Number of atoms	^{1}H NMR (δ ppm)		^{13}C NMR (δ ppm) Inulin (dahlia)
		Inulin (Sigma)	Inulin (dahlia)	
→1)-β-D-Fruf-(2→	H-1/C-1	3.58	3.58	60.84
	H-2/C-2	-	-	103.19
	H-3/C-3	4.09	4.09	76.91
	H-4/C-4	3.9	3.90	74.18
	H-5/C-5	3.71	3.72	81.03
	H-6/C-6	3.61	3.61	62.07
α-D-Glc*p*-(1→	H-1/C-1	5.28	5.28	92.81
	H-2/C-2	3.61	3.60	71.52
	H-3/C-3	3.31	3.31	72.08
	H-4/C-4	3.58	3.58	69.81
	H-5/C-5	3.61	3.61	79.90
	H-6/C-6	3.59	3.59	61.32

higher molecular weight of the experimental sample was indicated by a larger integral peak in the ^{1}H NMR spectrum. This is associated with the increased viscosity of the sample solution in comparison to that of the commercial inulin at the same solution concentration.

This structure is further confirmed by the ^{13}C NMR and DEPT-135 spectra. The ^{13}C NMR spectra of inulin extracted from dahlia tubers (Fig. 4a) showed only signals for fructose carbon. The anomeric carbon was observed at δ 103 ppm, corresponding to C-2, *i.e.* the carbon that is involved in the intra-chain linkage β-(2→1)-D-fructosyl-fructose bonds [24]. Five signals were observed for non-anomeric carbons. These were identified using DEPT-135 experiments (Fig. 4b) as the three methine carbon atoms C-3 (77 ppm), C-4 (74 ppm) and C-5 (81 ppm) and the two methylene carbon atoms C-1 (60 ppm) and C-6 (62 ppm). All chemical shifts (Table-3) were consistent with those seen in the literature [24]. These literature data confirm that the chemical structure of the polysaccharide-like inulin obtained from *Stevia rebaudiana*, *M. maritima* and *H. tuberosus* roots are composed of fructose units with β-(2→1) linkages with a terminal D-glucose molecule, which is a characteristic of inulin-type fructans obtained from plants [13,24,25]. In conclusion, this present study reports high yields of high-quality inulin from dahlia tubers by ultrasonic- and microwave-assisted extraction (UMAE) method that could be applied for industrial-scale inulin extraction from dahlia tubers.

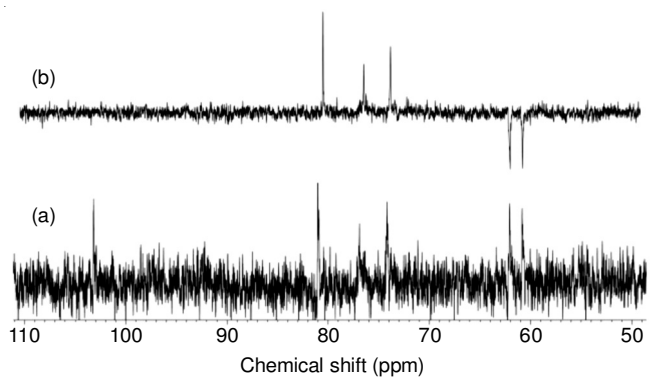

Fig. 4. ^{13}C NMR (a) and DEPT 135 (b) of inulin dahlia tubers

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