Microwave-Assisted Isolation and Acetylation of Inulin from Helianthus Tuberosus L Tubers

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ABSTRACT: Jerusalem artichoke (*Helianthus tuberosus* L.) tubers are industrial crop considered as a promising source for inulin production. "Green" method was performed for accelerated inulin extraction from *Helianthus tuberosus* L. tubers by the application of microwave irradiation. Further pretreatment of the water extract with acetone and ethanol yielded inulin (20%) with purity 89% and degree of polymerization 18. Jerusalem artichoke inulin was characterized by FTIR and NMR spectroscopy. For the first time eco-friendly synthesis of acetylated Jerusalem artichoke inulin was performed by the reaction with acetic anhydride, without toxic solvent, but only with sodium acetate as catalyst under microwave irradiation for 60 s. The degree of acetylation (DA=2.1) and the structure of inulin esters were confirmed by ^{1}H and ^{13}C NMR. The suggested microwave acetylation shortens significantly the esterification reaction.

KEWWORDS: Jerusalem artichoke, inulin, inulin acetates, microwave irradiation

1 INTRODUCTION

Jerusalem artichoke (JA) (*Helianthus tuberosus* L.) belongs to Asteracea family and it is an annual plant with high yields [1]. Its tubers present a rich source of bioactive compounds with great importance for human nutrition and production of biofuel and fermented products [1-3]. Significant amount of polysaccharide inulin (about 22% of fresh weight) is contained in its underground vegetal parts [1]. It is a biopolymer consisted mainly of β-D-fructose units connected by (2→1) linkages and terminated with one α-D-glucose residue linked to D-fructose by (1↔2) glyosidic linkage [4-6]. Industrial utilization of inulin depends on the degree of polymerization (DP). The increasing application of inulin in food technology as prebiotic, dietary fibers and fat replacer requires the use of effective approaches for its isolation [2]. Nowadays, many different extraction techniques of inulin from JA tubers as accelerated solvent extraction, ultrasound-assisted extraction and microwave irradiation have been reported [1, 3-9]. They have many advantages of the time-consuming conventional hot water extraction [4].

Moreover, inulin is cheap renewable plant polysacchride that can be modified to different polymers with a significant application in technics and pharmacy [10]. Inulin esters were evaluated as promising drug carriers, especially inulin acetates. Their use for production of colon targeting drugs and vaccine adjuvants has been recently published [11-15]. The antimicrobial activity of chicory inulin acetates has been demonstrated [16]. In addition inulin acetates are evaluated as eco-friendly biodegradable polymers [11, 16, 17]. The major drawbacks of inulin acetylation are the uses of toxic solvents and long reaction time [11-15, 17]. The need of abandant sources of inulin, together with "green" method for isolation and modification are from the great importance for pharmaceutical industry. Until now acetylation of inulin by microwave irradiation was not published. Therefore, the aim of the current study was to apply microwave irradiation for isolation and accelerated eco-friendly acetylation of inulin from Jerusalem artichoke tubers for production of bioactive biodegradable inulin polymers.

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2 MATERIALS AND METHODS

2.1 Materials

Jerusalem artichoke tubers were purchased from local market (Plovdiv, Bulgaria). They were washed with water, sliced and dried at 40°C. The dried tubers were finely ground to powder and stieved though 0.5 mm. All other chemical reagents were of the analytical grade.

2.2 Methods

2.2.1 Isolation of inulin from JA tubers

Inulin was extracted from the dry tubers using distilled water as a solvent (1:10 w/v). The

microwave-assisted extraction (MAE) was performed in duplicate in a microwave oven (Daewoo KOR, power 700 W and 2450 MHz frequency) for 5 min. The obtained extracts were precipitated with addition of four volume 95% (v/v) ethanol, then cooled at –18°C for 60 min and filtration was performed. The residue was washed with 95% (v/v) ethanol and acetone and vacuum-dried. Conventional extraction was performed as previously discribed [5, 6]. The obtained inulin from both methods was characterized by different spectral and chromatographic methods.

2.2.2 Characterization of inulin

Melting point of inulin was measured on a melting point apparatus Kofler. The reducing groups were determinated by PAHBAH method at 410 nm [20]. Total fructose content was defined by resorcinol-thiourea assay [5]. The purity of JA inulin was analysed by HPLC instrument Elite Chrome Hitachi with a Shodex® Sugar SP0810 (300×8.0 mm i.d.) with Pb^{2+} and a guard column at 85°C, coupled with refractive index detector (VWR Hitachi Chromaster, 5450, Japan). The mobile phase was distilled water with a flow rate 1.0 mL/min and the injection volume was 20 μL [19].

High performance size-exclusion chromatography (HPLC-SEC) analysis of JA inulin was carried out using HPLC chromatograph ELITE LaChrome (VWR Hitachi, Japan) on a column Shodex OH-pack 806 M (ID 8 mm and length 300 mm) (Shodex Co., Tokyo, Japan), operating at 30°C and a RI detector (VWR Hitachi Chromaster, 5450, Japan). Elution was performed with mobile phase 0.1 M $NaNO_3$ with a flow rate of 0.8 mL/min. Samples were filtered through 0.45 μm filter PTFE 45/25mm (Isolab, Germany) and they were injected (20 μl loop) at concentration 3 mg/ml. The standard curve built with different pullulans was use for calculation. Polydispersity index (X) of inulin was calculated as the ratio of the two molecular weights (Mw/Mn) [20].

2.2.3 Synthesis of inulin acetate

JA inulin (DP=17-18) isolated by microwave-assisted extraction was acetylated only with a catalyst. JA inulin (8 g) was vigorously stirred with 4.8 g anhydrous sodium acetate and 48 ml acetic anhydride (Figure 1) in a round bottom flask. The sample was irradiated in a microwave system (Daewoo KOR, power 700 W and 2450 MHz frequency) for 60 seconds. A tube containing anhydrous calcium chloride was fixed on top of the reflux to prevent access of water. Then the reaction mixture was poured into a 200 mL water-ice mixture, stirred vigorously and left at –18°C overnight. Inulin acetates were precipitated in an excess of cold water as a white solid, filtered and then washed with cold water. The acetyl ester was recrystallized by 95% (v/v) ethanol and re-precipited with water, and then was dried in a vacuum-oven.

2.2.4 *FTIR spectoroscopy*

Functional groups of JA inulin and inulin acetates were studied using FTIR spectoscopy. Samples (2 mg) were pelleted in KBr tablets. The infrared spectra were recorded on a Nicolet FTIR Avatar Nicolet (Thermo Science, USA) spectrometer in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} after 132 scans and the absorption was reported in wavenumbers (cm^{-1}).

2.2.5 *NMR spectroscopy*

^{1}H and ^{13}C NMR spectra were recorded using a Bruker AVIII 500 MHz spectrometer operating at a frequency of 500 MHz and 126 MHz, respectively. Inulin sample was dissolved in 99.95 % D_2O (20 mg/0.6 ml). Inulin acetate was dissolved in $CDCl_3$. The chemical shifts (δ) were expressed in ppm. Degree of polymerization (DP) of JA inulin was also estimated from ^{1}H NMR spectrum by taking the ratio of shift integral values of carbons in fructose units (X) to the shift integration values of the corresponding carbons in the glucose unit using equation DP n=((X-6)/7)+1 [21].

3 RESULTS AND DISCUSSION

3.1 Characterization of Inulin from JA

The physicochemical characteristics of JA inulin were summarized in Table 1. Inulin from JA tubers was obtained by conventional and microwave-assisted extraction and ethanol precipitation in yield of 18 and 20%, respectively. The isolated inulins contained high percent fructose (72-85%) and purity 89-83%. Glucose content expressed as reducing groups were higher in MAE inulin (4.5%). In comparison with chicory inulin, JA inulin isolated by conventional extraction showed similar molecular weights, purity and fructose content. Therefore, JA inulin could be successfully used as an alternative of chicory inulin in food industry. Inulin melting point was significant higher and it was near to the reported values for JA, chicory and dahlia inulin [3, 6, 11].

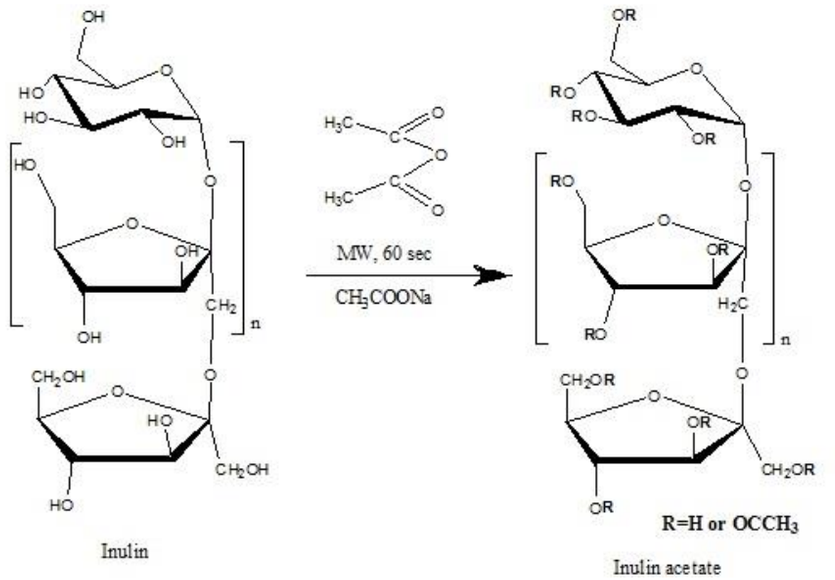


Figure 1 Microwave-assisted esterification of JA inulin with acetic anhydride.

Table 1 Characterization of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.).

Characteristics	JA inulin, Conventional extraction	JA inulin MAE	Chicory Inulin Raftiline HP (Reference)
Yield, % dry weight	18	20	-
Melting point, °C	178-179	178-179	180
Fructose content, %	85	72	90
Reducing groups, %	3.2	4.5	2.3
Purity, %	89	83	95
Mw, Da	3622	2714	3820
Mn, Da	3451	2602	3702
X	1.05	1.04	1.03
DP	23	18	24

Table 2 Characteristic bands of inulin from Jerusalem artichoke found in FTIR spectrum.

Bands, cm^{-1}	**Assignment**
3376	Hydrogen bonded O–H streching vibrations; H-bonds
2935	$\nu C–H_{as}(CH_2)$
2889	$\nu C–H_s(CH_2)$
1647	Absorption of water
1405	$\nu C–Hs(CH_2)$ in pyranosyl ring, $\beta o–н$ (OH)
1340, 1335	$\beta o–н$ (OH)
1245	$\beta o–н$ (OH)
1130	$\nu C\text{-}O\text{-}C_{as}$ (C–O–C), glycoside linkage
1031	νC–O (C–O)
986	νC–O (C–O)
937	α-D-Glcp residue in chain
871	ρCH_2 in ring, β-anomer bendings C1–H
818	2-ketose (pyranosyl of furanosyl ring)

HPSEC was performed to determine the molecular weight and polydispersity of JA inulin. Number avarage molecular weight (Mn) and weight average molecular weight (Mw) of JA isolated by MAE was 2714 and 2602 Da with polydispersity idex 1.04. Our results for polydispersity was in agreement with some reports from Praznik and Beck [22]. The molecular weight of inulin was higher than this obtained after ultrasonic irradiation [1]. In our previous research the molecular weight and DP of isolated inulins from JA was higher. That could be expalined with the harvest time, postharvest conditions, climate conditions [2, 3], extraction methods and sample pretreatment before extraction. The microwaves heating may cause hyperthermia and small degradation of inulin chain to reducing monosaccharides during extraction process in comparision with conventional extraction. This explained the higher amount of reducing groups and lower molecular weight of inulin after MAE (Table 1). However, MAE reduced sighificantly the time for inulin isolation to 10 min. In addition obtained JA inulin was characterized with similar physicochemicals parameters near to commercial chicory inulin.

3.2 Characterisation of Inulin Acetate

For the firt time in the current study JA inulin acetates were synthesized under microwave irradiation. The other advantage was the absence of toxic solvents (DMF or pyridine). JA inulin acetates presented odorless powders with white-yellowish tint, bitter taste, soluble in acetone, DMSO, 95% ethanol, methanol and insoluble in water. The yield was higher than 50%. Acetyl residue in JA inulin chain led to diffrent physicochemical properties in comparison to inulin, as lower melting points: 79-81°C. Similar reports were found for chicory inulin acetates [11, 16]. This approach for synthesis inulin acetate is proper for their future application in field of pharmacy, technique and food technology.

3.3 FTIR Spectroscopic Analysis of JA Inulin and Its Acetates

In the FTIR spectrum of JA inulin was observed typical bands for inulin-type fructan (Table 2) that coincided with some previous reports [3, 5, 6].

In FTIR spectrum of JA inulin acetates strong new bands at 1745 cm^{-1} attributed to stretching vibration of C=O groups from ester was appeared (Figure 2). The strong bands at 3335 cm^{-1} typical for the stretching vibrations of OH groups decreased significantly in the JA inulin acetates spectra. Additionally, bands at 1370 cm^{-1} due to bending of C-H from CH_3 and 1220 cm^{-1} due to stretching vibration of C-O which corresponding to the acetyl group were also appeared. Our report was in accordance with some reports for inulin acetates [11-17]. The bands at 817 cm^{-1} in both spectra proved that resulting ester contained β-D-fructose residues linked 2→1 glycoside bonds. Therefore, the hydrophobic character of inulin acetates can be explained with the substitution of free OH groups with acetyl residues.

3.4 NMR Studies of JA Inulin and Inulin Acetates

3.4.1 Degree of polymerization of JA inulin

The structure of JA inulin isolated by MAE was elucidated by ^{1}H and ^{13}C NMR studies (Figure 3(a)). Both spectra contained typical shifts for one glucose residue and fructose units in the polymer chain. Characteristic shifts corresponding to the skeleton protons of fructose ring at δ 4.11 (H3f), δ 4.27 (H4f) and in the region from 3.71 to 3.94 (H1f, H5f and H6f). Anomeric glucose signal at δ 5.45 corresponded to H1-Glc showed low intensity when compared with the high-intensity signals of fructose unit. The DP of JA inulin calculated by integration of glucose and fructose signals from ^{1}H NMR spectra was evaluated to be 17. These findings were in the accordance with some previous reports [1, 22]. In the ^{13}C NMR spectra of JA inulin (Figure 3(b)) was found chemical shifts referent to glucose (g) and fructose (f) residues: δ 103.65-103.05 (C2f), 92.44 (C1g), 81.03 (C5f), 76.73 (C3f), 74.23 (C4f), 72.40 (C5f), 71.14 (C2g), 69.18 (C6g), 62.08 (C6f), 60.85 (C1f), 60.44 (C6g) ppm. In anomeric region was found a shifts typical for C2f that was involved in linkage *β*-(2→1)-D-fructofyranosyl-fructose. In DEPT 135 spectra (Figure 3(c)) were identified methinic carbon atoms C1g, C5f, C3f, C4f, C3g, C5g, C2g, C4g and three methylene carbon C6f, C1f and C6g.

However, after acetylation in ^{1}H NMR spectum of inulin acetate was observed some new shifts at 2 ppm that were typical for methyl side chain of acetyl group (Figure 4). The efficiency of microwave-assisted acetylation was evaluated by the degree of acetylation (DA). After calculations described previously [14] we obtained JA inulin acetates synthesized by microwave-assisted acetylation with 2.1 number of acetyl group per fructose unit and DA 69% (Table 3). Until now, only chicory, dahlia and agave inulin were esterified with acetic anhydride, however in presence of solvents (Table 3). Wu and

Lee [11] reported that fast acetylation occured in the first hour, followed by a slower reaction; about 65% acetylation was completed within the initial 1.5 h. In our study, the degree of acetylation was 2.1 without presence of pyridine, as a toxic solvent. JA inulin acetates was characterized as highly acetylated ones.

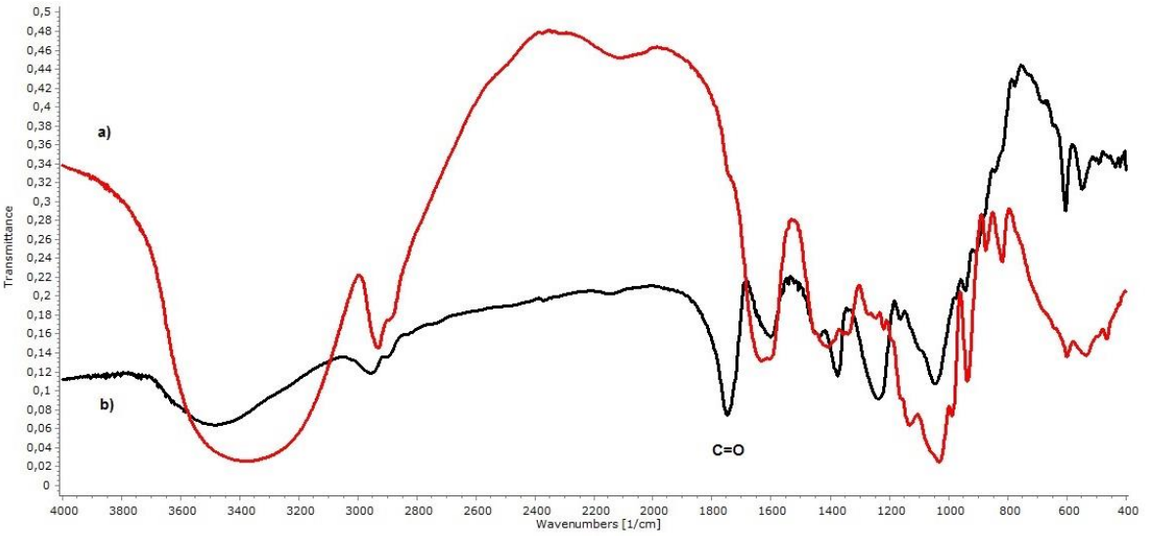
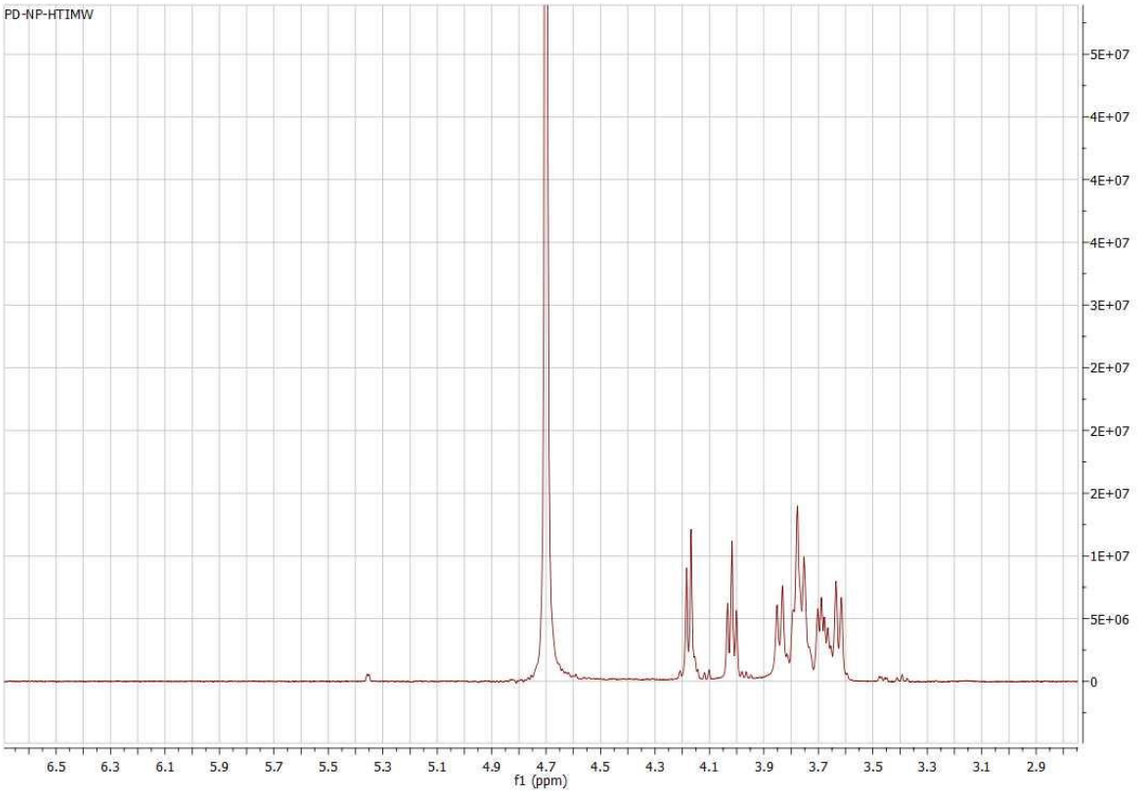
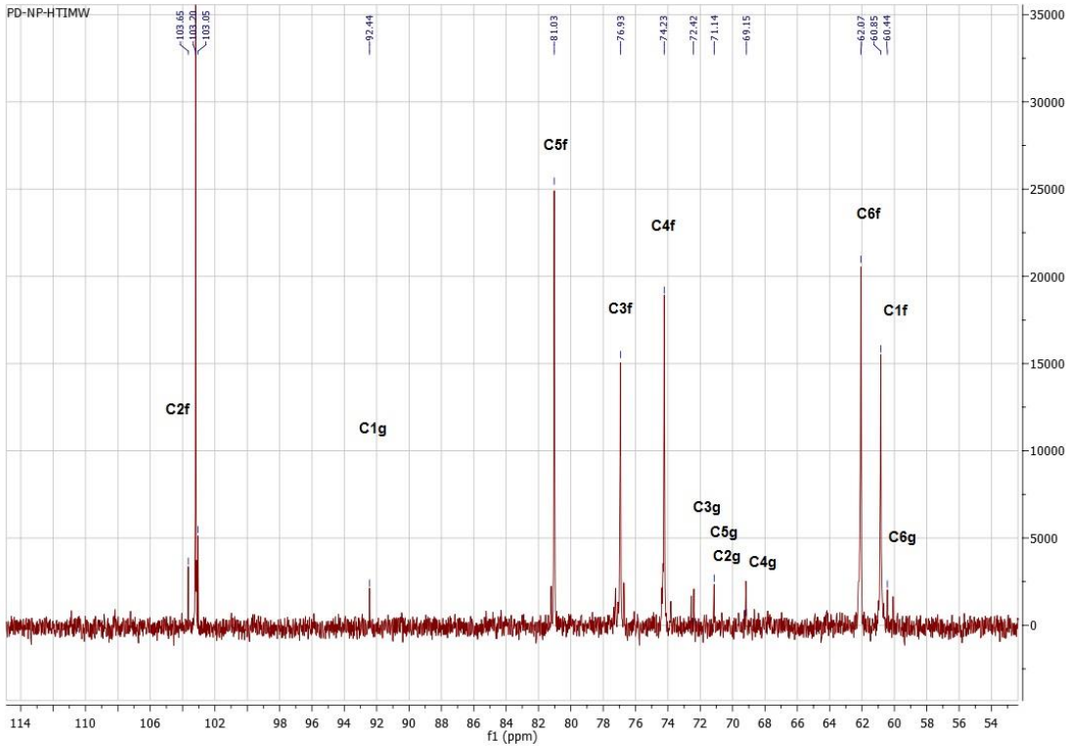


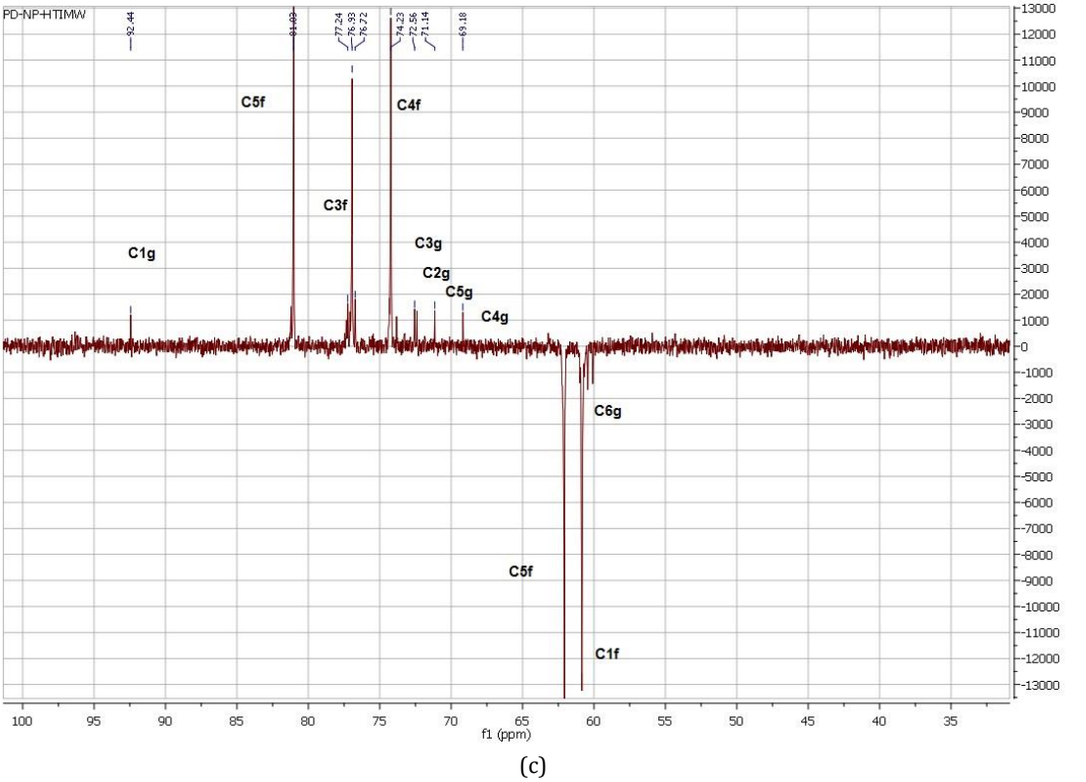
Figure 2 FTIR spectra of JA inulin (a) and inulin acetates (b) obtained after microwave irradiation.



(a)



(b)



(c)

Figure 3 ^{1}H NMR spectra (500 MHz, D_2O) (a),^{13}C NMR (126 MHz, D_2O) (b) and DEPT 135 experiment (c) of Jerusalem artichoke inulin isolated by MAE.

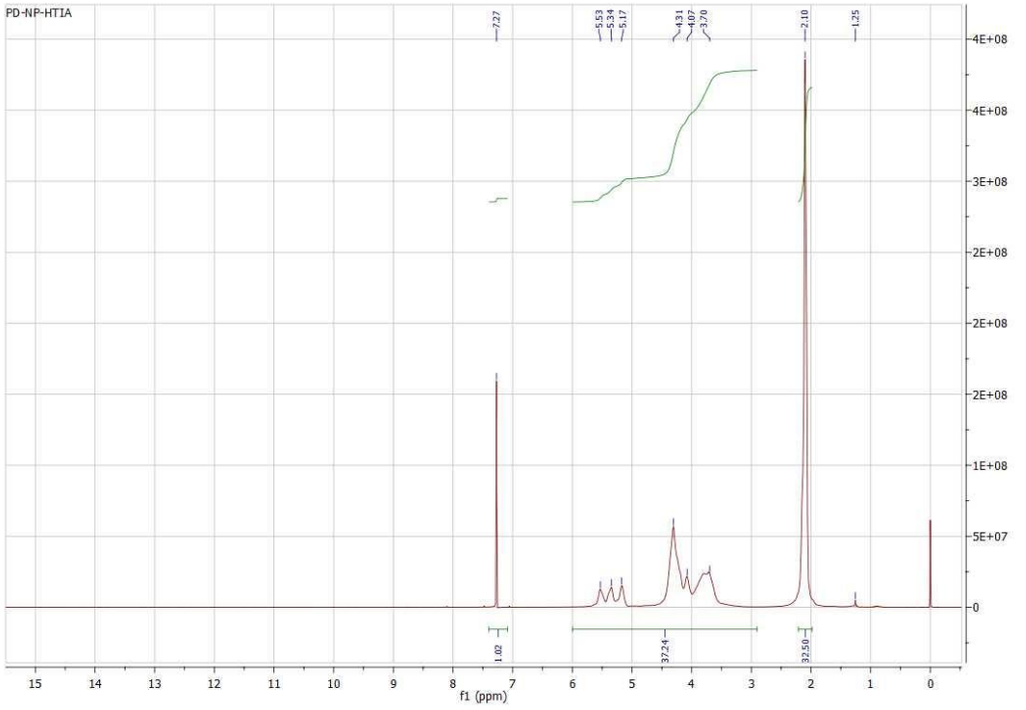


Figure 4 ^{1}H NMR spectra of JA inulin acetate.

Table 3 Acetylation of inulin derived from different plant sources.

Inulin acetate	Catalyst	Solvent	Conditions	DA	Reference
Agave Inulin acetates Dahlia Inulin acetates Chicory Inulin acetates	CH_3COONa	DMF	40°C for 24 h	1.6 45 % 1.6 (54%)	[13] [11] [14]
Chicory Inulin acetates	Py	Py	25°C for 24 h	1.6 to 2.8	[17, 23]
Chicory Inulin acetates	CH_3COONa	absent	boiling, 1 h, 140°C, 4 h	2.8-3.0 3.0	[16] [24]
Chicory Inulin acetates	NaOH	water	25°C	1.01	[24]
JA Inulin acetates	H_2SO_4	absent	heating	61–68%	[25]
JA Inulin acetates	CH_3COONa	absent	MW, 700 W, boiling, 60 s	2.1 (69%)	in this study

In comparison to the previous reports for acetylation of inulin (Table 3), the current research demonstrated fast and effective acetylation of JA inulin only for 60 s.

JA-Jerusalem artichoke; Py-pyridine, DMF-N,N-dimethylformamide. The structure of inulin acetates was additionally confirmed by the ^{13}C NMR spectrum. JA inulin acetate contained chemical shifts as follow: δ, 170.73, 170.22 (CH_2**C**O–) , 104.28 (C2), 103.50, 82.27, 77.28, 77.03, 76.77, 75.69, 64.88, 63.86, 61.90, 60.59, 20.74 (**C**H_3CO-) ppm. Two chemical shifts characteristic for acetyl carbonyl atoms were clearly appeared at 170 ppm. All the methyl carbons from the acetyl residue appeared at δ 20.7 (2×CO**C**H_3). Carbon atoms of the inulin moiety were found in the range of 62.66~103.50 ppm. The observed chemical shifts were in accordance of previous reports for acetylated chicory inulin [16, 17].

4 CONCLUSION

Jerusalem artichoke inulin (DP=18, purity 83%) was successfully extracted by microwave irradiation. For the first time microwave-assisted acetylation of JA inulin was performed and its advantages were demonstrated. Microwave irradiation accelerated the acetylation of JA inulin as reducing the reaction time to 60 seconds. Inulin ester was characterized as renewable material with degree of acetylation (DA=2.1). The current study demonstrated the efficiency of microwave irradiation for isolation and chemical modification of inulin from the Jerusalem artichoke tubers.

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REFERENCES

1. T. Barkhatova, M. Nazarenko, M. Kozhukhova and I. Khripko, Obtaining and identification of inulin from Jerusalem artichoke (Helianthus tuberosus) tuber. *Foods and Raw Materials* **3**, 13 (2015).
2. N. Petkova, I. Ivanov, P. Denev and A. Pavlov, Bioactive substance and free radical scavenging activities of flour from Jerusalem artichoke (Helianthus tuberosus L.) Tubers-a Comparative Study. *Türk Tarım ve Doğa Bilimleri*, 1773-1778 (2014).
3. M. Temkov, N. Petkova, P. Denev and A. Krastanov, Characterization of inulin from Helianthus tuberosus L. obtained by different extraction methods-Comparative study, *Proceeding of Scientific Works of University of Food Technologies* **LXII**, 461 (2015).
4. B. Srinameb, S. Nuchadomrong, S. Jogloy, A. Patanothai and S. Srijaranai, Preparation of inulin powder from Jerusalem artichoke (Helianthus tuberosus L.) Tuber. *Plant Foods for Human Nutrition* **70**, 221 (2015).
5. N. Petkova, M. Ognyanov, M. Todorova and P. Denev, Ultrasound-assisted extraction and characterisation of inulin-type fructan from roots of elecampane (Inula helenium L.). *ASN* **1**, 225 (2015).
6. Panchev, N. Delchev, D. Kovacheva and A. Slavov, Physicochemical characteristics of inulins obtained from Jerusalem artichoke (Helianthus tuberosus L.). *European Food Research and Technology* **233**, 889 (2011).
7. L.Y. Wei, J.H. Wang, X.D. Zheng, D. Teng, Y.L. Yang, C.G. Cai, T.H. Feng and F. Zhang, Studies of the extraction technical conditions of inulin from Jerusalem artichoke tubers. *Journal of Food Engineering* **79**, 1087 (2007).
8. S. Saengkanuk, S. Nuchadomrong, S. Jogloy, A. Patanothai and S. Srijaranai, A simplified spectrophotometric method for the determination of inulin in Jerusalem artichoke tubers. *European Food Research and Technology* **233**, 609 (2011).
9. J.F. Hu and S.Y. Qiu, Comparison of different inulin extraction technologies from Jerusalem artichoke. *Guizhou Agricultural Sciences* **10**, 181 (2009).
10. D. Vassilev, N. Petkova, M. Koleva and P. Denev, Ultrasound-assisted synthesis of sucrose and fructooligosaccharides esters as bioplasticizers. *Journal of Renewable Materials* **4**, 24 (2016).
11. X. Wu and P. Lee, Preparation and characterization of inulin ester microspheres as drug carriers. *Journal of Applied Polymer Science* **77**, 833 (2000).
12. N. Poulain, I. Dez, C. Perrio, M. Lasne, M. Prud'homme and E. Nakache, Microspheres based on inulin for the controlled release of serine protease inhibitors: preparation, characterization and in vitro release. *Journal of Controlled Release* **92**, 27 (2003).
13. R. Starbird, V. Zuniga, E. Delgado, B. Saake and G. Toriz, Design of microspheres for drug delivery to the colon from blue agave fructans. Part 1. Esterification of agave fructans. *Journal of Biobased Materials and Bioenergy* **1**, 238 (2007).
14. K. Jain, V. Sood, M. Bora, R. Vasita and Dh. S. Katti, Electrosprayed inulin microparticles for microbiota triggered targeting of colon. *Carbohydrate Polymers* **112**, 225 (2014).
15. S. Kumar, S. Kesharwani, B. Kuppast, M. Rajput, M. Bakkaria and H. Tummala, Discovery of inulin acetate as a novel immune-active polymer and vaccine adjuvant: synthesis, material characterization, and biological evaluation as a toll-like receptor-4 agonist. *Journal of Materials Chemistry B* **4**, 7950 (2016).
16. N. Petkova, Y. Tumbarski, I. Ivanov and P. Denev, Design of inulin acetates with potential antimicrobial activity. *BJVM*. **20**, Suppl. 1, 13 (2017).
17. F. Damian, G. Van Den Mooter, C. Samyn and R. Kinget, *In vitro* biodegradation study of acetyl and methyl inulins by Bifidobacteria and inulinase. *European Journal Pharmaceutics and Biopharmaceutics* **47**, 275 (1999).
18. M. Lever, A new reaction for colorimetric determination of carbohydrates. *Analytical Biochemistry* **47**, 273 (1972).
19. N. Petkova, R. Vrancheva, P. Denev, I. Ivanov and A. Pavlov, HPLC-RID method for determination of inulin and fructooligosacharides. *ASN* **1**, 99 (2014).
20. D. Murdzheva, N. Petkova, M. Todorova, I. Vasileva, I. Ivanov and P. Denev, Microwave-assisted synthesis of methyl esters of alginic acids as potential drug carrier, *International Journal of Pharmaceutical and Clinical Research* **8**, 1361 (2016).
21. T. Barclay, M. Ginic-Markovic, M. Johnston, P. Cooper and N. Petrovsky, Analysis of the hydrolysis of inulin using real time 1H NMR spectroscopy. *Carbohydrate Research* **352**, 117 (2012).
22. W. Praznik and R.H.F. Beck, Application of gel permeation chromatographic systems to the

determination of the molecular weight of inulin. *Journal of Chromatography A* **348**, 187 (1985).
23. S. Ehrhardt, A. H. Begli, M. Kunz and L. Scheiwe, Aliphatic carboxylate esters of inulin. *US Patent 5877144, Assigned to Suedzucker AG* (1996).
24. M. E. B. Bolkenbaas, H. W. C. Raaijmakers, H. C. Kuzee, D. H. Ar. Van and I. K. Haaksman, Bleach activator based on inulin. *WO Patent 200100771A1, Assigned to Cooperatie Cosun UA* (2001).
25. G. Khusenov, D. Rakhmanberdiev, Rakhimov and M. Khalikov, Obtaining inulin acetate. *European Scientific Review* **92** (2014).

