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Research Article

Optimization of inulin extraction from *Inula helenium* L. using response surface methodology followed by its MALDI-TOF and TLC-FLD based characterization

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ARTICLE INFO	ABSTRACT
	Background: Inulin, a prebiotic, is a mixture of linear chains β-2,1 fructans with a degree of polymerization (DP) of 2 to 60. Different DPs have various applications in the cosmetics, pharmaceutical, and food industries. **Objective:** This study aims to find the best method for DP determination. **Methods:** RSM was applied to optimize the extraction of inulin from *Inula helenium*. Four factors, including time, temperature, solvent-to-sample ratio, and pH and yield as response were selected. Inulin was purified using a hot water extraction followed by a slurry of calcium hydroxide and phosphoric acid. TLC-FLD, MALDI-TOF, and spectrophotometric methods were used to characterize and compare the DP of inulin. **Results:** RSM proposed a maximum yield (10.1 %) at a temperature of 79.6 °C, time of 31.9 min, the solvent-to-sample ratio of 39.9: 1, and pH of 7.7. The quality of extracted inulin was evaluated as follow: FT-IR spectra indicated typical bands at 820, 864, and 932 cm^{-1} that assigned the presence of 2-ketose, β-(2→1) fructofuranosyl unit, and α-D-glucopyranose residue. Inulin with DP (16) and molecular weight 2633 Da was determined in MALDI-TOF. Furthermore, TLC-FLD confirmed the approximate fructose and DP from (1-15). Also, the spectrophotometric method showed an approximate number of 22.3 ± 0.04 as the DP. **Conclusion:** In conclusion, the optimized isolation factors for inulin from the *Inula helenium* were proposed. In comparison with the spectrophotometric result, TLC-FLD quantitative result is much more confirmable to MALDI-TOF. TLC-FLD technique offered a simple and more precise than the spectrophotometric method for the quality of inulin.

1. Introduction

Inulin is a biopolymer from the fructan family. It is a carbohydrate composed of β-(2→1) fructofuranosyl unit and terminated with D-glucopyranose residue linked to D-fructofuranos by an α-(1→2) linkage [1].

Inula helenium L. belongs to the *Asteraceae* family which is distributed in Europa, Asia, and

Africa. The root of this plant has medicinal applications from ancient times and includes coumarin, flavonoids, polysaccharides (inulin 9-12 %), Fatty acid, and saponin [2-4].

The inulin DP is influenced by various factors such as biological origin, environmental growth conditions, plant harvest time, extraction method, and post-extraction processes [5]. Inulin's physicochemical qualities are determined by its DP, and various DPs may be employed in the pharmaceutical, cosmetics, and food sectors. Inulin with a high DP may be utilized as a fat alternative in the food business, whereas inulin with a low DP can be used as a sugar substitute [6, 7]. Inulin has been shown in trials to have health benefits such as reducing total cholesterol and triglycerides, avoiding colon cancer, relieving constipation, treating chronic inflammatory bowel disease, and decreasing appetite [8].

During the last decade, several studies about the extraction of inulin from *Inula helenium* by microwave-assisted extraction, ultrasound-assisted extraction, and characterization by FT-IR and colorimetric method, TLC and HPLC-RID were reported [2, 3, 9, 10]. Various methods, including colorimetry (phenol-sulfuric acid, dinitrosalicylic acid) [11], HPLC-ELSD [12], HPLC-RID, or CAD [13, 14] and HPAEC-PAD [15] were developed for the determination of DP and identification of inulin. Comparison among these methods indicates the colorimetric method can calculate the inulin DP from the ratio of total carbohydrate to reduced sugar [11]; this method requires different chemicals, and the specificity is poor. HPAEC is a sensitive method to determine DP, but it needs specific columns and instruments that are resistant to alkali [14]. Because RID cannot employ gradient elution, HPLC-RID procedures often have low resolution for high DP determination. ELSD, like RID, has a low sensitivity for inulin with high DP. CAD is

more sensitive than ELSD, around 2-6 times better, and it may be better suited to a higher DP [14]. In some studies, MALDI-TOF was used as a high-precision method to determine high DP. Fructan in *Lolium perenne* L and *Chicory intybus* was characterized by MALDI-TOF in linear positive ion mode [16]. In addition, TLC analysis in the fluorescence mode of inulin was carried out by Lucia et al. [17].

There are no reports on the characterization of inulin from *Inula helenium* L. by MALDI-TOF and TLC-FLD. The conditions for hot water extraction of *Inula helenium* were optimized. RSM was designed to optimize pH, temperature, solvent to sample ratio, and time on maximum yield. Moreover, this study is considered the first attempt aiming: a) to determine inulin DP from *Inula helenium* by MALDI-TOF and TLC-FLD b) to compare MALDI-TOF, TLC-FLD and colorimetric method for DP determination.

2. Materials and Methods
2.1. Preparation of Inula helenium roots powder
Inula helenium roots collected from agricultural field Shahid Beheshti University, Tehran, Iran. A voucher specimen of *Inula helenium* L. (MPH-802) has been deposited at the Herbarium of Medicinal Plants and Drugs Research Institute (MPH), Shahid Beheshti University, Tehran, Iran. The *Inula helenium* roots were washed and sliced.

2.2. Chemicals
Phenol, potassium sodium tartrate, 3,5-dinitro-2-hydroxybenzoic acid, phosphoric acid, aluminium chloride, fructose, calcium hydroxide, sodium sulfite, acetic acid, and silica gel F_{254} were obtained from Merck Company (Darmstadt, Germany). Sulfuric acid and n-butanol were purchased from CARLO ERBA Company (D71, 27100 Val-de-Reuil, France). Inulin was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and fiberrific inulin was

obtained from Pure-lē Natural (545 Welham Road, Barrie, Ontario, Canada L4N8Z6). Most chemicals used in this experiment were obtained in the analytical grade.

2.3. Experimental design

A CCD was constructed by software Design Expert Version 7.0.0 (Stat-Ease Corporation, Minneapolis, MN, USA). RSM was used to determine the effect of independent variables on yield (%). The independent variables for this research were X_1 (extraction temperature), X_2 (extraction time) X_3 (solid: liquid ratio) and X_4 (pH) encoded in five levels (Table 1). The design included 30 sets of test that consist of 5 levels

with six replicated center points. Maximum and minimum experimental levels were selected using the literature reports and performing some screening tests.

2.4. Hot water extraction

The extraction of *Inula helenium* roots was performed under the obtained conditions from RSM. 1.5 g of dried roots were extracted under the conditions of the independent variable indicated in Table 1. Each experiment was performed individually. The treatment was filtered, and the supernatant was separated by centrifuge at 4500 rpm for 10 minutes at 25 °C.

Table 1. Independent variable and their levels in RSM

Independent variable	Symbol	Levels				
	coded	-2	-1	0	1	2
Temperature (C)	X_1	35	50	65	80	95
Time (min)	X_2	5	14	23	32	41
Ratio of solvent to solid (V/W)	X_3	10	20	30	40	50
pH	X_4	2	4	6	8	10

2.5. Purification of inulin

The sample was extracted under the optimal extraction condition and then purified as follow: the solution after extraction was concentrated, and then 5 % Ca (OH) $_2$ at 50-60 ℃ for 30 min was added to remove impurities (pectin, protein, and cell wall materials). By this method, the pH of the solution rose from 5-6 to 10-12. The solution was centrifuged at 4000 rpm for 15 min, and precipitation was thrown away, then the yellow supernatant mixed with 10 % phosphoric acid (pH = 8-9) at 60 ℃ for 2-3 h. The method was repeated twice. In the next step, to remove coloured material, the solution was mixed with active carbon at 40 ℃ for 30 min. The treated solution was filtered (Whatman No. 1) and dried by rotary evaporation [18, 19].

2.6. Characterization of inulin
2.6.1. Chemical characterization

The total carbohydrate was determined with the phenol-sulfuric acid method [20].

The sample (1 ml) was mingled with 1 ml 5 % phenol and 5 ml sulphuric acid. The mentioned mixture was left in Bain-marie at 30 °C for 20 min. After incubation, the absorbance of this solution was measured at 490 nm (Shimadzu, UV-2501PC) with inulin (sigma) as standard. The reducing sugar was determined with DNS reagent [21]. Solution extract (3 ml) was mixed with DNS reagent (3 ml). The mention mixture was vortexed and left in a Bain-Marie for 10 min at 90 °C. After the mixture reached to room temperature, the solution's absorbance was measured at 575 nm with D-fructose as a

standard. The amount of inulin was determined by the difference between total sugar and reducing sugars [22, 23].

Inulin content = total sugar – reducing sugar (1)

The purity and yield were measured as follow [11, 18]:

Yield = (inulin content / amount of root powder) $\times$ 100 (2)

Purity = (inulin content / dry matter content of the extracted liquid) $\times$ 100 (3)

The average polymerization degree was calculated as follow:

Total carbohydrate/total reducing sugar (4)

2.6.2. FT-IR spectroscopy

The FT-IR spectra of purified inulin were recorded on the FT-IR spectrometer (Tensor 27, Bruker, USA) with KBr pellets at the frequency range of 4000 - 400 cm.$^{-1}$

2.6.3. TLC

The chain length of inulin was detected using TLC Linomat 5 applicator (CAMAG, Switzerland), twin trough chamber (20 $\times$ 10 cm; CAMAG, Switzerland), microsyringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz, CAMAG, Switzerland). Ten microliters of solution were applied on 10$\times$10 silica gel 60 F_{254} plates (Merck, Germany). BuOH:i-Pro:H_2O: CH_3COOH (7:5:4:2) (v/v/v/v) was used as the ascending development solvent (three times), which at final repeat, the solvent was allowed to pass through TLC for 15 min[22]. TLC plates were dried and derivatized by dipping 10 % aluminium chloride (85:15) ethanol-water to detect in fluorescence mode and scanned by TLC scanner IV (CAMAG, Switzerland), winCATS version 1.4.6 software (CAMAG, Switzerland) at 366 nm. Also, The sugars were visualized by spraying diphenylamine-aniline-H_3PO_4–acetone

(1:1:5:50) (w/v/v/v) and incubated at 90 °C for 20 min [22].

2.6.4. MALDI

Mass spectrum of purified inulin was recorded on the MALDI-TOF reflector mass spectrometer (Bruker Daltonics GmBH, Bremen, Germany). Inulin was initially mixed 1:1 (v/v) with 2,5-dihydroxybenzoic acid matrix (20 g/L matrix in 100 % acetonitrile/ 0.1 % trifluoroacetic acid, Agilent technologies, Palo Alto, USA) and applied immediately on to a target plate (Scout 384, Bruker Daltonics GmBH, Bremen, Germany), and left to air-dry. Nitrogen laser was used in wavelength at 337 nm, and a linear mass analyzer was used to record inulin mass spectra in positive mode.

2.7. Statistical analyses

The response variable was fitted to a second-degree polynomial model using the following equation:

$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x^2_1 + b_{22}x^2_2 + b_{33}x^2_3 + b_{44}x^2_4 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4$

Where y is the yield response; x_1, x_2, x_3 and x_4 are the independent variable; b_0, bi, bii, bij are the constant, linear effect, quadratic effect and interaction effect respectively.

In this study, independent variables were permitted to be at each level within the range of the CCD. All experiments were performed in triplicate. The significant terms in the CCD model were found by ANOVA for yield response. In addition, R2, adj-R2, CV, Lack of fit, and significant probabilities were determined to check the model adequacy. The above quadratic equation was applied to build surfaces for the variables. The software Design Expert Version 7.0.0 was used to analyze the results.

3. Results

3.1. Optimization of inulin extraction

3.1.1. Effects of the independent variable on the yield of inulin

The independent variables, including solid: liquid ratio, pH, extraction time, and extraction temperature, were optimized based on the CCD for maximum inulin yield. The results of the statistical analysis are shown in Table 2.

The polynomial model's determination coefficient ($R^2 = 0.9887$) revealed that the model was very significant. The adjusted determination coefficient (adj R^2) was used to assess the model's suitability. The adj R^2 was 0.9848, indicating a very significant regression model. Furthermore, the CV illustrates how widely the data were scattered. CV for inulin extraction yield was in the acceptable range (4.09). Since CV expresses standard deviation as the percentage of the mean, the small value of CV indicates that variation in the mean is low and develop an adequate response model, while the high CV shows the high variation in the mean value and does not develop a suitable model[24]. The lack-of-fit test that measures ANOVA model's fitness, did not result in a significant F-value in yield, showing that the ANOVA model is accurate to predict the response variable.

The yield results in Table 2 showed that linear coefficients of sample to solid ratio, extraction temperature, and pH were significant (less than 0.0001 level). Moreover, the extraction time was significant at 0.0001. Quadratic terms of temperature and pH were significant at less than 0.0001 level. At the 0.0874 level, the interaction terms of sample to solid and temperature were significant. According to the sum of squares (Table 2), extraction duration, extraction temperature, pH, and sample to solid ratio in linear terms, temperature and pH in quadratic terms, and sample to solid/temperature in interaction terms all had a significant impact on yield.

Table 2. Analysis of variance (ANOVA) with the predicted values for responses variable (%)

Yield response						
Source	Coefficient estimate	df	SS	MS	F-value	P-value
model	-16.87935	7	120.12	17.16	250.89	< 0.0001
X_1	+0.25861	1	40.98	40.98	599.11	< 0.0001
X_2	+0.028426	1	1.57	1.57	22.97	0.0001
X_3	+0.050000	1	24.44	24.44	357.36	< 0.0001
X_4	+2.68750	1	37.50	37.50	548.27	< 0.0001
X_{12}	ns	1	ns	ns	ns	ns
X_{13}	+7.83333E-004	1	0.22	0.22	3.23	0.0874
X_{14}	ns	1	ns	ns	ns	ns
X_{23}	ns	1	ns	ns	ns	ns
X_{24}	ns	1	ns	ns	ns	ns
X_{34}	ns	1	ns	ns	ns	ns

Table 2. Analysis of variance (ANOVA) with the predicted values for responses variable (%) (Continued)

Source	Coefficient estimate	df	SS	MS	F-value	P-value
Yield response						
X^2_1	-1.50000E-003	1	3.24	3.24	47.37	<0.0001
X^2_2	ns	1	ns	ns	ns	ns
X^2_3	ns	1	ns	ns	ns	ns
X^2_4	-0.17187	1	13.44	13.44	166.57	<0.0001
Residual		20	1.37	0.068		
Lack of fit		17	1.31	0.077	4.59	0.1174
Pure error		3	0.052	0.017		
Core total		29	121.92			
C.V	4.09					
R2	0.9887					
AdjR2	0.9848					

Fig. 1 shows three-dimensional (3D) plots for inulin yield as a function of sample to solvent ratio, extraction time, extraction temperature, and pH. Results indicated that, the value of the yield raised as the extraction temperature increased from 50 to 80 °C. Because of the higher temperatures, the yield response was greater. The cell walls totally break down as the temperature increases, and the inulin extracts more. Increased temperature, on the other hand, lowered viscosity and increased solubility [18, 22, 25, 26]. The yield indicated a tendency to increase when the amount of liquid was increased. It is due to the availability of more solvent that increases the driving force of carbohydrates out of the *Inula helenium* root [18, 22, 27]. The results showed that the response variables raised as the time of extract increased from 14 to 32 min. It can be due to the time needs for a contact of inulin to the release medium that the solvent penetrated in to the *Inula* powder, dissolved the inulin and diffused out from *Inula helenium* root [11].

Moreover, the data indicated that maximum yield is obtained at approximately pH 7. Also, increasing the pH on the alkaline side had almost no effect on yield, but the pH on the acidic side reduced the yield [28].

3.1.2. Optimization of variable conditions and validation of results

Based on the results, the optimum point of inulin extraction was the sample to solvent ratio (1:39.9) extraction temperature at 79.6 °C, extraction time of 31.9 min, and pH at 7.7. The predicted inulin yield at the optimum point was 10.1 %. While the experimental yield was 10.0 ± 0.06. So, there was an acceptable match between the estimated and experimental values, indicating a suitable fit between the regression models to the experimental data. The purity and DP value after the optimum point purification were 81.02 ± 0.03 % and 22.3 ± 0.04, respectively. This might be in terms of the reduction of impurities and increase in the inulin content.
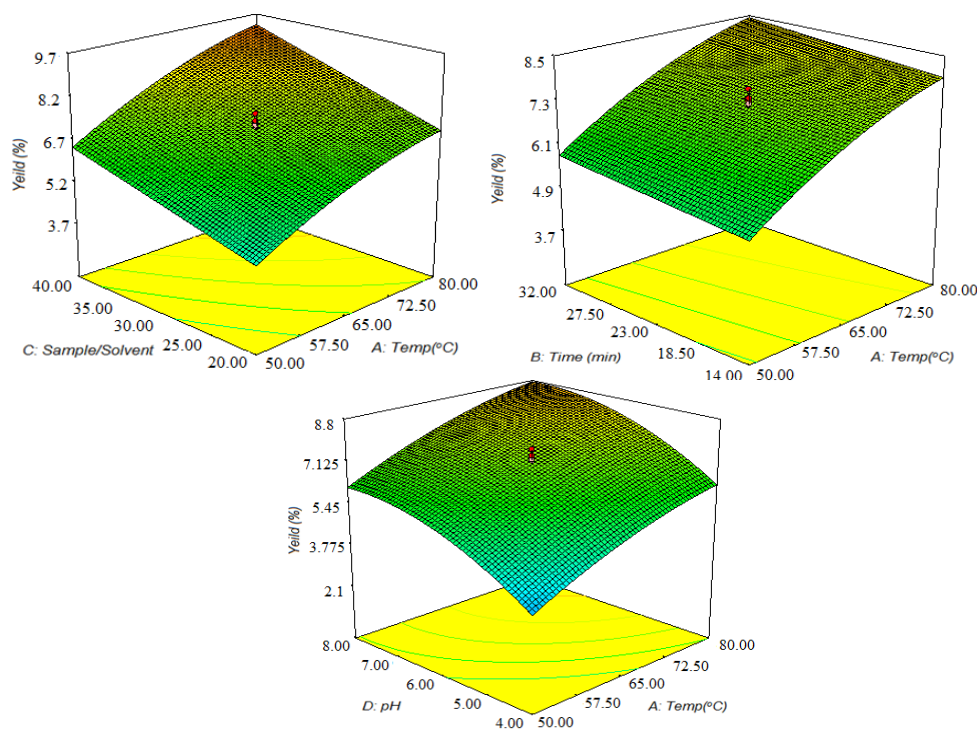
Fig. 1. Three-dimensional (3D) plots for the extraction yield of inulin

3.2. Characterization of Inulin

3.2.1. FT-IR spectroscopy

FT-IR spectra of inulin (Fig. 2) displayed a broad peak at 3402.11 cm^{-1} and a weak absorption peak at 2930.09 cm^{-1} which was attributed to the O-H stretching vibration and C-H stretching vibration of the CH2 groups. The Absorption of water was shown at 1618.41 cm^{-1} and, the absorption band at 1420.28 was assigned C-H vibration. Absorption peaks 1125.18 cm^{-1} and 1029.66 cm^{-1} were due to the C-O-C ring stretching vibrations and C-O stretching vibrations [29, 30]. The band at 932.56 cm^{-1} was assigned α-D-Glcp residue. Two absorption bands at 864.49 cm^{-1} and 820.41 cm^{-1} were attributed to the presence of *β*-type glycosidic linkage and 2-ketofuranose of sugar. [7]. Assignment results were matched to the previous report about *Inula helenium* inulin [1-3].

3.2.2. TLC analysis

The chromatographic approach for analyzing inulin with high polarity and low UV absorbance is difficult. TLC-FLD, a unique approach, may evaluate inulin using a detecting reagent of 10 % aluminium chloride (85:15) ethanol-water on a fluorescence detector. Aluminum-carbohydrate complexes are formed when hydroxy groups in inulin are bonded to aluminum chloride (as Lewis acid). Aluminum-carbohydrate, as Organo-metal compounds, contain fluorescence absorption and can be detected at 366 nm [31].

Fig. 3a indicated inulin on derivatization with diphenylamine-aniline-phosphoric acid-acetone (1:1:5:50) (w/v/v/v). This chromatogram showed different DP in visible mode that contains fructose, sucrose, and other DPs [2]. A study on TLC in visible mode reveals fructose, sucrose, kestose and other DP for inulin from *Jerusalem artichoke* tubers [22]. Inulin and aluminium chloride reagent (Fig. 3b) indicated different DP in florescence mode that contains fructose, sucrose, and other DP. Fig. 3c,d indicated different peaks that correspond to the different DP of standard inulin (1-16) and extracted inulin (1-15) in the fluorescence mode. The results of extracted inulin are nearly consistent with standard inulin.
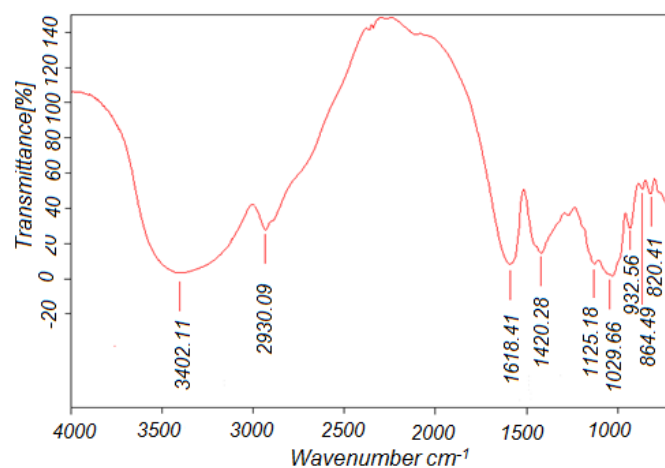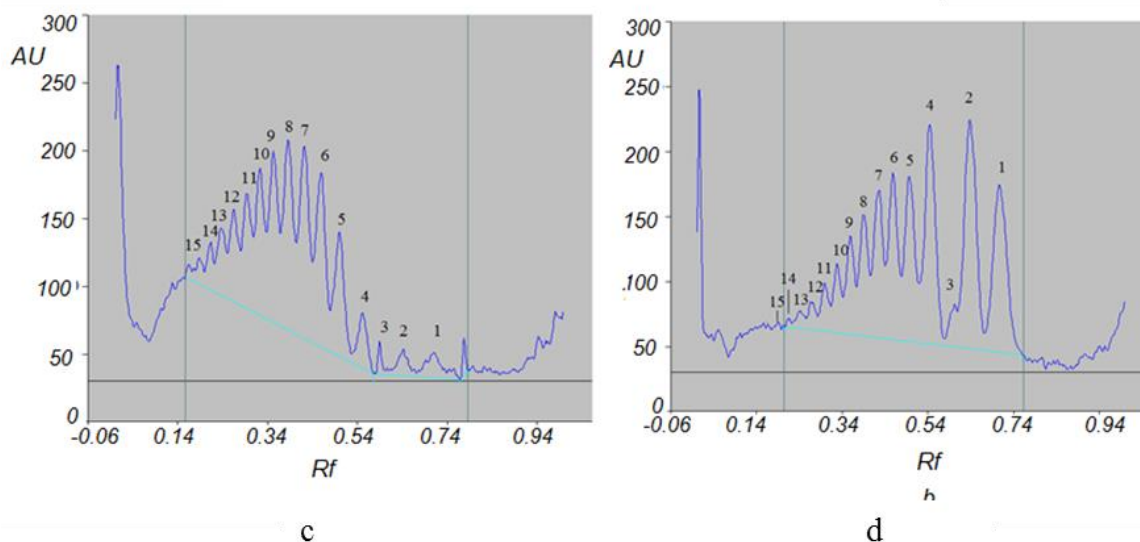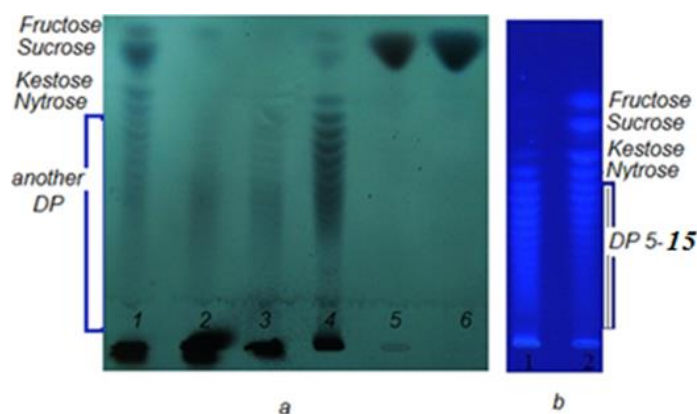
Fig. 2. Inulin FT-IR spectrum

Fig. 3. a) TLC analysis of inulin in visible mode (1. extracted inulin, 2, 3. sigma standard, 4. fiber rific standard, 5. Fructose, 6. sucrose); b) TLC analysis of inulin in fluorescence mode (1. fiber rific standard, 2. extracted inulin); c) TLC analysis of fiberrific standard in fluorescence mode (1. fructose, 2. sucrose, 3. 1- kestose, 4. Nystose and 5-15. Oligomers (DP = 5-15)); d) TLC analysis of extracted inulin in fluorescence mode (1. fructose, 2. sucrose, 3. 1- kestose, 4. Nystose and 5-15. Oligomers (DP = 5-15))
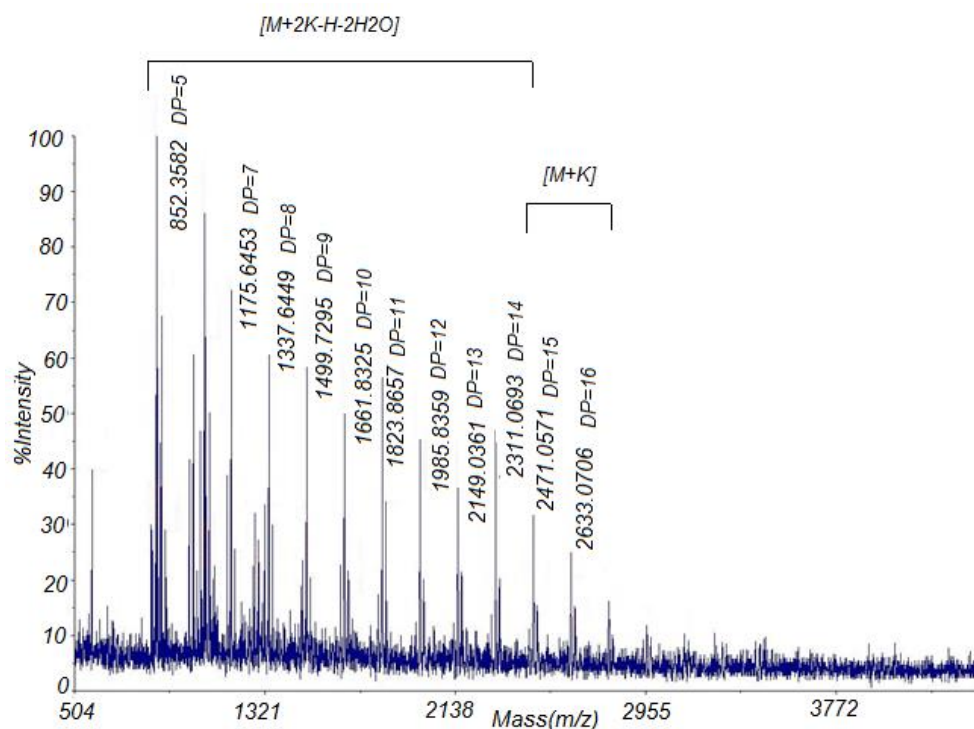
Fig. 4. MALDI-TOF mass spectrum of purified inulin

3.2.3. MALDI-TOF/MS spectrum

MALDI-TOF/MS spectrum of the purified inulin is shown in Fig. 4. Mass profiles of inulin in positive mode was determined by a pattern of $[M+K]^+$ and $[M+2K-H-2H_2O]^+$ signals [32]. The observed masses were 852.3582, 1175.6443, 1337.6449, 1499.7292, 1661.8325, 1823.8657, 1985,8369, 2149.0361, 2311.0693, 2471.0571 and 2633.0706 that corresponding to DPs 5, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16 respectively. However, the theoretical masses were 851.8611, 1176.1423, 1338.2829, 1500.4235, 1662.5641, 1824.7047, 1986.8453, 2148.9859, 2311.1256, 2471.2073 and 2633.3479 that indicated an error mass 0.4971, -0.498, -0.638, -0.6943, -0.7316, -0.839, -1.0084, 0.0502, -0.0563, -0.1502 and -0.2773 respectively. The peak intensity decreases by increasing the molecular weight. The molecular weight of inulin was 2633.0706 Da, and it contains 16 monomers; it means the purified inulin polymerization degree is 16.

4. Discussion

Many articles on optimization and characterization of inulin from different genus were published, and studies were performed on the effects of independent variable on the yield of inulin. The result of optimum point yield of inulin from *Inula helenium* was in agreement with the yield reported in the literature [33]. The kind and the amount of independent variables are in agreement with the study on the conventional extraction inulin by RSM from the *Eremurus spectabilies*, proposed extraction time 31.95 min, extraction temperature 80 °C, and solvent to solid ratio 40 (v/w) [11]. Besides, W. Lingyun et al. indicated that neutral pH is the best condition for inulin extraction [22].

The polymerization degree of pure inulin was compared using chemical, TLC-FLD, and MALDI techniques. The MALDI-TOF device revealed distinct masses that corresponded to varying degrees of polymerization. Purified inulin has a molecular weight of 2633.0706 Da according to MALDI, indicating a

polymerization degree of 16. Based on the TLC-FLD results, inulin DP was 15. TLC-FLD and MALDI results almost confirm each other. The chemical method gave the highest polymerization degree in purified inulin (22.3 ± 0.04), and this result is approximately in agreement with the study on the polymerization degree of inulin from Dahlia tuber (Table 3), DP 20 by HPLC-RID and 23 using

spectrophotometric analysis [1]. This result may indicate that the spectrophotometric method contains a positive error. Comparing the results of the polymerization degree of the inulin from *Inula helenium* with those reported on Table 3 revealed some discrepancies. These differences are due to environmental growth conditions, plant harvest time, extraction method, and post-extraction processes [5].

Table 3. Some previous researches on determination of DP

Plant	Extraction method	Various method to determined DP	DP	Ref
Inula helenium root	ultrasound-assisted extraction	HPLC-RID	30-33	2
Inula helenium root	Ethanol extraction	TLC	8	3
Inula helenium root	ultrasound-assisted extraction (methanol) Microwave-assisted extraction (methanol)	TLC	7-9	8
Dahlia tuber	ultrasound-assisted extraction	HPLC-RID	20	1
		Spectrophotometric analysis	23	1
		NMR analysis	17	1

5. Conclusion

RSM was an efficient strategy to optimize inulin extraction from *Inula helenium* root on the extraction yield. Different independent variables, such as extraction temperature, extraction time, solvent to solid ratio, and pH impacted the yield. FT-IR spectra confirmed the inulin structure. Since there are no reports on the determination of inulin DP from *Inula helenium* L by MALDI-TOF and TLC-FLD; this study is considered to determine inulin DP by MALDI-TOF and TLC in fluorescence mode using alumina chloride reagent and compare MALDI-TOF, TLC-FLD and colorimetric method for the DP determination. The study's key finding is that MALDI is an accurate apparatus for determining DP, and that TLC-FLD may be more efficient than a chemical approach for determining DP. As

a result, TLC-FLD provided a low-cost, easy, and more exact approach for determining inulin quality than spectrophotometric methods.

Authors Contributions

E.A.: participated to the all experimental section and manuscript preparation. H.R. and M.M.F.: participated to the concepts, definition of intellectual content, literature search, experimental studies, data acquisition, data analysis and final manuscript revision.

Conflict of Interest

The authors report no conflicts of interest.

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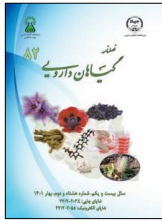
مقاله تحقیقاتی

بهینه‌سازی استخراج اینولین از ریشه گیاه زنجبیل شامی با استفاده از روش سطح پاسخ و آنالیز آن با روش‌های واجذب–یونش لیزری به کمک ماتریس و کروماتوگرافی لایه نازک

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اطلاعات مقاله	چکیده
گل واژه‌ها: اینولین MALDI-TOF اسپکتروفتومتر زنجبیل شامی کروماتوگرافی لایه نازک فلورسانس	**مقدمه:** اینولین به عنوان یک پری بیوتیک شامل مخلوطی از زنجیره‌های خطی بتا–۱،۲ فروکتان با درجات مختلف پلیمریزاسیون ۲ تا ۶۰ است. درجات مختلف پلیمریزاسیون کاربردهای متفاوتی در صنایع آرایشی و بهداشتی، دارویی و غذایی دارند. **هدف:** این مطالعه سعی دارد بهترین روش برای ارزیابی درجات پلیمریزاسیون را پیدا کند. **روش بررسی:** بهینه‌سازی استخراج اینولین از ریشه گیاه زنجبیل شامی با استفاده از روش سطح پاسخ انجام شد. چهار فاکتور زمان، دما، نسبت حلال به ماده جامد و pH بر راندمان اینولین استخراج شده بررسی گردید. خالص‌سازی اینولین با آب داغ، و سپس با استفاده از کلسیم هیدروکسید و فسفریک اسید انجام گردید. میزان درجه پلیمریزاسیون اینولین با استفاده از روش‌های اسپکتروفتومتری، MALDI-TOF و TLC-FLD ارزیابی شد. **نتایج:** بر اساس آزمایش‌های انجام شده شرایط بهینه استخراج اینولین، با بازده ۱۰/۱ درصد؛ دمای ۷۹/۶ °C، زمان ۳۱/۹ دقیقه، نسبت حلال به ماده جامد ۳۹/۹: ۱ و ۷/۷ pH تعیین گردید. طیف FT-IR سه جذب ۸۲۰، ۸۶۴ و ۹۳۲ cm^{-1} که به ترتیب متعلق به ۲–کتوز، واحد بتا–۱،۲ فروکتوفورانوزیل و α-D گلوکوپیرانوز است را نشان داد. مقدار درجه پلیمریزاسیون اینولین در طیف MALDI-TOF معادل با ۱۶ و وزن مولکولی ۲۶۳۳ دالتون بود. طیف TLC-FLD درجه پلیمریزاسیون ۱ تا ۱۵ را نشان داد همچنین در روش اسپکتروفتومتری میزان درجه پلیمریزاسیون تقریبا معادل با ۰/۰۴ ± ۲۲/۳ بدست آمد. **نتیجه‌گیری:** مقدار فاکتورهای مناسب برای استخراج اینولین از گیاه زنجبیل شامی پیشنهاد شد. درجه پلیمریزاسیون بدست آمده از روش TLC-FLD نتایجی قابل قبول‌تر و با دقت بیشتر را نسبت به روش اسپکتروفتومتری در مقایسه با MALDI-TOF نشان داد.