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Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers

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Abstract

Inulin is widely used in functional foods throughout the world for its health-promoting and technological properties. Jerusalem artichoke is cultivated widely in the northern part of China for environment protection. Jerusalem artichoke tubers with 14–19% inulin can be a valuable source of inulin. To optimize conventional extraction of inulin, various combinations of pH, time, temperature, and solvent:solid ratio were used. Experiment design employed fractional factorial design (FFD), path of steepest ascent, central composite design (CCD) and response surface methodology (RSM). The empirical model developed by RSM was adequate to describe the relationships between the studied factors and the response of inulin extraction yield. Based on canonical analysis, the optimal conditions for maximizing inulin extraction yield (83.6%) were at natural pH for 20 min at 76.65 °C and solvent:solid ratios of 10.56:1 (v/w). Moreover, comparison of conventional extraction, direct sonication extraction, indirect sonication extraction showed the indirect sonication extraction is a suitable method for inulin extraction.

Keywords: Inulin; Extraction; Jerusalem artichoke; Response surface methodology; Ultrasound-assisted extraction

1. Introduction

Inulin, a non-digestible oligosaccharide, can preferentially stimulate the growth and activity of one or a limited number of desired bacteria in the colon, and thus improves host health (Gibson & Roberfroid, 1995). And more, positive effects on blood glucose attenuation, lipid homeostasis, mineral bioavailability and immunomodulation effects, along with the ability to add texture and improve rheological characteristics and nutritional properties of food allows inulin to be termed a functional food (Niness, 1999). Inulin has been increasingly used in various foods due to its beneficial nutritional attributes.

Inulin is a linear polymer of D-fructose joined by β(2 → 1) linkages and terminated with a D-glucose molecule linked to fructose by an α(1 → 2) bond, as in sucrose (Modler, 1994). Inulin serves as storage polymers in many members of the Compositae such as *Cichorium intybus* (chicory), *Inula helenium* (elecampane), *Taraxacum officinalis* (dandelion), and *Helianthus tuberosus* (Jerusalem artichoke) (Watherhouse & Chatterton, 1993).

Although today, chicory is the major crop used for the industrial production of inulin, the endeavouring to improve the extraction quality of other crops does not bring to a stop. Jerusalem artichoke tubers with 14–19% inulin can be a valuable source of inulin too (Van, Cummings, Delzenne, Hoebregs, & Smits, 1995).

The northern part of China is drought all the time and cold in winter. Jerusalem artichoke is a suitable crop in the circumstance so that Jerusalem artichoke has been cultivated widely for environment protection (Jiang, 1999).

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Most of the tubers were not harvested because the purification technology of inulin is undeveloped. At present, there is no rapid and economic method for processing a larger amount of the tubers to avoid the high cost of storage.

Several methods for inulin extraction from Jerusalem artichoke have been developed. A pretreatment step involving boiling water-extracting for 10–15 min of the ground tubers had been used (Laurenzo et al., 1999). Precipitation by alcohol is efficient and widely used in laboratory; however, it was deemed uneconomical and unsuitable on an industrial scale. One of a powerful tool for extraction from various plant tissues is ultrasound-assisted extraction. Ultrasound-assisted extraction is the application of high-intensity, high-frequency sound waves and their interaction with materials (Luque-Garcia & Luque de Castro, 2003; Vinatoru, Toma, & Mason, 1999). The propagation and interaction of sound waves alters the physical and chemical properties of materials that are subjected to ultrasound (Mason & Lorimer, 1988). In the case of raw plant tissues, ultrasound has been suggested to disrupt plant cell walls thereby facilitating the release of extractable compounds and enhance mass transport of solvent from the continuous phase into plant cells (Vinatoru, 2001). It seems that the application of ultrasound would be a promising method to enhance the extraction process (Hromádková & Ebringerová, 2003; Li, Pordesimo, & Weiss, 2004; Wang & Zhang, in press; Wu, Lin, & Chau, 2001).

The objective of this study was to develop a set of optimum extraction conditions for Jerusalem artichoke tubers with an aim towards improving inulin extraction yield and reducing cost. Based on previous work, temperature and pH of extraction medium, extraction time, and solvent:solid ratio were considered to be important factors affecting the extraction yield and inulin quality. These factors were investigated by RSM, which consists of a series of statistical analyses of a process that may be controlled by numerous factors. In addition, comparison of ultrasound-assisted extraction and conventional extraction techniques for the inulin extraction was preformed.

2. Materials and methods

2.1. The powder preparation of Jerusalem artichoke tubers

The Jerusalem artichoke tubers were obtained from Dongying, Shandong and were harvested at maturity, then dried for preservation to prevent them from brown rot. The Jerusalem artichoke powder was obtained by peeling, slicing, drying, milling until the whole sample passed through a 0.125 mm sieve. The prepared sample with approximately 5.61% moisture content was stored in dry container for further use.

2.2. Experimental design of conventional extraction

Response surface methodology (RSM) was employed to optimize multiple variables to predict the best performance conditions with a minimum number of experiments (Box, Hunter, & Hunter, 1978; Kong, He, Chen, & Chen, 2004; Shi, Chang, Schwarz, Wiesenborn, & Shih, 1996).

In this research, a series of statistically designed studies were performed to investigate the effect of each of the independent variables (temperature and pH of extraction medium, extraction time, and solvent:solid ratio). The optimization process first entails identifying the most important factors in the extraction using the fractional factorial design, then focusing on the critical subset of factors. The steepest ascent design was used to determine the direction toward predicted higher responses. Finally a central composite design was performed to optimize the critical factors and maximize the extraction yield.

A simplified two-level fractional factorial design method was used for the initial test to find the effective test range of the variables (extraction condition) which affected the response (inulin extraction yield) and allowed the fitting of a first-order model to the data. A 2^{4-1} fractional factorial design leading to 8 sets of experiments was used to verify the most significant factors affecting the extraction of inulin. The variables were coded according to the following equation:

$$x_i = (X_i - X_0)/\Delta X_i \qquad (1)$$

where x_i is the coded value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the center point, and ΔX_i is the step change value. The inulin extraction yield was considered as the dependent variable or response.

After the FFD, the next experiment, if the optimal condition is not in the domain of our expected experimental scope, will then be carried out along the path of steepest ascent, that is, the direction at right angles to the contour lines representing equal yield, which showed the relative amounts by which the factors have to vary to attain a maximum increase of response.

To describe the nature of the response surface in the optimum region, a central composite design with five coded levels was performed. The quadratic model for predicting the optimal point was expressed according to following equation:

$$y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \sum b_{ij} x_i x_j \qquad (2)$$

where y is the response variable, b is the regression coefficients, and x is the coded level of the independent variable. Data were analyzed using the response surface regression procedure (SAS Institute Inc, Cary, NC, USA).

2.3. Ultrasound-assisted extraction

After the optimum processing condition was identified by the models derived by RSM, the extraction treatment of the Jerusalem artichoke tubers was performed under these conditions. Three 135 ml plastic tubes of 5.0 cm in diameter were prepared. The 10 g Jerusalem artichoke powder was diluted at the optimum solvent:solid ratio and kept at the optimum temperature with an additional electrothermic filament in each plastic tube. Conventional extraction method of heating the solvent in a bath was used as a control for comparison with ultrasound-assisted extraction methods. For indirect sonication, the sample tube was immersed in an ultrasound cleaning bath. The sample tube in the bath was shaken periodically with an orbital shaker and the liquid level inside the tube was about 1.0 cm below the liquid surface in the bath. The ultrasound cleaning bath was a Model SK2200H ultrasonic cleaning instrument (Shanghai Kudos ultrasonic instrument Co., Ltd., Shanghai, China) at working frequency was 59 KHz. For direct sonication, a sonicator probe horn was fitted into the sample tube with its tip dipped into the solvent. The sonicator probe horn (with a 3 mm diameter tip) was connected to a 150 W JY96-II ultrasound microprocessor (Scientz, Ningbo, China) having a frequency of 20 kHz. Each extraction was performed for 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40 min.

The extraction liquid was diluted for the following thin layer chromatographic analysis and high-performance anion exchange chromatographic analysis of the inulin.

3. Analysis of inulin

3.1. Determination of inulin content

The extraction liquid was centrifuged at 4500 g for 10 min to remove suspended particles. The supernatant was diluted before determination.

Total carbohydrate was determined by the phenolsulphuric acid method of Dubois et al. using inulin (Raftiline®GR) as standard (1956). Reducing sugar was determined by the dinitrosalicylic acid method using D(−)-Fructose ($M_w = 180.16$, Fluka) as standard (Miller, 1959). The pH value was measured with pH meter.

The inulin content was measured with the difference between total carbohydrate and reducing sugars.

Inulin extraction yield (%) = (inulin content × volume of extraction liquid/mass of artichoke powder) × 100.

3.2. Thin layer chromatographic identification

Thin layer chromatography to examine the inulin extraction was carried out using GF254 plates (Qingdao Haiyang Chemical Co.). Butanol–isopropanol–water–acetic acid (7:5:4:2) is the ascending development solvent. After two runs, the sugars were visualized as blue spots by spraying aniline–diphenylamine–phosphoric acid–acetone (1:1:5:50) and heating at 85 °C for about 10 min (Wang, 2005; Yokota, Enomoto, & Tomita, 1991; Yokota et al., 1991).

3.3. High-performance anion exchange chromatographic analysis with pulsed amperometric detection

To determine the oligofructan pattern in more detail, high-performance anion exchange chromatographic analysis with pulsed amperometric detection (HPAEC-PAD) was used. Typically, the extraction liquid was centrifuged at 4500 g for 10 min to remove suspended particles. The supernatant was diluted 10 times before analysis. The supernatant was analyzed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD ICS2500, Dionex, Sunnyvale, CA, USA) using a 4 × 250 mm CarboPac PA-100 column. The gradient was established by mixing eluant A (150 mM NaOH) with eluant B (500 mM sodium acetate in 150 mM NaOH) using a flow rate through the column of 0.25 ml min^{-1}. Peak areas could be converted to quantities with the appropriate standards, whereas, for Dionex analysis, only the relative concentrations can be obtained, as standards are not available (Wang, 2005).

4. Results and discussion

4.1. Conventional extraction with response surface methodology

4.1.1. The fractional factorial design and analysis

The screening experiments were designed to evaluate the impact of four factors, temperature and pH of extraction medium, extraction time, and solvent:solid ratio. The independent variables, the coded variables, and their levels in this study are presented in Table 1.

A two-level fractional factorial design was employed and the results of the fractional factorial design were showed in Table 2. The inulin extraction yield varied markedly in a range of 52.5–65.7%. The lowest values of inulin extraction yield were obtained when minimal level of

Table 1
Range of values for fractional factorial design (FFD)

Independent variable	Symbol	Coded levels		
		−1	0	1
Temperature (°C)	X_1	30	40	50
Washing time (min)	X_2	15	20	25
Solvent:solid ratio (v/w)	X_3	4	5	6
pH	X_4	7	8	9

Table 2
Experimental results from fractional factorial design (FFD)[a]

Treatment no.	x_1	x_2	x_3	x_4	Yield, %
1	−1	−1	−1	−1	52.6
2	−1	−1	1	1	54.8
3	−1	1	−1	1	52.5
4	−1	1	1	−1	63.3
5	1	−1	−1	1	57.9
6	1	−1	1	−1	65.5
7	1	1	−1	−1	56.2
8	1	1	1	1	65.7
9	0	0	0	0	56.5
10	0	0	0	0	57.9

[a] $x_1 = (X_1 - 40)/10; x_2 = (X_2 - 20)/10; x_3 = (X_3 - 5)/1; x_4 = (X_4 - 8)/1.$

temperature and minimal levels of solvent:solid ratio were used. These results suggested that these variables significantly affected the inulin extraction yield. According to the results of the FFD regression analysis, the temperature (x_1) and the solvent:solid ratio (x_3) were found to be significant at the probability level of 95% for inulin extraction yield and have positive effects, especially the solvent:solid ratio. The effects of temperature, extraction time, solvent:solid ratio and pH value on inulin extraction yield were also analyzed by multiple regression techniques. The predicted regression Eq. (2) for the yield of inulin extraction yield (y) was given in the following equation Eq. (3) as a function of the coded values:

$$y = 0.58286 + 0.02754x_1 + 0.00876x_2 + 0.03746x_3 - 0.0084x_4 \tag{3}$$

The results of t-test for variance between average of observation of two-level experiment and center point showed that the difference was not significant ($P > 0.05$). This result indicated that optimum point was not in the domain of our experiment. Experimentation of steepest ascent path is necessary to reach the optimum domain.

4.1.2. The steepest ascent experiment and analysis

The results from the fractional factorial design clearly showed that the optimal region was beyond the current design range. In this situation, a directional search method, like steepest ascent, can be used to determine the next set of experiments. The path of steepest ascent begins at the center of the current design space and stretches well outside the design space. A sequence of equally spaced locations along the path is then selected which form a set of experiments. Thus the path was to increase the temperature (°C) and solvent:solid ratio (v/w) in order to improve the inulin extraction yield. Extraction time was fixed at 20 min. The variable in pH was non-significant coefficient thus pH was kept natural. The values of inulin extraction yield obtained in these experiments were summarized in Table 3. The data clearly showed that the inulin extraction yield increased when the temperature and solvent:solid ratio increased. After the sixth step on the path, further experimentation cannot increase the inulin extraction yield. The highest inulin extraction yield was achieved in the sixth step. These results indicated that the experimentation is approximating the neighborhood of the optimum inulin extraction yield.

Table 3
Trial made along path of steepest ascent and experimental results

Treatment no.	X_1 (temperature)	X_3 (solvent:solid ratio)	Yield, %
1	40	5	57.7
2	47	6	66.1
3	54	7	69.9
4	61	8	71.7
5	68	9	74.5
6	75	10	82.2
7	82	11	79.7
8	89	12	75.5

4.1.3. The central composite design and response surface analysis

A response surface design is appropriate when the optimal region for running the process has been identified. Further optimization of inulin extraction yield was carried out by using a Box–Wilson central composite design with four star points and five replicates at center point for each of two factors. Table 4 showed the design of this experiment and the results. Regression analysis was performed to fit the response function with the experimental data. The statistical significance of the second-order model equation was checked by an F-test (ANOVA) and data shown in Table 5. The fit value, termed R^2 (determinant coefficient), of the polynomial model was calculated to be 0.8305, indicating that 83.05% of the variability in the response could be explained by the second-order polynomial prediction equation given below (Eq. (4)). The ANOVA results showed that this model is appropriate. It also suggested that extraction yield was primarily determined by the linear and quadratic terms of temperature and solvent:solid ratio of the model and no significant interaction existed between the two factors. The equation for the inulin extraction yield showed positive linear effect and negative quadratic effect:

$$y = 0.823460 + 0.015384x_1 + 0.039430x_3 - 0.057624x_1^2 + 0.006575x_1 \times x_3 - 0.036324x_3^2 \tag{4}$$

Table 4
Experimental design and results from central composite design (CCD)[a]

Treatment no.	Coded levels		Yield, %	
	x_1	x_3	Observed	Predicted
1	−1	−1	69.0	68.1
2	−1	1	71.9	74.7
3	1	−1	70.9	69.9
4	1	1	76.4	79.1
5	−1.41421	0	69.6	68.6
6	1.414214	0	73.8	73.0
7	0	−1.41421	67.8	69.5
8	0	1.414214	84.2	80.7
9	0	0	83.1	82.3
10	0	0	84.7	82.3
11	0	0	86.6	82.3
12	0	0	80.1	82.3
13	0	0	77.2	82.3

[a] $x_1 = (X_1 - 75)/10$; $x_3 = (X_3 - 10)/1$.

Table 5
Analysis of variance for central composite design (CCD)

Regression	DF	Type I sum of squares	R^2	F value	$Pr > F$
Linear	2	0.014331	0.2737	5.65	0.0346
Quadratic	2	0.028972	0.5534	11.43	0.0062
Crossproduct	1	0.000173	0.0033	0.14	0.7228
Total model	5	0.043476	0.8305	6.86	0.0126

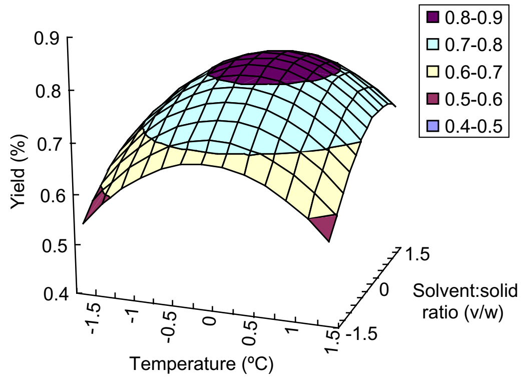

Fig. 1. The response surface plot of inulin extraction yield as a function of temperature (x_1) and solvent:solid ratio (x_3).

The three-dimensional graph obtained from the calculated response surface was showed in Fig. 1. Three-dimensional response surface plots of temperature and solvent:solid ratio against inulin extraction yield can further explain the results of the statistical and mathematical analyses. It is evident from the plot that inulin extraction yield reached its maximum at a combination of coded level 0.1653 (x_1) and 0.5577 (x_3). This is a reconfirmation that the fitted surface has a maximum point which is 76.65 °C (X_1) and 10.56 solvent:solid ratio (v/w) (X_3). The model predicted a maximum response of yield = 83.6% for this point.

4.2. Comparison of ultrasound-assisted extraction and conventional extraction techniques

The Jerusalem artichoke powder was extracted in the sonicator for different periods of time in order to determine the contact time required to achieve the maximum yield of inulin. Fig. 2 showed the typical trends of inulin yield against extraction time period for the Jerusalem artichoke powder. The extraction yields increased significantly with the conventional period extended from start to 16 min, but increased slightly or leveled off from 16 to 40 min. The results suggest that the conventional extraction period for approaching maximum yield of inulin from the Jerusalem artichoke powder is about 16 min. Otherwise, indirect sonication approached maximum yield at about 10 min. Direct sonication approached maximum yield at about 8 min. This means that the extraction rate of the direct ultrasound-assisted process was about 2 times faster than that of conventional method. Ultrasound-assisted extraction decrease the extraction time and raise the speed of extraction.

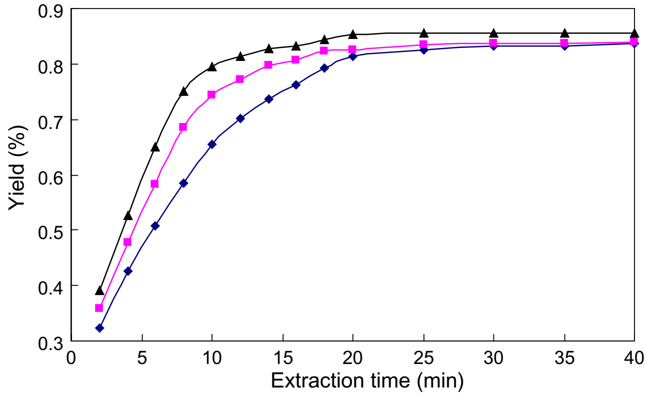

Fig. 2. The inulin yields of three extraction techniques: diamonds, conventional extraction; squares, indirect sonication; triangles, direct sonication.

As seen in Fig. 3, the chromatogram of the extracts obtained by indirect and direct ultrasound treatments showed similar patterns as that of the hot water extract. However, inulin is a polydisperse carbohydrate material. The thin layer chromatography could not quantify the contents of different oligosaccharides. It is known that ultrasound can degrade polysaccharides to parts (Machova, Kogan, Alfoldi, Soltes, & Sandula, 1995). It needed more evidence to evaluate the effect of ultrasound during inulin extraction.

As shown by Fig. 4, peaks from retention time 8 to 20 min were detected as glucose, fructose, sucrose, kestose and nystose. The total Rel. Area of these peaks was 62.48% (Fig. 4a), 63.75% (Fig. 4b), 74.34% (Fig. 4c), respectively.

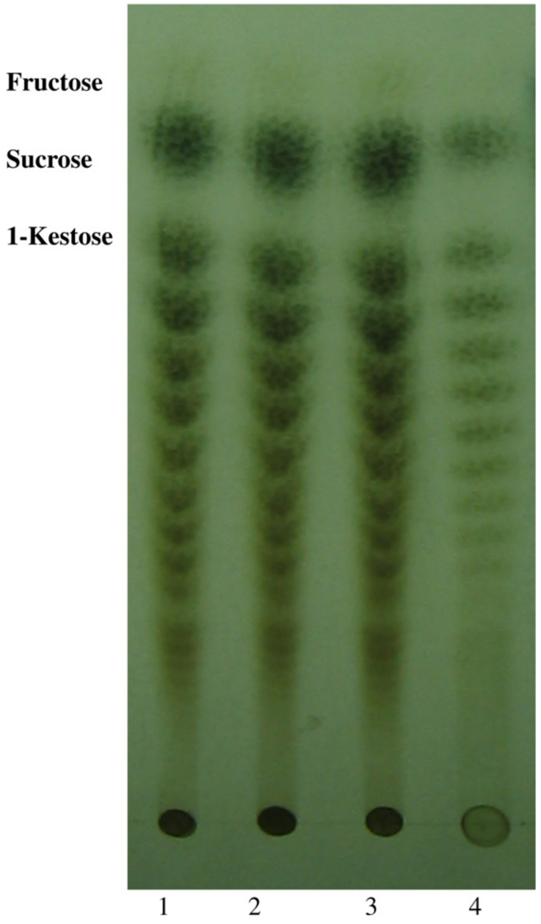

Fig. 3. Thin layer chromatography of inulin extraction: Lane 1, direct sonication; Lane 2, indirect sonication; Lane 3, conventional extraction; Lane 4, purified inulin.

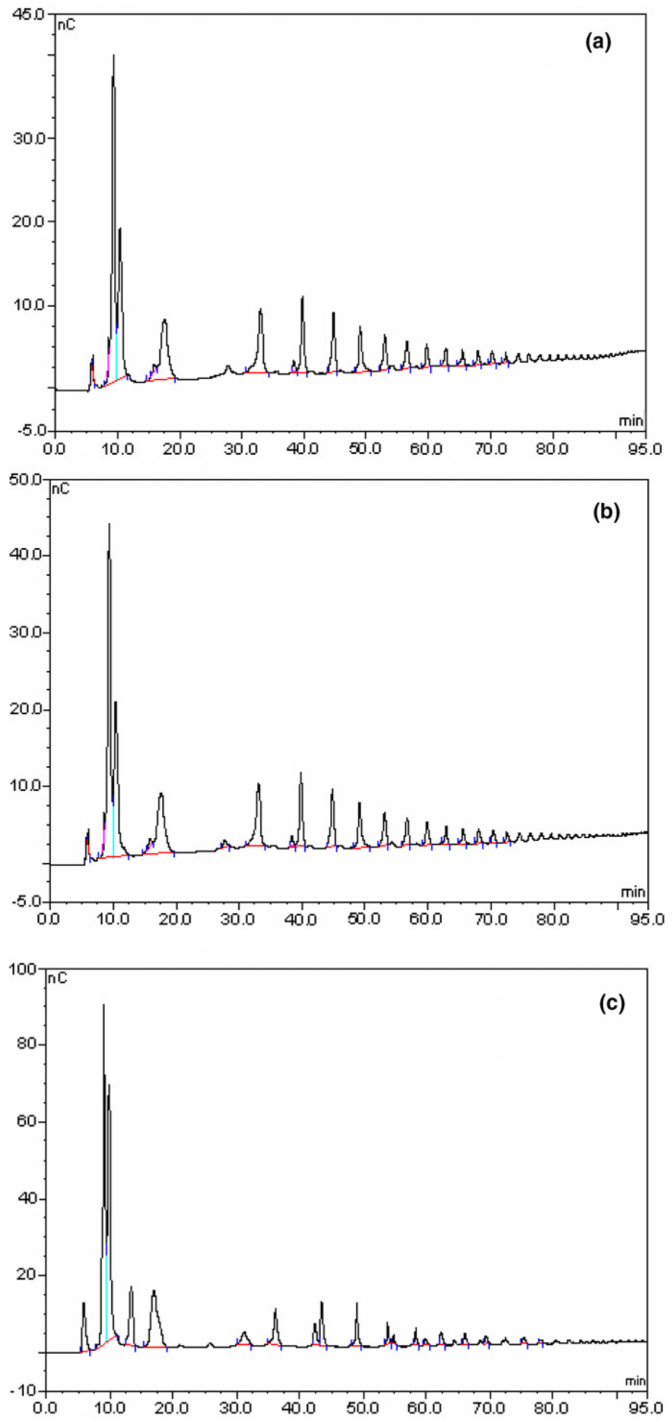

Fig. 4. High-performance anion exchange chromatogram of inulins from conventional extraction (a), indirect sonication (b), direct sonication (c).

Direct sonication obviously increased the oligosaccharide yield. And inulin with a high degree of polymerization decreased correspondingly. It indicated direct sonication could degrade inulin partly. The presence of peak at retention time 13.32 min (Fig. 4c) implied that some low-molecular-weight fragments had formed by the action of ultrasound, changing the chemical composition of the extracted inulin. Therefore direct sonication is unsuitable in inulin extraction. But it can be used in depolymerization of inulin to get a diffluent short inulin. More experiments have to be done for further study.

5. Conclusion

For conventional extraction, the response surface methodology (RSM) was useful in optimizing the inulin extraction process from Jerusalem artichoke tubers. With the aid of ultrasound, the extraction efficiency of inulin would be effectively enhanced. Direct sonication using the probe horn combined with mechanical agitation may be more efficient than indirect sonication. However, sonication with the cleaning bath is non-destructive to the sample which will eliminate the possible contamination and loss of the extract. Moreover, the cleaning bath is usually much quieter than the probe horn during the operation. Therefore, an ultrasonic cleaning bath might be more convenient and efficient for the inulin extraction from the Jerusalem artichoke tubers.

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