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ORIGINAL ARTICLE

Characterization of fructan extracted from *Eremurus spectabilis* tubers: a comparative study on different technical conditions

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Abstract The fructans, inulin and oligofructose, were known to possess many physiologic properties. In the present study, the Box-Behnken design was used to determine the optimum extraction conditions of fructan from *Eremurus spectabilis* root powder (Serish) with water extraction, direct and indirect ultrasound assisted extraction methods that gave the maximum yield. Sonication amplitude (20–100 %), sonication temperature (30–70 °C) and sonication time (5–40 min) were considered variables of direct and indirect ultrasound extractions while for conventional extraction the following variables were water to solid ratio (30–50 v/w), temperature (40–90 °C) and time (5–40 min). A second-order polynomial model was fitted to each response and the regression coefficients were determined using least square method. There was a good agreement between the experimental data and their predicted counterparts. In addition to establishing the difference of these extraction methods, the scanning electron microscopy, Fourier-transform infrared spectroscopy, zeta potential and particle size analysis have been shown to be useful tools to investigate, approximate and predict characteristics of extracted fructan. Moreover, comparison of conventional extraction, direct sonication extraction, indirect sonication extraction showed the indirect sonication extraction is a suitable method for fructan extraction.

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Keywords Extraction yield · Fourier-transform infrared spectroscopy · Fructan · Scanning electron microscopy · Serish

Introduction

A prebiotic is defined as non-digestible food ingredient useful for the host by selectively stimulating growth and/or activity of one or a limited number of bacterial species in the colon and thus improving host health (Roberfroid 2002). Fructans are an important product of the industry of prebiotics. In addition to their interesting nutritional and health benefit properties, fructans are also used in food formulations for their techno-functional properties such as fat substitute, bulk agent, water retention, etc (Blecker et al. 2001; O'brien et al. 2003). They are mixtures of molecules consisting of fructose moieties linked to each other by β (2→1) bonds. Glucose molecules may be linked to the end of the chain by an α (1→2) bond as in sucrose. The degree of polymerization (DP) varies from two to several hundred, with the major components of fructans being inulin (mostly DP 2–60) and oligofructose (DP 2–10). These two components occur in significant amounts in many fruits and vegetables (Flamm et al. 2001).

Serish (*Eremurus spectabilis*) belongs to the family of Liliaceae and geographically distributed in the region of South Asia and Central Asia, including Iran, West Pakistan, Afghanistan, Iraq, Turkey, Palestine, Lebanon, Syria and Caucasus. Their roots accumulate high levels of fructans during their growth and are traditionally used to cure jaundice, liver disorders, stomach irritation, pimples and bone fractures and even as a glue for industrial application (Crockett 1972; Brickell 1996; Brayan 1989; Dashti et al. 2005).

Recently, the attention of researchers has focused on the fructan extraction from plants and several methods for fructan extraction have been proposed among which hot-water treatment (Laurenzo et al. 1999). It should be noted that hot-water extraction of fructan is correlated with long extraction time and high energy consumption. Precipitation by alcohol is efficient and widely used in laboratory; but it is uneconomical and unsuitable for industrial purposes. It is desirable to find out a method for fructan extraction with high yield and activities (Laurenzo et al. 1999). One powerful tool for extraction from various plant tissues is ultrasound-assisted extraction. In the case of raw plant tissues, ultrasound has been suggested to disrupt plant cell walls thereby facilitating the release of extractable compounds and enhancing mass transport of solvent from the continuous phase into plant cells (Vinatoru 2001; Toma et al. 2001; Chemat and Khan 2011).

To the best of our knowledge, there are no reports on the fructan extraction from Serish. The present study is considered the first attempt aiming: (a) to examine the effects of conditions of both conventional and ultrasound assisted extraction i.e. time, temperature, water to solid ratio and sonication amplitude on yield; (b) to determine the optimal conditions for water and ultrasound assisted extraction; (c) to check the validity of Box-Behnken design to analyze the synergistic and/or antagonistic effects of solvent and ultrasound assisted extraction on the yield; (d) to compare ultrasound assisted extraction and conventional extraction techniques for the fructan extraction.

Materials and methods

Preparation of Serish roots powder

The Serish root powders were obtained from the local medical plant market, Mashhad, Iran. Then, the prepared samples were passed through a 50 μm sieve and stored in dry container for further use.

Chemical characteristics of Serish roots powder

Moisture, ash, fat and total dietary fiber were determined according to standard AOAC methods (2005). Crude protein content was measured using Kjeldahl method and considering 6.25 as the conversion rate of nitrogen to crude protein. Carbohydrate content was estimated by difference. All variables were examined in triplicate.

Experimental design

A Box-Behnken design was constructed using the software Design Expert Version 6.0.10 (Stat-Ease Corporation, Minneapolis, MN, USA) and was used for estimating the effect of independent variables on the extraction yield (%). Three extraction variables considered for this research were x_1 (extraction time), x_2 (extraction temperature) and x_3 (water: solid) for conventional extraction method and x_1 (sonication time), x_2 (sonication temperature) and x_3 (sonication amplitude) for ultrasound-assisted extraction methods (Table 1). The design consists of 17 sets of test conditions for each extraction method where three levels were attributed to each factor at high, central, and low levels, with additional four replicated center points. Maximum and minimum treatment levels were chosen by carrying out preliminary screening tests and according to the literature reports and instrumental aspects.

Water extraction procedure

Conventional solvent extraction was carried out in a water bath (model WB/0B7-45, Memmert Company, Schwabach, Germany). Ten gram of Serish root powder was suspended with distilled water under treated condition shown in Table 1. The suspension was then filtered through muslin cloth to remove the insoluble residues. The extracted slurry was centrifuged at 4,500×*g* for 10 min to collect the supernatant. The resulting solution was diluted with water to obtain a 1 % solution and kept at 4 °C until the day of experiment.

Ultrasound-assisted extraction

Indirect sonication

The extraction treatment of Serish tubers was also performed under indirect sonication. Five gram of Serish powder was diluted at the optimum solvent:solid ratio and exposed to extract for different sonication amplitude, time and temperature (Table 1). 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 cm below the liquid surface in the bath. The temperature was kept constant throughout sonication by circulating water through the jacket of chamber. The ultrasound cleaning bath (model Ultrasonix OS 280R, Schuder Schal GmbH & Co., KG, Germany) operating at a frequency of 25 kHz with input power of 500 W.

Direct sonication

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 an ultrasonic processor (model UP 200 H, Dr. Hielscher GmbH, Treptow, Germany) having a frequency of 24 kHz and input

Table 1 Variables and levels used in Box-Behnken design

Water extraction					Ultrasound-assisted extraction				
Variable	Symbol	Levels			Variable	Symbol	Levels		
		−1	0	1			−1	0	1
Extraction time (min)	x_1	5	22.5	40	Sonication time (min)	x_1	5	22.5	40
Extraction temperature (°C)	x_2	40	65	90	Sonication temperature (°C)	x_2	30	50	70
Water to solid ratio	x_3	30	40	50	Sonication amplitude (%)	x_3	20	60	100

power of 200 W. A circulating bath was used to maintain the temperature constant during the sonication. The experimental conditions were similar to indirect method (Table 1).

Analytical methods

Determination of total carbohydrate

Total carbohydrate was assayed colorimetrically using the Phenol–sulphuric acid method (Paseephol et al. 2007). Sample weights were adjusted to obtain a reading of 10–70 μg. Sample solution (1 ml) was mixed with 1 ml of 5 % phenol and 5 ml of sulphuric acid. The mixture was incubated in a water bath at 30 °C for 20 min. The solution appeared as yellow-orange colour and its absorbance was measured at 490 nm using UV–VIS spectrophotometer (model S2000, WPA Lightwave, England). A series of D (-)-Fructose (M_w=180.16, Fluka) solutions of known concentration were used to establish a standard curve.

Determination of reducing sugar

The concentrations of soluble reducing sugars were measured using a 3,5-dinitrosalicylic acid (DNS) method (Miller 1959). After extraction, the solution was diluted 10 times with water, and 3 mL of the diluted sample was transferred to a test tube where it was mixed with 3 mL of the DNS reagent. After mixing with a vortex, samples were heated in water bath at 90 °C for 10 min followed by rapid cooling to ambient temperature. The absorbance of all samples was measured at 575 nm using a UV–VIS spectrophotometer. Calibration curve was prepared using D(-)-Fructose as standard.

Determination of fructan content (extraction yield)

The fructan content was measured by the difference between total carbohydrate and reducing sugars. The percentage fructan yield (%) was evaluated based on following equation (Lingyun et al. 2007):

$$\text{Fructan extraction yield}\,(\%) = (\text{fructan content} \times \text{volume of extracted liquid/mass of Serish root powder}) \times 100 \quad (1)$$

Purity determination

The purity was evaluated as follow (Lingyun et al. 2007):

$$\text{Purity value}\,(\%) = (\text{fructan content/dry matter content of the extracted liquid}) \times 100 \quad (2)$$

Dry matter content was determined by a refractometer (model RFM340, Bellingham and Stanley, Bellingham,UK).

Degree of polymerization

As an index of degree of polymerization, the average chain length, was calculated according to (Lingyun et al. 2007):

$$\text{Degree of polymerization} = \text{total amount of carbohydrate/total amount of reducing sugar} \quad (3)$$

Particle size

The particle size distribution of the different fructan dispersions was determined by LASER scattering using particle size analyzer (VASCO 3, Cordouan Technologies, Pessac, France). A sufficient amount of fructan dispersion was introduced into the sample cell of the particle size analyzer under moderate stirring. The refractive index used was 1.33. Measurements were performed at room temperature in triplicate. The average particle size, expressed in nm, refers to the median diameter in volume.

Zeta potential

Zeta-potentials were deduced at pH 5.5 and 25 °C using a laser zetameter (ZetaCompact Z8000, CAD Instrumentation, France) under a 7 V/cm electric field. Results were based on an automated video analysis of about 100 particles. An electric field of 7 V/cm was applied (Chronakis et al. 2004).

Appearance and morphology

The morphology of samples was observed using scanning electron microscope (Muir et al. 2007). For this purpose, a 10 µl drop of each sample was deposited on a circular platform, dried and sputter-coated with gold film in a sputter coater (SC7620, Polaron Sputter Coater, Quorum Technologies, Newhaven, England). Observations were made using the scanning electron microscope (LEO 1450 VP, Carl Zeiss, Germany) at an accelerating voltage of 30 kV. All the samples were examined at magnification of 5,000 (Mokarram et al. 2009).

FT-IR analysis

Fourier-transform infrared spectroscopic (FT-IR) studies were performed in transmission mode on a spectrophotometer (Paragon 1000, Perkin Elmer, USA). A thin uniform layer of the liquid was placed between two salt cells of KBr and exposed to infrared beam. This method was chosen for its express execution and qualitative information for certain functional groups. Spectra were obtained at 4 cm^{-1} of resolution from 4,000 cm^{-1} to 400 cm^{-1}. The interference of water and CO_2 from air was deducted during scanning (Nikolic and Cakic 2007).

Statistical analyses

Modelling of variables

For each of the response variables, a second-degree polynomial model was used to fit the data using the following equation:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + \varepsilon \quad (4)$$

where Y is the predicted response; x_1, x_2 and x_3 the variables; b_0 a constant; b_1, b_2 and b_3 the linear effects; b_{11}, b_{22} and b_{33} the quadratic effects; and b_{12}, b_{13} and b_{23} the interaction effects.

In this study, predictor variables were permitted to be at any level within the range of the design. All experiments were carried out in triplicate. Statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. In addition, Lack of fit, coefficients of determination (R^2), adj-R^2, coefficient of variation (CV) and significant probabilities were calculated to check the model adequacy. The above quadratic equation was used to build surfaces for the variables. The software Design Expert Version 6.0.10 was used to analyze the results.

Optimization and validation of the model

Besides explaining the behavior of variables by the contour curves, the models fitted in this study could also be utilized for optimization purposes using the desirability function. This approach consists in first converting each response variable into a desirability function d_i, that varies from 0 to 1 (Wu and Hamada 2000). That is, if we want to find the factor levels that take to a maximum response variable value, we need to set d_i=1 for high values and d_i=0 for low values of this response variable. In case, we want a minimum response variable value, we need to set d_i=0 for high values and d_i=1 for low values of this response variable. The idea is that this desirability function acts as a penalty function that leads the algorithm to regions where we can find the desired response variable values. The factor levels that take to a maximum or a minimum of the response variable are called "optimum points". Equation 5 expresses the global desirability function, D, defined as the geometric mean of the individual desirability functions. The algorithm should search for response variable values where D tends to 1.

$$D = (d_1d_2\ldots d_m)^{1/m} \quad (5)$$

where m is the number of response variables (Derringer and Suich 1980).

Optimization was based on generation of the best results for fructan extraction yield of different extraction methods. The extraction yield was determined under optimal conditions. Design Expert Version 6.0.10 was used to optimization. In order to determine the validity of the model, the experimental and predicted values were compared by paired t-test using Minitab 15 (Minitab Inc., State College, PA, USA) software.

Comparison of different extraction methods

In order to assess significant differences among different extraction methods, a completely randomized design was performed using the Minitab 15. Duncan's new multiple range test was used to describe means with 95 % confidence.

Results and discussion

Chemical characteristics of Serish roots powder

The Serish powder had 6.2±0.36 g/100 g moisture, 7.18±0.24 g/100 g protein, 7.23±0.17 g/100 g fat, 6.09±0.09 g/

100 g ash, 73.3±1.12 g/100 g carbohydrate, 57.76±0.51 g/100 g total fiber. It has a marked amount of protein and fats. The ash value is relatively high, suggesting an important mineral content. The composition of total fiber suggest its possible use as a source of dietary fiber for enrichment of foods. In addition, results show that Serish root powder is a polysaccharide-rich material. A study on fructan extraction (Abbasi and Farzanmehr 2009), reveals that 20–70 g/100 g of polysaccharides are related to fructan. Thus, it can be expected that 14.66–53.31 g/100 g fructan will be extracted from Serish powder showing its potential as an industrial resource of fructan extraction.

Conventional extraction

The results of ANOVA for extraction yield of fructan with the corresponding coefficients of multiple determinations (R^2) are shown in Table 2. The regression model was highly significant with satisfactory coefficient of determination (R^2=0.981). Therefore, it is more appropriate to use an adj-R^2 to evaluate the model adequacy. The value of the adjusted determination coefficient (adj R^2=0.975) also confirmed that the model was highly significant. Moreover, coefficient of variation (CV) describes the extent to which the data were dispersed. The CV for conventional extraction yield was within the acceptable range (3.31). Since CV is a measure of expressing standard deviation as a percentage of the mean, the small value of CV give better reproducibility. In general, a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model (Daniel 1991). The lack-of-fit test, which measures the fitness of the model, did not result in a significant F-value, indicating that the model is sufficiently accurate for predicting the yield of conventional extraction.

The results in Table 2 indicated that the linear coefficients of extraction time, temperature and water to solid ratio were significant. Quadratic term of extraction time was significant at less than 0.001 level. The other term coefficients were not significant (P>0.05).

Based on the sum of squares, the importance of the independent variables on yield could be ranked in the following order: the linear term of extraction temperature (x_2), linear term of water to solid ratio (x_3), the quadratic term of extraction time (x_1 x_1) followed by linear term of it (x_1).

Three-dimensional (3D) plots for extraction yield as a function of extraction temperature and time at different water to solid ratio are given in Fig. 1. The data were generated through keeping one variable at centre value of the testing ranges and varying the other two within the experimental range.

Results showed that, the yield raised as the extraction time increased from 5 to 22.5 min and approached its peak value (45.61 %) at this time (Fig. 1a). This might be due to the time requirement for contact of fructan to the release medium where the liquid penetrated into the Serish powder, dissolved the fructan and subsequently diffused out from the root (Milani et al. 2011). On the other hand, when extraction time goes by certain threshold (22.5 min), the yield started to decrease. This may be ascribed to degradation of fructan to free sugar and enhancement of impurities extraction at higher times. The yield showed a large tendency to increase when the extraction temperature was increased. This is maybe due to the enhancement of the mass transfer resulting from the increased solubility of fructan and the decreased viscosity of the solvent (Shi et al. 1996). It is clear from Fig. 1a that yield was increased by portion of water to solid. This might be attributed to the availability of liquid that increases the driving force of fructan out of the root (Lingyun et al. 2007).

Indirect ultrasonic extraction

The values of the regression coefficients give an idea as to what extent the control variables affect the responses quantitatively (Table 2). Analysis of variance (ANOVA) shows that the selected quadratic model adequately represented the data obtained for extraction yield by indirect sonication method. The regression model were highly significant with satisfactory coefficient of determination (R^2=0.854). Moreover, the CV was within the acceptable range (3.12) and the lack-of-fit tests did not result in a significant F-value, indicating that the model is sufficiently accurate for predicting the extraction yield.

The results in Table 2 indicated that both linear and quadratic terms of sonication time, temperature and ultrasound amplitude were significant. The interaction terms exhibited no obvious significant effect on the yield.

Based on the sum of squares, the importance of the independent variables on yield could be ranked in the following order: sonication temperature (x_2), sonication amplitude (x_3) and sonication time (x_1) followed by the quadratic terms of time (x_1x_1), temperature (x_2x_2) and amplitude (x_3x_3).

As it can be seen in Fig. 1b, the response surfaces showed that the sonication time had a quadratic significant effect on the yield. This effect is probably due to time dependent process of extraction that increased the yield until 22.5 min. Conversely, yield decrease might be due to extraction of non-reducing sugars and disruption of fructan branch to reducing sugars (Rezzoug et al. 2008; Sepúlveda et al. 2007).

According to Fig. 1b, extraction yield of indirect method increased with the elevation of the temperature. This is because the improvement of mass transfer with increasing temperature due to the solubility increase and viscosity decrease. On the other hand, increase of sonication temperature probably resulted in reduction of the cavitation bubbles and also depolymerization of fructan into the free sugars that lead to decrease of yield (Tungland and Meyer 2002; Li et al. 2007; Milani et al. 2011).

Table 2 Analysis of variance for the predicted quadratic polynomial models for yield of different extraction methods

Source	d.f.	Water extraction		Indirect sonication		Direct sonication	
		Coefficient	Sum of squares	Coefficient	Sum of squares	Coefficient	Sum of squares
Model	6	−13.505***	1,000.52	26.034***	181.76	33.034**	181.76
Linear							
b_1	1	0.702**	22.34	0.422**	23.46	0.422*	23.46
b_2	1	0.372***	693.70	0.672***	69.28	0.672***	69.28
b_3	1	0.515***	212.37	0.193**	29.71	0.193*	29.71
Quadratic							
b_{11}	1	−0.013***	72.11	−0.007**	20.46	−0.007*	20.46
b_{22}	1	ns	ns	−0.005**	18.73	−0.005*	18.73
b_{33}	1	ns	ns	−0.001*	15.60	−0.001*	15.60
Interaction							
b_{12}	1	ns	ns	ns	ns	ns	ns
b_{13}	1	ns	ns	ns	ns	ns	ns
b_{23}	1	ns	ns	ns	ns	ns	ns
Lack of Fit	3	ns	0.87	ns	13.90	ns	13.90
Pure error	4		18.42		17.27		17.27
Total	16		1,019.81		212.92		212.92
C.V.		3.31		2.32		2.78	
R^2		0.981		0.913		0.854	
adj R^2		0.975		0.861		0.766	

ns no significant effect at level <0.05

* $p<0.05$

** $p<0.01$

*** $p<0.001$

CV coefficient of variation

When the amplitude increased from 20 % to 60 %, the extraction yield increased exponentially but decreased afterwards. This is due to the effect of ultrasonic waves on the vegetal materials that break the cells and releases its content into the extraction medium (Vinatoru 2001). The implosion of cavitation bubbles generates macro-turbulence, high-velocity inter particle collisions, and perturbation in micro-porous particles of the biomass, which accelerates the eddy diffusion and internal diffusion increases (Vilkhu et al. 2008). Consequently, the fructan can easily diffuse through the cell walls and the extraction yield. Moreover, the cavitation near the liquid–solid interface sends a fast moving stream of liquid through the cavity at the surface. Similar trend has also been reported by Entezari et al. (2004) and Zhu et al. (2010) who also concluded that the extraction efficiency of date syrup and *Hizikia fusiformis* polysaccharides increased at higher sonic power. The reduction of extraction yield at amplitudes higher than 60 % might be caused by the depolymerization of part of fructan into the free sugar.

Direct ultrasonic extraction

Quadratic model was fitted for yield of direct ultrasonic assisted extraction of Serish fructan (Table 2). Analysis of variance (ANOVA) shows that the selected quadratic model is well adjusted to the experimental data. The regression model was highly significant with satisfactory coefficient of determination (R^2=0.854). Moreover, coefficient of variation was within the acceptable range (2.78). The lack-of-fit tests did not result in a significant F-value, indicating that the model is sufficiently accurate for predicting the extraction yield.

The results in Table 2 indicated that trend of changes is similar to the method of indirect sonication. Both linear and quadratic terms of sonication time, temperature and ultrasound amplitude were significant. The interaction terms exhibited no obvious significant effect on the yield. Based on the sum of squares, the importance of the independent variables on yield could be ranked in the order of sonication temperature (x_2), sonication amplitude (x_3) and sonication time (x_1) followed by the quadratic terms of time (x_1x_1), temperature (x_2x_2) and amplitude (x_3x_3).

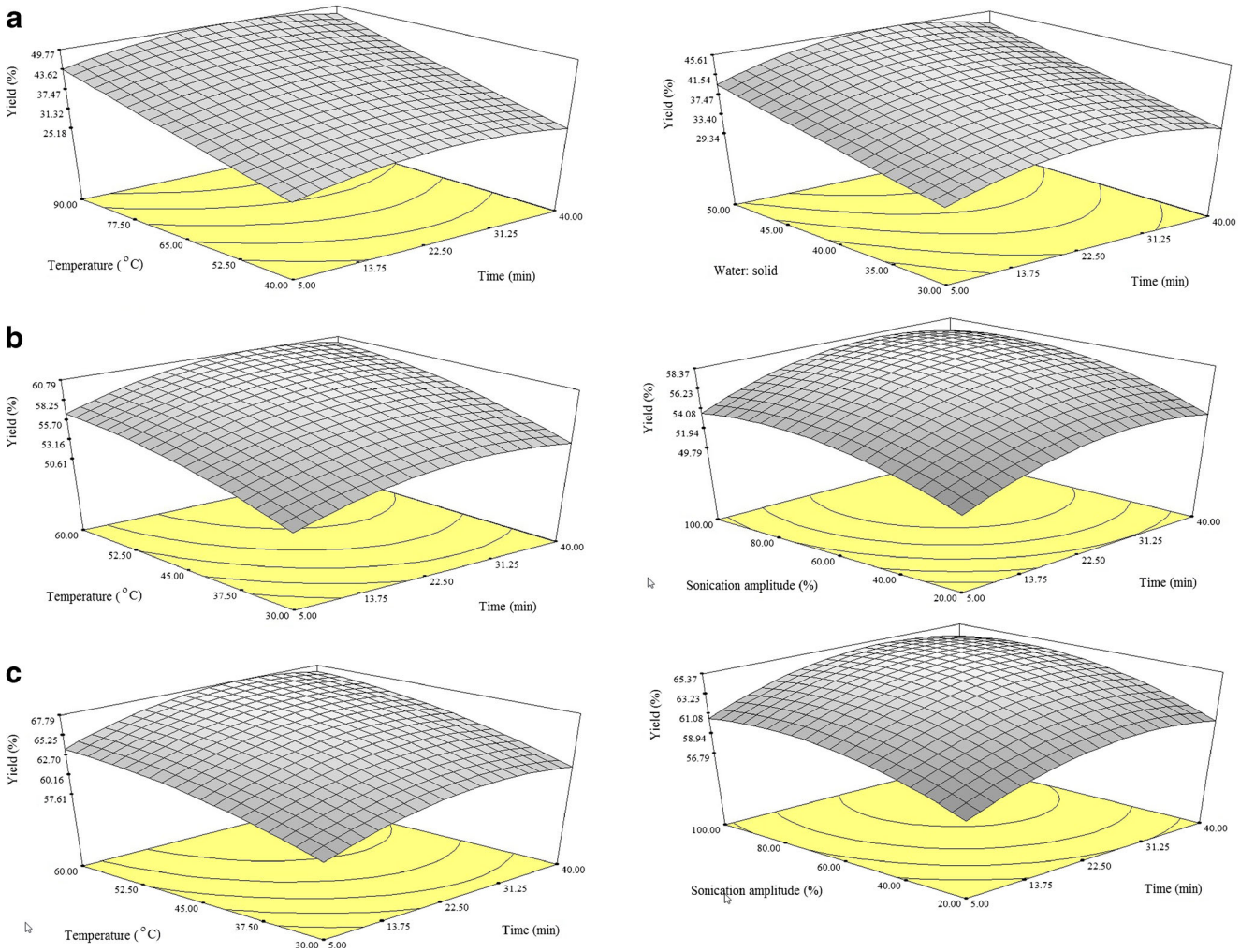

Fig. 1 Response surface plots for the extraction yield of fructan in the conventional method (**a**), indirect sonication method (**b**), direct sonication method (**c**); other variables are held at medium level

Table 3 Optimum conditions, predicted and experimental values of the response variables

Parameters	Extraction method			
	Conventional	Indirect sonication	Direct sonication	
Variables	Time (min)	28.38	29.30	29.27
	Temperature (°C)	87.99	60	60
	Water: solid	50	–	–
	Sonication amplitude (%)	–	79.97	80
Characteristics	Predicted yield (%)	54.10	61.28	63.60
	Experimental yield (%)	52.37 ^{c}±0.22	59.97 ^{b}±0.31	61.83^{a}±0.19
	Purity (%)	32.58^{c}±0.18	33.31^{b}±0.21	36.56 ^{a}±0.16
	Degree of polymerization	5.12^{c}±0.11	6.26 ^{a}±0.12	5.75^{b}±0.17
	Particle size (nm)	1,202.52^{a}±6.50	956.61^{b}±5.42	854.43^{c}±5.53
	Zeta potential (mV)	−32.20 ^{c}±0.23	−20.13 ^{b}±0.18	−1.19 ^{a}±0.05

The similar letters in the same rows are not significant at $p<0.05$

As it can be seen in Fig. 1c, the response surfaces showed that the sonication time had a quadratic significant effect on the yield. This might be due to the disruption of fructan branch to reducing sugars because of sonication time increasing after peak time (22.5 min) (Rezzoug et al. 2008). According to Fig. 1c, extraction yield of direct method increased with the elevation of the temperature. This is because the improvement of mass transfer with increasing temperature due to the solubility increase and viscosity decrease (Tungland and Meyer 2002; Li et al. 2007; Milani et al. 2011). When the amplitude increased from 20 to 60 %, the extraction yield increased exponentially but decreased afterwards. This is due to the effect of ultrasonic waves on the vegetal materials that break the cells and releases its content into the extraction medium (Vinatoru 2001). The reduction of extraction yield at amplitudes higher than 60 % might be caused by the depolymerization of part of fructan into the free sugar.

Optimization procedure and validation of results

Multiple response optimizations were performed to measure the optimum levels of independent variables to achieve the desired response goals. Extraction yields were desired maximal. Then, the optimal conditions were extracted by Design Expert software.

The final results for these optimizations are shown in Table 3 and suggested that an extraction method using these conditions could be good combination of these components in order to achieve the best extraction yield of three examined methods. These new extraction conditions were submitted to the same experimental procedures applied as those from the beginning of this study. There was no significant difference between the estimated and observed values ($P<0.05$), suggesting a good fit between the models to the experimental data.

Comparison of different methods for extraction of fructan

Characteristics of fructan, obtained by direct and indirect sonication under optimal conditions, were compared with those obtained by conventional extraction (Table 3).

Direct sonication gave the highest amount of extraction yield and purity while causing a decrease in the degree of polymerization when compared to the indirect sonication. Thus, direct sonication is more effective than indirect sonication in maximizing yield and purity because its cavitation effect is stronger, thus causing an intensification of mass transfer. In addition, the decrease of degree of polymerization in direct sonication might be due to its stronger cavitation effect that leads to break fructan chain and subsequently decrease the average chain length. These results are in agreement with a study on the optimization of extraction conditions for active components from *Hypericum perforatum* and inulin from *Jerusalem artichoke* tubers using response surface methodology (Liu et al. 2000; Lingyun et al. 2007).

Comparison of the data indicates that particle sizes of ultrasound treated samples were significantly lower than conventional extracted samples. It might be due to the fact that

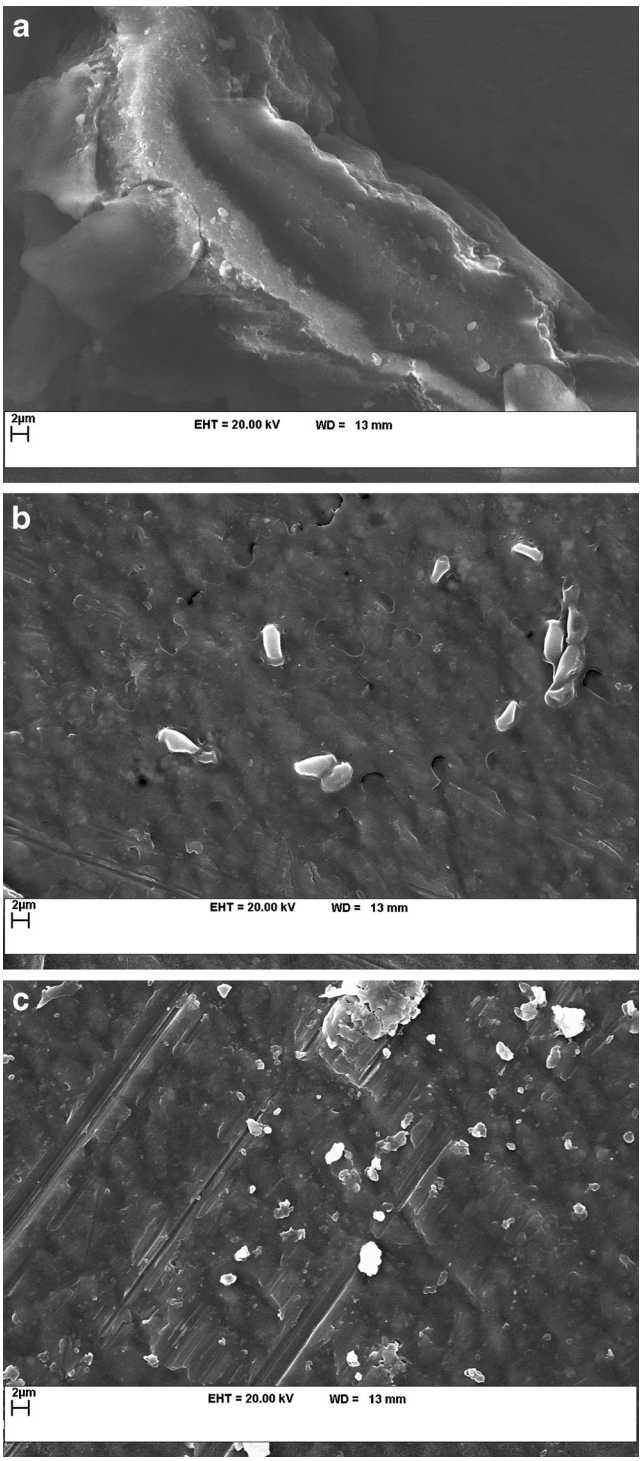

Fig. 2 Scanning electron microscope (SEM) photographs (×5,000) of the extracted fructans in the conventional method (**a**), indirect sonication method (**b**), direct sonication method (**c**)

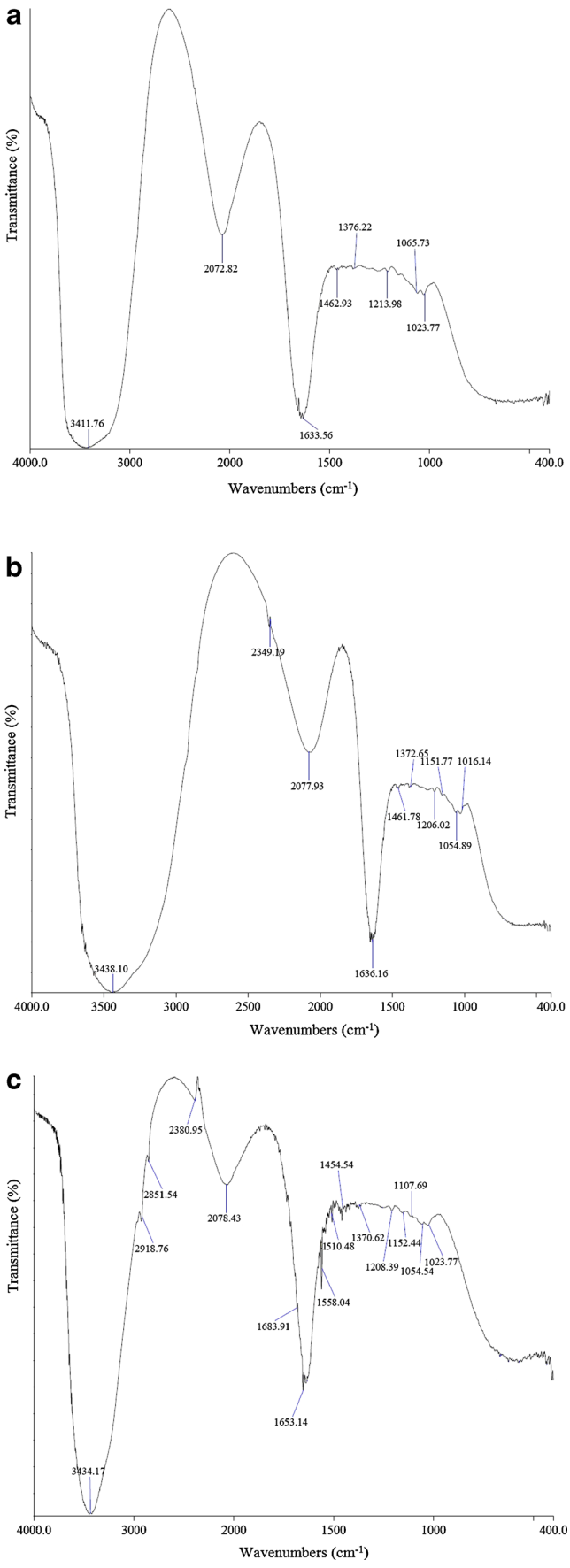

Fig. 3 FT-IR spectra of the extracted fructans in the conventional method (**a**), indirect sonication method (**b**), direct sonication method (**c**)

cavitation on the surface of the source material causes impingement by micro-jets resulting in surface peeling, erosion and particle breakdown (Paniwnyk et al. 2009). The stronger cavitation effect of direct sonication resulted in the more particle size decrease than the indirect method.

The application of high-intensity ultrasound generally resulted in an increase in the magnitude of the zeta potential (Table 3). These measurements revealed that application of ultrasound causes irreversible changes in fructan structure and conformation. This phenomenon can be attributed to the reduction of the fructan reactivity after sonication which might be due to some heterogeneous sonochemical interactions and structural changes that took place during the sonication process. Polysaccharide reactivity is governed by the distribution and number of functional groups attached to the polymerized sugar units that form the backbone of the polysaccharide (Weiss et al. 2011). Indeed, polysaccharides subjected to high-intensity ultrasound can undergo a large number of sonochemical reactions such as glycosylation, acetalyzation, oxidation, C-heteroatom, and C–C bond formations (Kardos and Luche 2001), which may lead to elimination of the negatively charged groups. Besides, the difference between strength of direct and indirect sonication methods became evident by comparing their zeta potentials.

Scanning electron microscopy images of conventional and ultrasound assisted extracted fructans clearly indicated the development of microfractures and disruption of cell walls, which confirms the existence of mechanical effects generated due to the cavitation phenomena (Fig. 2). In addition, it is evident that micro fractures appeared in the fructan granules after application of ultrasound and the surface morphology of fructan granules changed giving nature that is more porous. Similar results were obtained for oil extraction from autoclaved almond powder and extraction gingerols from ginger (Zhang et al. 2009; Balachandran et al. 2006).

The infrared spectroscopy is a fast and convenient method for the investigation of functional groups of polysaccharides (Cakié et al. 2002). The FTIR spectra of extracted fructans by different methods are shown in Fig. 3. The spectra are basically similar to the previously analyzed spectra of other examined systems (Zhao et al. 2011; Nikolic and Cakic 2007). In the IR area of about 3,400 cm^{-1}, there is a wide intense band. This band can be assigned to the O-H stretching vibrations of CH-OH groups from a fructo-furanose unit. The absorption peaks in the spectra of these three samples also showed some differences that reflect the effect of ultrasound. The absorbance around 1,100–900 cm^{-1} was slightly higher and sharper in sonicated samples than the water extracted one, possibly

suggesting a more ordered conformation with fewer conformations (and consequently smaller distribution of bond energies) in the water extracted sample. Some weak absorption peaks of about 2,851.54 – 2,918.76 cm^{-1} for C-H stretching vibrations, were observed in the spectra of direct sonicated samples. The relatively strong absorption peak at around 1,633.16 cm^{-1} and 1,636.56 cm^{-1} reflected the absorption of the C=O group which was part of glycosides. This is a characteristic of monomers (fructose and glucose). This probably means that during extraction a part of the fructans where hydrolyzed to fructose or glucose (Zhao et al. 2011). Other peaks, at about 1,151 cm^{-1} and 1,054 cm^{-1} are resulted from C-O bond and C-C bond, the peak at about 1,370 cm^{-1} represents the angular deformation of C-H (CH_3 group) (Shi et al. 2012). In addition, a series of common spectra at about 1,335 and 1,455 cm^{-1} appeared in the spectra of fructans, and they were related to bending vibrations and internal deformations of the methylene CH_2–OH group from the fructose ring (Panchev et al. 2011).

Conclusion

Response surface methodology was an efficient statistical tool to model the influence of extraction conditions of fructan from Serish root powder on the extraction yield. These results also suggested that by modifying the proportion of these components, a large range of variations may be obtained. Based on these models, the optimum treatments with water extraction, direct and indirect ultrasound assisted extraction were obtained. The effectiveness of the proposed conditions was tested, yielding satisfactory results. The main conclusion of this report is that direct sonication may be more efficient than indirect sonication for extraction of pharmacological useful compounds from Serish. However, sonication with the cleaning bath is non-destructive to the sample that will eliminate the possible contamination and loss of the extract. Moreover, the cleaning bath is usually much quiet than the probe horn during the operation. Thus, an ultrasonic cleaning bath might be more suitable and effective for the fructan extraction from the *Eremurus spectabilis* tubers.

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