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Microwave-assisted extraction of jujube polysaccharide: Optimization, purification and functional characterization

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ABSTRACT

The operational parameters involved in microwave-assisted extraction (MAE) of jujube polysaccharide including microwave power, water to raw material ratio and extraction temperature and time were optimized by RSM. MAE at 400 W, 75 °C, 60 min, using 30 g water/g powdered jujube was the best condition for maximum yield (9.02%) of polysaccharide. Two novel water-soluble polysaccharides (JCP-1 and JCP-2) with average molecular weights of 9.1×10^4–1.5×10^5 Da in term of the symmetrical narrow peaks were identified using the analytical purification procedures. The JCP-1 and JCP-2 mainly composed of glucose, arabinose, galactose and rhamnose in molar ratios of 1.4:2.1:4.2:0.9 and 1.2:1.8:4.1:1.1, respectively. The use of 1.5% JCP-1 led to a high emulsifying stability (95.5%) in a model oil-in-water type emulsion with a reduced surface tension (44.1 m N/m) and droplet size (1.32 μm), and an increased apparent viscosity (0.13 Pa s) during 21-day cold storage. The antioxidant activities were increased in dose-dependent manners (25–200 μg/mL).

1. Introduction

Jujube as a native plant of China belongs to the genus *Ziziphus* Mill and family *Rhamnaceae*. China with more than 700 cultivars of this fruit is the only country known to be exporting dried jujube fruits (Gao, Wu, & Wang, 2013). It has been demonstrated that jujube fruits formulated into paste, puree, syrup, and confection are consumed for digestion improvement and general health maintenance due to the presence of various constituents, including triterpenic acids, flavonoids, cerebrosides, amino acids, phenolic acids, mineral constituents and polysaccharides (Zhao, Liu, & Tu, 2008; Choi, Ahn, Kozukue, Levin, & Friedman, 2011; Gao et al., 2013). Wang et al. (2012) reported that the adequate consumption of *Ziziphus jujube* and its polysaccharide extracts might has a favorable effect on improving or maintaining antioxidant systems and the liver functions of the host.

Hot-water extraction (HWE) method of polysaccharides is commonly associated with long extraction time and high temperature. It thus is necessary to find a novel method for extracting polysaccharides economically that could avoid the disadvantage of HWE. Microwave-assisted extraction (MAE) as a potential alternative to conventional extraction techniques has been recently applied to extract target compounds from various matrices mainly due to the significant savings in processing time, solvent consumption and energy and the enhancement of extraction efficiency (Zeng, Zhang, Gao, Jia, & Chen, 2012). Microwave energy as a nonionizing radiation with a spectral frequency between 300 and 300,000 MHz produces a volumetrically distributed heat source due to molecular friction resulting from dipolar rotation of polar solvents and from the conductive migration of dissolved ions, accelerating the mass transfer of target compounds (Rodriguez-Jasso, Mussatto, Pastrana, Aguilar, & Teixeira, 2011).

Response surface methodology (RSM) as a collection of statistical and mathematical techniques is useful for developing, improving and optimizing processes and is used to determine the efficient relationship between one or more response variables and a set of quantitative experimental variables or factors (Gharibzahedi, Razavi, & Mousavi, 2014b). However, to the best of our knowledge, there is no data about the optimization of MAE conditions of water-soluble polysaccharides from *Z. jujube* using RSM. Hence, the aim of this research was to investigate optimization of the mucilage yield during MAE, purification process, preliminary chemical characteristics and, emulsification and antioxidant potential of polysaccharides extracted by a combination of chemical and instrumental analysis.

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2. Materials and methods

2.1. Plant materials and chemicals

The fruits of *Z. jujube* Mill. were collected from the capital of Southern Khorasan province in Eastern Iran (Birjand city) in April of 2015. They were dried at 60 ± 2 °C in an oven for 2 days after removal of seeds, and crushed into powder using a laboratory mill (Sunny, SFP-820, Tehran, Iran). All the chemicals used were of analytical grade and purchased from Merck Chemical Co. (Darmstadt, Germany).

2.2. Microwave-assisted extraction process

A microwave apparatus using vessel system (MLS Ethos 1600 Microwave System, MLS, Leutkirch, Germany) was used to perform MAE process of jujube polysaccharides under the different conditions. Extractions were carried out with various microwave powers (250–450 W), extraction temperatures (45–85 °C), extraction times (30–70 min) and ratio of water to raw material (25–45 w/w). The preliminary range of the extraction variables was determined using a single-factor-test. The vessel after the extraction was allowed to cool at room temperature and debris fragments of polysaccharide extracts were removed by centrifugation at 5000 rpm for 15 min. Then it was precipitated for three cycles by adding three times of volume of 95% (v/v) ethanol at 4 °C for 48 h. The refined pellets were completely dissolved in suitable volume of water and dialyzed for 4 days against water (cut-off Mw 8000 Da). Deproteinization of the retentate portion was carried out by the freeze-thaw process for repeating 10 times followed by filtration (Wang et al., 2012). The obtained extracts were centrifuged under the above conditions in order to remove insoluble materials. The supernatant was lastly lyophilized in the freeze-dry apparatus to obtain the refined polysaccharide.

2.3. Yield calculation

Yield of the extracted polysaccharide (Y) was determined as the dry weight of the polysaccharide powder (Z) relative to the weight of jujube fruit powder (X; Eq. (1)):

$$Y = \left\langle \frac{Z}{X} \right\rangle \times 100 \quad (1)$$

2.4. Process optimization study

Extraction process of jujube polysaccharide was performed using a central composite rotatable design (CCRD), as a function of microwave power (250–450 W, X_1), extraction temperature (45–85 °C, X_2), extraction time (30–70 min, X_3), and ratio of water to raw material (25–45 w/w, X_4). The dependent variable was extraction yield (Y) of jujube polysaccharide. With CCRD, five levels for each factor are applied which enables to fit second-order polynomials to the experimental data points. Hence, curved surfaces can be fitted to the experimental data. A total of 30 experiments were conducted in CCRD (Table 1): sixteen factorial points (coded levels as (+1) and (−1)); eight axial points (coded as ($-\alpha$) and ($+\alpha$)) and six center points (coded as 0).

The software Design-Expert (trial version 8.1.6, Stat-Ease Inc., Minneapolis, USA) was used to assess the RSM-CCRD results. The linear and quadratic and interaction effects of the investigated four variables on the extraction yield of jujube polysaccharide were evaluated. Their significance was evaluated by analysis of variance (ANOVA). The results of experimental design were fitted by a second-order polynomial equation in order to correlate the response to the independent variables. The general equation to predict the optimal point was described as follows (Eq. (2)):

$$Y = \alpha_{k0} + \sum_{i=1}^{4} \alpha_{ki} x_i + \sum_{i=1}^{4} \alpha_{kii} x_i^2 + \sum_{i<j=2}^{4} \alpha_{kij} x_i x_j \quad (2)$$

where Y is the predicted response (extraction yield); α_{k0}, α_{ki}, α_{kij} and α_{kii} represent regression coefficients; and, x_i and x_j are the coded independent factors. The goodness-of-fit of the polynomial model was checked accounting for R^2 and adjusted-R^2, coefficient of variation (CV) and adequate precision (Gharibzahedi, Razavi, & Mousavi, 2015a; Gharibzahedi, Rostami, & Yousefi, 2015b).

The RSM predictive equations were used to determine optimal conditions for achieving the highest extraction yield for jujube polysaccharide. Five additional confirmation tests were conducted to verify the accuracy of statistical experimental design. In order to determine the validity of the models, the experimental and predicted values were finally compared.

2.5. Purification process of the extracted polysaccharide

The crude polysaccharide extracted at optimal conditions was sequentially purified via a columns of diethylaminoethyl-cellulose (DEAE-cellulose) 52 (3 cm × 30 cm) and Sephadex G-100 (2.6 × 50 cm) as previously described by Qiao et al. (2009). 15 mg/mL of the crude polysaccharide solution was applied to DEAE-Cellulose 52 column. Subsequently, the column was stepwise eluted using a linear gradient of 0–0.7 M NaCl solutions at a flow rate of 1 mL/min to arrive at homogenous preparation. Eluate (10 mL/tube) was automatically collected and after the analyzing carbohydrates based on the phenol–sulfuric acid procedure (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956), two polysaccharide fractions were obtained, concentrated, dialyzed and further purified using Sephadex G-100 column (JCP-1 and JCP-2). Purified JCP-1 and JCP-2 fractions were finally lyophilized for further investigations.

2.6. Molecular weight measurement

The molecular weight of purified samples was determined high-performance gel permeation chromatography (HPGPC; Waters Division Millipore, Milford, MA, USA) equipped with two serially linked Ultrahydrogel™ linear columns (I.D. = 7.8 mm, L = 300 mm), a Waters model 410 refractive index (RI) detector. The purified sample was dissolved in distilled water (2.0 mg/mL) and passed through a 0.45 μm filter, applied to the gel-filtration column, and eluted with distilled water at a flow rate of 0.8 mL/min. The calibration curve for the determining molecular weight was made using a series of Dextran standards with known molecular weights and the software empower was applied for the calculation of average molecular weights.

2.7. Chemical analysis of the extracted polysaccharide

The contents of protein, total carbohydrate and uronic acid of purified jujube polysaccharides were respectively analyzed by the colorimetric methods of Bradford with bovine serum albumin as the standard (Bradford, 1976), phenol-sulfuric acid with D-glucose as the standard (DuBois et al., 1956), and *m*-hydroxydiphenyl with D-galacturonic acid as standard (Blumenkrantz & Asboe-Hansen, 1973).

2.8. Analysis of monosaccharide composition

The method of Zeng et al. (2012) with small modification was used to analyze monosaccharide composition of jujube