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Response surface optimization of mucilage aqueous extraction from flixweed (*Descurainia sophia*) seeds

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ABSTRACT

The effects of four factors of pH (4–8), temperature (50–95 °C), weed–seed ratio (W/S, 15–45), and time (1–4 h) on the yield of mucilage extraction of *Descurainia sophia* seeds were investigated using response surface methodology-Box-Behnken design (RSM-BBD). Results showed that a second-order model for the studied response was adequately fitted with a coefficient of determination of 98.7% ($p < 0.0001$). The optimum conditions to achieve the highest yield (10.45%) were extraction time of 2.9 h, extraction temperature of 94.32 °C, pH of 7.55 and the W/S ratio of 44.2. The extracted mucilage at the optimal point effectively scavenged DPPH free radical, and more concentrations of this polysaccharide indicated potent antioxidant activity in a dose-dependent manner.

1. Introduction

Mucilage naturally is a complex polymeric carbohydrate molecule, belongs to hydrocolloids group with a highly branched structure [1]. They usually are consisting of different magnitudes of L-rhamnose, L-arabinose, D-galactose, and D-xylose, and also various proportions of galacturonic acid [2]. In general, the mucilage structure is formed two individual water-soluble fractions including pectin with gelling attributes with Ca^{2+} and a mucilage component without gelling characteristics. Mucilages by forming colloidal and high viscous suspensions or swelled solutions can be used for the thickening, gelling, film forming and stabilizing purposes in the formulation of many food products such as syrups, sauces, instant foods, ice cream, beverages and confectionaries [3].

Hydrocolloids obtained from plants have the higher advantages in comparison to animal types because they have a friendly image toward consumers [4]. This fact can play a leading role in substantial markets for mucilages obtained from plant seeds to substitute them with some hydrocolloids produced from animal sources.

Flixweed (*Descurainia sophia*) with an erect and branched stem belongs to *Brassiaceae* family and produces large numbers of seeds from early to late summer [5]. This plant is native to temperate and tropical Asia (Afghanistan, Armenia, Azerbaijan, China, Georgia, Iran, and Iraq) and Europe. The seeds containing mucilaginous substance are dull red to light brown with 0.7–1.5 mm long [6]. Lee et al. [7] reported that the transparent mucilage of this seed is thin and there is in less than about 20% of the seed width. Since this mucilage type is used as a traditional medicine in many countries, it can be applied as a bioactive component in the fortification of many foods. Aqueous extraction is the most common procedures used to extract the seed mucilaginous material [8,9]. However, the various parameters like extraction conditions or cultivar type can lead to the different yields and rheological properties [10]. It was demonstrated that extraction time, temperature, pH and ratio of water to seed (W/S) among all the effective factors might have considerable influence on the yield, purity and relative viscosity of the produced crude polysaccharides [4]. Koocheki et al. [11] investigated the effects of temperature, processing time, pH and water to seed ratio on mucilage extracted from *Lepidium perfoliatum* seeds. Samavati and Manoochehrizade [12] investigated the effects of extraction temperature, extraction time, the ratio of water to raw material on extraction yield of crude polysaccharides from the leaves of *Malva sylvestris*.

Statistical experimental designs are powerful tools for searching the key factors rapidly from a multivariable system. Response surface methodology (RSM) has been reported to be an effective statistical technique to optimize a process by reducing the number of experimental trials when the independent variables have a combined effect on the desired response [13]. Many researchers have

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used RSM to optimize the extraction conditions of different hydrocolloids [4,14–17], but no specific information is available about the polysaccharides from *D. sophia* seeds. Therefore, the aim of this research was to optimize the effects of operating factors in aqueous extraction process including extraction time, temperature, pH and W/S ratio on the extraction yield of *D. Sophia* seeds using RSM method.

2. Materials and methods

2.1. Chemicals

The *D. sophia* seeds were purchased from a local market of medical plants (Karaj, Iran). This seed is from a wild plant in Iran and has not got a special variety. This seed harvested from mountains of Mashhad city which was placed in east north of Iran with a hot and dry weather. The seeds were manually cleaned to remove all foreign matter such as dust, dirt, stones and chaff. Ethanol was purchased from Merck Chemical Co. (Darmstadt, Germany). All the chemicals used in this study were of analytical grade.

2.2. Mucilage extraction

Mucilage extraction of the seeds was carried out according to the applied method by Koocheki et al. [4]. Briefly, *D. sophia* seed mucilage (10 g) was extracted by water in a designed extraction temperature (45–95 °C), extraction time (1–5 h), number of extraction (1–5), and W/S ratio (5–45). After determining the preliminary range of the extraction variables which was led to the highest extraction yield, for further studies the main process carried out with the variable parameters in the range of W/S ratio 15–45, pH 4–8, temperature 50–95 °C and the extraction period 1–4 h. The pH was continuously monitored using a pH-meter (GLP 22 Grison, Germany) and adjusted by 0.1 N NaOH and 0.1 N HCl. Also, temperature of the water bath ranged from 50 to 95 °C. The seed-water slurry was stirred with an electric mixing paddle through the extraction period until absolute water absorption was occurred. The seeds were separated from the liquid using a 27-cm basket centrifuge (Universal 320, Hetttich zentrifugen, Germany) lined with a 1-mm mesh. The seed slurry was poured into the basket while the centrifuge was running at 1200 rpm. The mucilage was recovered from the extract with precipitation in three volumes of 95% ethanol. The precipitates were collected and dried overnight in a vacuum oven (Model 4567, Kimya Pars Co., Iran).

2.3. Analysis of the extraction yield

According to the Eq. (1), the yield was calculated as the dry weight of the mucilage powder relative to the seed weight [18]:

$$\text{Mucilage extraction yield (\% w/w)} = \frac{\text{Dried mucilage extraction weight (g)}}{\text{Seed weight (10 g)}}. \quad (1)$$

2.4. Measurement of antioxidant activity

The procedure of Yang et al. [19] with minor modification was used to measure the free radical scavenging activities of the extracted mucilages before and after the process optimization. The various volumes of the mucilage solution (1 mg/mL) were added to 2 mL DPPH solution (800 mM in dehydrated ethanol) and the final reaction volume was prepared up to 4 mL with 70% ethanol. After their shaking, the resulted mixtures were incubated in the dark at 25 ± 2 °C for 30 min. The absorbance was read using an UV/Vis spectrophotometer (Jasco, V-630, Tokyo, Japan) at 517 nm. Distilled water was used as the blank. The synthetic antioxidant of butylated hydroxytoluene (BHT) was applied as the positive control compounds. The scavenging activity of mucilages and BHT on DPPH free radical was calculated using the following equation (Eq. (2)):

$$\text{Scavanging activity} = \left(\frac{A_0 - A_i}{A_0}\right) \times 100\% \quad (2)$$

where A_i is the absorbance of the sample and A_0 is the absorbance of absorbance of control (without sample).

2.5. Experimental design and statistical analysis

RSM was used to estimate the effects of four parameters of pH (4–8, X_1), extraction temperature (50–95 °C, X_2), W/S ratio (15–45, X_3), and extraction time (1–4 h, X_4) on the extraction yield (Y). A Box-Behnken design (BBD) was employed for designing the experimental data (Table 1). The Design Expert (Trial Version 8.0.7.1 Stat-Ease Inc., Minneapolis, MN, USA) software was applied for regression and graphical analyzes of the obtained data. The experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The generalized regression second-order polynomial model proposed for predicting the response variables is given as (Eq. (3)):

$$Y = C_{k0} + \sum_{i=1}^{4} C_{ki}x_i + \sum_{i=1}^{4} C_{kii}x_i^2 + \sum_{i<j=2}^{4} C_{kij}x_i x_j \quad (3)$$

where Y is the predicted response (mucilage extraction yield); C_{k0}, C_{ki}, C_{kii}, and C_{kij} represent regression coefficients; and, x_i and x_j are the coded independent factors.

The quality of the fit of polynomial model was expressed by the coefficient of determination (R^2), adjusted-R^2 (R^2_{adj}), predicted-R^2 (R^2_{pred}), the prediction error sum of squares (PRESS), coefficient of variation (CV) and adequate precision (ADP) as previously stated by Gharibzahedi et al. [20]. The statistical significance and regression coefficient significance were determined with F-value and p-value, respectively. The correlation between the response and independent variables can be readily seen in the response surface and contour plots. These plots show the simultaneous interaction of two factors on the responses and find the location of optimum experimental variables [21].

3. Results and discussion

3.1. Preliminary studies

Extraction process to achieve the highest amount of mucilage yield was carried out in the effect of different temperatures using the different extraction temperatures of 45, 60, 70, 80, 90 and 95 °C (Fig. 1**a**). The extraction time, pH, number of extraction and the W/S ratio were fixed at 3.5 h, 7, 3 and 35, respectively. As shown in Fig. 1**a**, the maximum extraction yield was observed when the extraction temperature was 95 °C. This tendency is in agreement with other reports to extract plant polysaccharides [22,23]. Although the extraction yield of polysaccharides can also be high at temperatures more than 95 °C, from an industrialization view, an increase in the temperature will bring about the increase in cost for the extraction process. The increase of the polysaccharides diffusion coefficient and the enhanced solubility of the polysaccharides in the extracting solvent at higher temperatures caused the increase of the polysaccharides mass going out from the seeds into the solution [24]. The extraction coefficient increased with increasing the extraction temperature due to the increase of the polysaccharides solubility [25].