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Plantago major seed mucilage: Optimization of extraction and some physicochemical and rheological aspects

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ABSTRACT

The effect of different extraction procedures on functional properties of mucilage extracted from *Plantago major* seed were investigated using response surface methodology. Extraction at 75 °C, using 1:60 water:seed ratio at pH 6.8 was the best condition for maximum yield (15.18%), emulsion stability (67.4%), foam stability (88.4%), solubility (97.36%) and water absorption capacity (39.74 g/g) of mucilage. At this optimum point, PMSM had, on average 82.85% carbohydrate, 76.79 mgGAE/g_{dry} total phenol content, 97.8 mg g^{-1} total flavonoid content and 915.54 μg ml^{-1} antioxidant activity. The results indicated that PMSM had average molecular weight of 1.2×10^6 Da. FTIR analysis demonstrated the presence of carboxyl, hydroxyl and methyl groups and glycoside bonds. The chain flexibility parameter, activation energy, ζ-potential and droplet size for PMSM were determined as 946.09, 0.78×10^7 J/kgmol, 15.23 mV (at neutral pH) and 448.56 nm, respectively. Intrinsic viscosity for PMSM in deionized water was 14.24 dl g^{-1}.

1. Introduction

Most seeds contain polysaccharide polymers with specific functional characteristic as a useful source of commercial hydrocolloids (Koocheki, Mortazavi, Shahidi, Razavi, & Taherian, 2009a; Koocheki, Taherian, Razavi, & Bostan, 2009). Mucilage is a type of hydrocolloid, a long-chain substance of high molecular weight polysaccharide. It is widely used as a food additive, including thickening agent and gelling agent in the food industry. Plant seeds are customary and old sources of mucilage (Campos, Ruivo, da Silva Scapim, Madrona, & Bergamasco, 2016). *Plantago major*, also known as Ribwort, is a plant seed which is a species of a flowering plant in the *plantaginaceae* family. The plant produces a large amount of seeds. It is one of the most abundant and widely distributed pharmaceutical crops throughout the world ubiquitously found in many parts of the world; its seeds have been applied for a long time as an anti-infective, immune-modulating, anti-inflammatory, analgesic, anti-microbial, anti-ulcerogenic, antioxidant and anti-cancer agent, as well as for wound curing purposes (Reina et al., 2013).

The coating of seeds has carbohydrate polymers that absorb water and form mucilage with a high viscosity. These polysaccharides consist of xylose, arabinose, galactorunic acid, glucuronic acid, rhamnose, galactose, and glucose (Samuelsen, 2000). The seeds are small with an ellipsoidal shape (0.4–0.8 × 0.8–1.5 mm) and a slight bitter taste. The seeds are placed in capsules (8–16 per capsule) and become gummy in sultry weather owning to the swelling of the polysaccharides present in the seed coat (Qadry, 1963).

Aqueous extraction is the most common method for the extraction of mucilage from seeds. Extraction of mucilage was done through conventional hot-water extraction (HWE) which is time-temperature dependent (Koocheki, Mortazavi et al., 2009; Koocheki, Taherian et al., 2009). Our screening experiments showed that extraction temperature, pH, and water:seed ratio could have remarkable effect on crude polysaccharides properties. Many studies have been reported on different seeds and the extraction conditions resulting in different amounts of yield, rheological and functional properties from one cultivar of the seeds to another. Thus, in order to achieve the highest yield and quality polysaccharides, it is necessary to optimize the extraction procedure. There are several factors affecting the extraction. Furthermore, to determine the optimum extraction conditions the likelihood of interactions between the independent factors should be taken into account (Koocheki, Mortazavi et al., 2009; Koocheki, Taherian et al., 2009; Panyoo Akdowa et al., 2014).

Response surface methodology (RSM) has been declared to determine how the independent variables have an interactive effect on dependent variables. RSM is a combination of statistical and mathematical techniques successfully utilized to develop,

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improve and optimize such processes (Rostami & Gharibzahedi, 2016). However, to the best of our knowledge, there is no data about the optimization of HWE conditions of water- soluble polysaccharides from *P. major* seed using RSM. Because of difference in gum structure, the rheological behavior is quite different from one gum solution to another (Hesarinejad, Koocheki, & Razavi, 2014). To the best of our knowledge, no study has gained insight into the physicochemical and rheological characterization of PMSM. Hence, the aim of this research was to investigate optimization of the mucilage yield(EY), emulsion stability(ES), foam stability(FS), solubility and water absorption capacity(WAC) during HWE, preliminary chemical characteristics, molecular weight, zeta potential, size distribution, antioxidant potential, surface tension and FTIR spectrum of polysaccharides extracted by combination of chemical and instrumental analysis. In addition, the effect of temperature (25–65 °C) on some dilute solution properties (e.g. intrinsic viscosity; molecular conformation and chain flexibility) of this novel hydrocolloid was investigated to explore its potential applications in food and pharmaceutical systems.

2. Material and methods

2.1. Extraction optimization

2.1.1. Sample preparation

P. major seeds were supplied from a local market in Mashhad, Iran. The cleaned *P. major* seeds were wrapped in plastic bags, sealed and kept in a dry and cool place. All chemicals used were of analytical grade.

2.1.2. Extraction procedure

P. major seed mucilage (PMSM) was extracted from whole seeds using deionized water with the water:seed ratio of 20:1–60:1 at pH 4–10. The pH was regulated with 0.1 M HCl or NaOH, and the temperature of the water bath ranged from 45 to 75 ± 2.0 °C. Water was preheated to a certain temperature before adding the seeds. Using an electric mixing paddle, the seed-water slurry was stirred during the entire process (1.5 h) based on preliminary tests. In order to separate the mucilage from the swollen seeds, the seeds were passed through an extractor with a rotating plate scrapping the mucilage from the surface of the seeds. Afterwards, the collected mucilage was filtered, dried in an oven (45 °C overnight), milled and screened with a mesh 18 sieve. The dried powder was then packed and kept in cool and dry conditions.

2.1.3. Analytical methods

The percentage of extraction yield (EY) was computed as the ratio of the powdered mucilage dry weight to the seed weight (Koocheki et al., 2010).

The ES against high temperature (kept in an 80 °C water bath for 30 min) was calculated as the ratio between the emulsion final and initial volumes. The emulsions were prepared at room temperature by adding sunflower oil (6 mL) to PMSM solutions (60 mL) gradually and mixed by high speed stirrer at 2000 rpm for 10 min and then homogenized at 9600 rpm for 1 min (Ultra Turrax T-25,IKA,Germany) (Sciarini, Maldonado, Ribotta, Pérez, & León, 2009).

The FS were determined using the method proposed by Sciarini et al. (2009). Aliquots (30 mL) of 1%w/w suspensions were whipped at 20,000 rpm for 2 min with a homogenizer (Ultra Turrax T-25,IKA,Germany). The FS was measured as the foam volume after 30 min, relative to the total suspension volume.

Solubility percentage was measured based on Koocheki, Razavi, and Hesarinejad (2012). In brief, the mucilage suspensions (1%w/v) were located in a water bath at 30 °C for 30 min. Next, they were centrifuged at 800g for 15 min. Aliquots (10 mL) of the supernatant were dried in a hot-air oven at 125 °C overnight until reaching a constant weight. Solubility percentage was computed using the following equation:

$$Solubility\,(\%) = \left(\frac{\text{Initial weight} - \text{Final weight}}{10} \times 30\right) \times 100 \qquad (1)$$

WAC was calculated based on the method suggested by Sciarini et al. (2009). The mucilage (0.25 g) was completely hydrated with deionized water in a tube and then centrifuged at 1,600g for 10 min. The supernatant was then discarded and the swollen sample was weighed. WAC (g/g) was calculated as:

$$WAC = \left(\frac{\text{swollen sample weight} - \text{sample weight}}{\text{sample weight}}\right) \qquad (2)$$

2.1.4. Experimental design and statistical analysis

RSM was utilized to examine the effect of the three independent variables (water:seed ratio, x_1; extraction temperature, x_2 and pH, x_3) on the response variables. RSM is an efficient strategy for optimizing a multivariable process, due to its practical use in their optimization. The technique provides mathematical and statistical procedures to study relationships between one or more responses (dependent variables) and a number of factors (independent variables) (Diniz & Martin, 1996). A central composite rotatable design (CCRD) was employed to create the experimental design. RSM was applied to the experimental data using a commercial statistical package, Design-Expert version 6.0.4 (Statease Inc., Minneapolis, MN). The experiments were randomized to minimize the unexplained variability effects in the responses because of extraneous factors. The experimental design contained axial points, and six center points to determine the reproducibility of the method (Montgomery, 2001). The response variables (Y) included EY(%), ES(%), FS(%), solubility(%) and WAC(g/g). These values were related to the coded variables (x_i,i = 1–3) by a second order polynomial using the below equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_1\beta_1 X_1^2 + \beta_2\beta_2 X_2^2$$
$$+\beta_3\beta_3 X_3^2 + \beta_1\beta_2 X_1 X_2 + \beta_1\beta_3 X_1 X_3 + \beta_2\beta_3 X_2 X_3 + \varepsilon$$

The coefficients of the polynomial model are represented by β_0(constant term), β_1,β_2 and β_3(linear effects),β_{11},β_{22} and β_{33}(quadratic effects), and β_{12},β_{13} and β_{23}(interaction effects). Statistical significance of the model terms was also investigated. The significant terms of the model were determined through analysis of variance (ANOVA) for each response. The adequacy of the models was verified using model analysis, lack-of fit test, coefficient of determination (R^2) and adjusted-R^2 analysis. The terms found statistically non-significant ($p > 0.05$) were omitted from initial models and the experimental data were refitted to develop the final reduced model. It should be taken into account that some non-significant variables ($p < 0.05$) were added again to the model because of quadratic or interaction effects. The correlation between the response and independent variables can be observed in the response surface and contour plots. These plots indicate the simultaneous interaction between two factors on the responses and illustrate the location of the optimum experimental variables (Baş & Boyacı, 2007). RSM outputs, namely contour and 3D graphic surface plots provide the optimum and most effective variables for hydrocolloid extraction.

2.2. Physicochemical properties of optimized PMSM

2.2.1. Chemical analysis

The moisture, ash, fat, protein content of the seeds was measured (AOAC, 2005) and the carbohydrate content was measured according to two methods. One of them was described by