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Stepwise extraction of *Lepidium sativum* seed gum: Physicochemical characterization and functional properties

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ABSTRACT

Cress seed gum (CSG) was fractionated using stepwise extraction with water, yielding three fractions (F1, F2, F3) whose average molecular weights ranged from 863 to 1080 kDa. The chemical composition (monosaccharide, ash, moisture, CHN and uronic acid contents) and molecular weight of the fractions varied significantly. Generally, the major identical peaks of FT-IR spectra for three fractions and whole cress seed gum were similar. The results of DSC and TGA indicated that F3 had the highest thermal stability and considering the initial decomposition temperature, the decreasing order of thermal stability was F3 > F2 > F1. Cress seed gum and its fractions exhibited non-Newtonian shear thinning behavior which the Herschel-Bulkley model was successfully described the steady shear flow behavior of samples, and apparent viscosity followed the order of F3 > F2 > F1. F3 exhibited the best surface tension reducing ability compared to other fractions and CSG. All the samples had good emulsifying capability (>97%) and stability (>96%). The emulsion capacity increased slightly along the series of F1, F2 and F3, whereas, emulsion stability decreased along the same series. CSG and F3 showed the highest and the lowest foaming capacity and stability, respectively.

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1. Introduction

Hydrocolloids are broadly used as functional ingredients in the food and pharmaceutical systems, and nowadays there is a great interest for natural hydrocolloids with low cost and proper functionality. Hydrocolloids from plants have an advantage over those of animal origin due to their friendly image towards consumers [1–3]. *Lepidium sativum* (Garden cress) belongs to the Cruciferae family and grows widely in the Middle East, Europe and USA [4]. Cress seeds have been used in traditional medicine to treat asthma, hypertension, hepatotoxicity, hyperglycemia, enuresis and fractures [1,5,6]. The seeds contain a large amount of mucilageinous substances, which are a good source of hydrocolloids with high molecular weight [4,7,8].

Bigoniya et al. showed the presence of testa (1–2 layered and appeared yellowish brown), tegmen (attached to inner side of testa layer and appeared as single layer), alleurone layer, endosperm and embryo (as innermost structure surrounded by endosperm cells) in the transverse section of *L. sativum* seed [9]. Mucilage is generated from the outer testa [10]. Extraction optimization, some physicochemical and functional properties of the mucilage extracted from cress seeds as a new source of hydrocolloid have been recently studied [1,4,7,11–17]. *L. sativum* seed gum (CSG) had a weight-average molecular weight of 540 kDa and radius of gyration 75 nm, the majority of the CSG was carbohydrate with a sugar content of nearly 77%. Acid equivalent weight, intrinsic viscosity and FT-IR measurements confirmed polyelectrolyte nature of the CSG, which relates to the carboxyl groups carried by galacturonic acid and glucuronic acid unit. The cress seed gum showed a strong shear thinning behavior in steady shear measurements and a weak gel type behavior in dynamic tests [4,11,13]. Extraction conditions for maximum values of yield and viscosity and minimum protein content of hydrocolloid extract from cress seed were investigated using response surface methodology by Karazhiyan et al. [12]. Data analysis exhibited that temperature (25–85 °C), pH (3–10), extraction time (10–25 min) and water to seed ratio (10:1–80:1) significantly ($p < 0.05$) affected the extraction yield and viscosity, whereas the effect of water to seed ratio on protein content was

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not significant ($p > 0.05$) [12]. To the best of our knowledge, there is no published report about fractionation of cress seed gum. Many studies have been conducted to investigate fractionation of hydrocolloids by different methods and the results indicated that the fractions had different physicochemical and functional properties [18–28]. Therefore, the objectives of the present study were to (i) fractionate cress seed gum using stepwise extraction and (ii) investigate the physicochemical (elemental analysis, sugar composition, uronic acids content, functional groups (FTIR), molecular weight, thermogravimetric analysis, differential scanning calorimetry) and functional characteristics (steady shear rheological properties, surface tension, emulsifying and foaming properties) of the whole cress seed gum and its fractions.

2. Materials and methods

2.1. Materials

Seeds of *L. sativum* were obtained from a local market in Tehran, Iran. The seeds were cleaned manually to remove all foreign matters and broken seeds. Ovalbumin was obtained from Sigma-Aldrich Co. (A5503, lyophilized powder). All chemicals used in this study were of analytical reagent grade.

2.2. Cress seed gum extraction and fractionation

Aqueous cress seed gum was extracted from whole seeds using distilled water (water to seed ratio of 30:1, pH 7) at room temperature (21 °C) and based on the procedure as described by Karazhiyan et al. [12]. The seed-water slurry was slowly mixed throughout the extraction period (15 min). Separation of the gum from the swollen cress seeds was achieved by passing the seeds through an experimental extractor (Pars Khazar 700P, Rasht, Iran) equipped with a rotating plate that scraped the gum layer on the seed surface. This procedure produced whole cress seed gum (CSG).

Three fractions (F1, F2 and F3) were obtained by dividing of the water volume (water to seed ratio of 30:1) and extraction time (15 min) into three parts (water to seed ratio of 10:1 and extraction time: 5 min) according to the following procedure:

Step 1: The cleaned cress seeds (100 g) were soaked in 1000 mL distilled water (pH 7, extraction time: 5 min) at room temperature (21 °C). The gum was separated from the swollen seeds by passing the seeds through the extractor. The extracted gum was termed fraction F1.

Step2: The seeds (which was passed through the extractor in step 1) were collected from the extractor and soaked in 1000 mL distilled water (pH 7, room temperature, extraction time: 5 min). The seed-water slurry was passed through the extractor and the sample obtained was denoted as F2.

Step 3: The seeds were collected from the extractor again and soaked in 1000 mL distilled water (pH 7, room temperature, extraction time: 5 min). Separation of the gum from the swollen seeds was achieved by passing the seeds through the extractor, and the gum obtained was termed fraction F3.

All samples (CSG, F1, F2 and F3) were purified by alcoholic precipitation based on the work done by Razavi et al. [29] with some modifications. After extraction, three volumes of 96% ethyl alcohol were added to one volume of the extracted sample and left for 2 h at room temperature. The collected precipitate was dried at room temperature for 24 h and the dried gum was ground and used for analysis.

2.3. Yield, moisture and total ash measurements

The yield (%) was calculated as the dry weight of the gum powder relative to the seed weight. The moisture content of the dried samples was quantified from weight loss upon heating at 105 °C for 3 h in an oven and the ash content was estimated by heating in a muffle furnace at 550 °C for 3 h until constant weight was achieved [4].

2.4. CHNS analysis and protein determination

Carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) contents of the samples were determined by using an elemental analyzer (Elementar, Vario EL Series III, Germany). The protein content (%) was calculated by multiplying the nitrogen content (%) by 6.25 [4].

2.5. Sugar analysis

Monosaccharide composition of the samples (CSG, F1, F2 and F3) were determined after acid hydrolysis (2 M trifluoroacetic acid, 2 h, 121 °C) and by use of separate recovery factors for each carbohydrate [30]. The monosaccharide analysis was done using an ICS3000 ion chromatography system containing a GS50 gradient pump, an ED50 electrochemical detector and an AS50 chromatograph coupled to an AS50 autosampler (Dionex Corp., Sunnyvale, CA). Separations were performed using a CarboPacTM PA20 (3 mm × 150 mm) analytical column (Dionex Corp., Sunnyvale, CA) and an elution program as described previously by Balaghi et al. [31]. The monosaccharides were separated with adequate resolution using a two-eluent system consisting of deionised water and 500 mM aqueous NaOH. Neutral monosaccharides were eluted isocratically with 2.5 mM NaOH for 20 min followed by a second isocratic elution at high NaOH (500 mM) for 10 min to elute any acidic monosaccharides present. This high concentration of NaOH simultaneously washed the column. Before each injection (10 μL), a column re-equilibration program was run for 5 min with 100 mM NaOH followed by 5 min with 2.5 mM NaOH. A mixture of D-(+) fucose, α-L-rhamnose, L-(+) arabinose, D-(+) galactose, D-(+) glucose, D-(+) xylose, D-(+) mannose, D-(+) galacturonic acid, D-(+) glucuronic acid (concentration of each component 0.0010–0.0500 g/L) was used as an external standard. Sample concentration was $5\,\text{g}\,\text{L}^{-1}$.

2.6. Molecular weight measurement

The molecular weight (M_W) measurements were performed using Zetasizer Nano ZS (model: ZEN3600, Malvern Instruments, UK) at 25 °C. The instrument was calibrated using toluene with a known R_θ of $1.35 \times 10^{-5}\,\text{cm}^{-1}$ at 633 nm. Molecular weight was determined using the following equations [32].

$$\frac{Kc}{R_\theta} = \frac{1}{M_w} + 2A_2c \tag{1}$$

$$K = \left(\frac{2\pi^2}{\lambda_0^4 N_A}\right)(1 + \cos^2\theta)\left(\frac{dn}{dc}\right)^2 \tag{2}$$

$$R_\theta = R_{\theta,solution} - R_{\theta,solvent} \tag{3}$$

where K is a calibration constant obtained with toluene, c is concentration, λ_0 is the incident beam wavelength (633 nm), N_A is the Avogadro's number, θ is scattering angle (=90°), n is the refractive index of solvent, and dn/dc is the specific refractive index increment. A_2 (the second virial coefficient) and M_W (The weight-average molecular weight) could be obtained from the slope and the reciprocal of the intercept of Debye plot (Kc/R_θ vs. c), respectively.

2.7. FTIR analysis

FTIR spectra of the samples were recorded on a Perkin-Elmer spectrometer (MODEL: Spectrum one, USA) in a range from 4000

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