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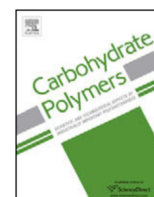

# Protein-free cress seed (*Lepidium sativum*) gum: Physicochemical characterization and rheological properties

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Protein-free cress seed gum (PFCSG) was obtained by precipitation of crude cress seed gum (CSG) with ethanol followed by treatment with protease. Molecular weight, moisture, ash and uronic acids content decreased after elimination of protein. Elimination of protein improved significantly rheological properties and thermal stability of cress seed gum. Mechanical spectra of the CSG and PFCSG were classified as weak gels and PFCSG showed stronger and more elastic network structure. The gum dispersions exhibited strong shear-thinning behavior which was described satisfactory by the Herschel-Bulkley and Moore models. Protein-free cress seed gum had higher apparent and intrinsic viscosities than the crude gum. CSG indicated lower hysteresis loop area, but degree of structural recovery of the samples showed no significant difference. The main decomposition of PFCSG started above 213 °C with two peaks (at 261.72 °C and 306.58 °C) and initial decomposition temperature of CSG was 190.21 °C with one peak at 258.28 °C. DSC results coincided with those observed by thermogravimetric analysis. Enzyme treatment lowered the surface activity of CSG.

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## 1. Introduction

Hydrocolloids from plants have an advantage over those of animal origin due to their friendly image towards consumers, and nowadays there is a great interest for natural hydrocolloids with low cost and proper functionality (Behrouzian, Razavi, & Karazhiyan, 2014; Dickinson, 2003; Vardhanabhuti & Ikeda, 2006). *Lepidium sativum* (Garden cress) seeds have been used in traditional medicine to treat asthma, hypertension, hepatotoxicity, hyperglycemia, enuresis and fractures (Behrouzian et al., 2014; Ghante, Badole, & Bodhankar, 2011; Gokavi, Malleshi, & Guo, 2004), and contain a large amount of mucilageinous substances, which are a good source of hydrocolloids with high molecular weight (Karazhiyan et al., 2009; Naji & Razavi, 2014; Razavi, Farhoosh, & Bostan, 2007). Extraction optimization, some physicochemical, rheological and functional properties of cress seed gum (CSG) have been recently studied (Behrouzian, Razavi, & Karazhiyan, 2013; Behrouzian et al., 2014; Karazhiyan et al., 2009; Karazhiyan, Razavi, & Phillips, 2011; Karazhiyan, Razavi, Phillips, Fang et al., 2011; Naji & Razavi, 2014; Naji, Razavi, & Karazhiyan, 2012; Naji, Razavi, Karazhiyan, & Koocheki, 2012; Naji, Razavi, & Karazhiyan, 2013; Razavi, Bostan, Niknia, & Razmkhah, 2011). The majority of the CSG was carbohydrate (77%) and it showed a weight-average molecular weight of 540 kDa and radius of gyration 75 nm (Karazhiyan et al., 2009). CSG behaved as a typical polyelectrolyte owing to the presence of carboxyl groups, with the intrinsic viscosity decreasing with increasing NaCl concentration (Karazhiyan, Razavi, Phillips, Fang et al., 2011). Shear thinning behavior in steady shear measurements and a weak gel type behavior in dynamic tests were observed for 1% solution of cress seed gum, and its rheological properties was depended on many factors such as shear rate, temperature, time, pH, biopolymer concentration, concentration and type of salts and sugars (Behrouzian et al., 2013; Karazhiyan et al., 2009; Naji & Razavi, 2014).

Gum extraction methods usually result in solutions containing a mixture of components (i.e., other polysaccharides as well as non-carbohydrate material), which have to be further purified to isolate the specific polysaccharide of interest (Cui, 2005). Also, the

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presence of protein in polysaccharides can induce an inflammatory response in tissues and this response may inhibit the pharmacological use of materials based on polysaccharides (Cunha, Paula, & Feitosa, 2007). The purification of polysaccharides removes unacceptable flavors, impurities and endogenous enzymes of the crude gums and the purified gums give clearer and more stable solutions. There are several methods for the purification of crude gums and precipitation with ethanol was largely used (Bouzouita et al., 2007; da Silva & Gonçalves, 1990). Non-carbohydrate materials, such as proteins, are often removed by digestion with proteolytic enzymes, and low molecular weight soluble fragments generated during hydrolysis can be removed by dialysis, or the remaining carbohydrate polymers may be precipitated with ethanol (Cui, 2005).

Cunha et al. (2007) reported that crude and treated guar gum with porcine pancreatin enzyme showed different physicochemical and rheological properties. Youssef, Wang, Cui, and Barbut (2009) also indicated similar results for crude and protein-free fenugreek gums. Amid and Mirhosseini (2012) showed that purification methods significantly affected protein content and monosaccharide composition of durian seed gum, thus influenced rheological properties of the purified gum. Different surface activities were observed for protein-free fraction of basil seed gum and the crude gum by Naji-Tabasi, Razavi, Mohebbi, and Malaekeh-Nikouei (2016).

With respect to the impressive effect of elimination of impurities and protein fraction on gum properties, different physicochemical and rheological characteristics are expected for protein-free CSG (emphasizing the polysaccharide fraction characteristics). To the best of our knowledge, there is no published report investigating protein-free cress seed gum. Therefore, the objective of this study was to evaluate the physicochemical (included: elemental analysis, sugar composition, uronic acids content, functional groups (FT-IR), molecular weight, thermogravimetric analysis, differential scanning calorimetry and surface tension) and rheological properties (included: steady shear and dynamic rheology, thixotropy and intrinsic viscosity) of protein-free cress seed gum in comparison with crude cress seed gum.

## 2. Materials and methods

### 2.1. Materials

Seeds of *Lepidium sativum* were procured from a local market in Tehran, Iran, and were cleaned manually to remove all foreign matters and broken seeds. Protease from Streptomyces griseus was obtained from Sigma-Aldrich Co. (P5147). Ethanol (96%) was purchased from Noor Zakariya Razi Company (Isfahan, Iran), and the other chemicals used in this study were of analytical reagent grade (Merck Company, Darmstadt, Germany).

### 2.2. Cress seed gum extraction

Aqueous *L. sativum* seed gum was extracted from whole seeds based on the method as described by Karazhiyan, Razavi, and Phillips (2011) using distilled water (water to seed ratio of 30:1, pH 7, soaking period: 15 min) at room temperature. The seed-water slurry was slowly mixed throughout the soaking period (15 min) and separation of the gum from the swollen seeds was achieved by passing the seeds through an extractor (Pars Khazar 700P, Rasht, Iran) equipped with a rotating plate that scraped the mucilage layer on the seed surface. Then, the extracted gum was dried at room temperature and the dried gum was ground and denoted as crude cress seed gum (CSG).

### 2.3. Preparation of protein-free cress seed gum

It was carried out according to the procedure described by Brummer, Cui, and Wang (2003), with some modifications. After extraction, three volumes of 96% ethyl alcohol were added to one volume of the extracted gum and left for 2 h at room temperature and the collected precipitate was dried (at room temperature) and ground. A 0.2% (w/v) solution of the dried precipitate gum was prepared by dispersing the gum in distilled water and stirring (500 rpm) with a magnetic stirrer for about 2 h at ambient temperature until full dissolution and then it was incubated for 150 min at $37 \pm 0.1\,^{\circ}$C with protease enzyme. After that, the gum was precipitated with ethanol (three volumes) and left for 30 min at room temperature. After drying the collected precipitate (at room temperature), the dried gum was ground and termed as PFCSG.

### 2.4. Moisture, ash and CHNS analysis

The moisture content of samples was quantified from weight loss upon heating at 105 °C for 3 h in an oven.The ash content was estimated by heating in a muffle furnace at 550 °C for 3 h until constant weight was achieved (Karazhiyan et al., 2009). Carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) contents of CSG and PFCSG were determined by using an elemental analyzer (Elementar, Vario EL Series III, Germany). The protein content (%) of the samples was calculated by multiplying the nitrogen content (%) by 6.25 (Karazhiyan et al., 2009).

### 2.5. Molecular weight measurement

The $M_w$ measurements were carried out using Zetasizer Nano ZS (model: ZEN3600, Malvern Instruments, UK) at 25 °C. The instrument was calibrated using toluene with a known $R_\theta$ of $1.35 \times 10^{-5}\,\mathrm{cm}^{-1}$ at 633 nm. Molecular weight was determined using the following equations (Mohammad Amini & Razavi, 2012):

$$\frac{Kc}{R_\theta} = \frac{1}{M_w} + 2A_2c \qquad (1)$$

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

$$R_\theta = R_{\theta,\mathrm{solution}} - R_{\theta,\mathrm{solvent}} \qquad (3)$$

where K is a calibration constant obtained with toluene, c is concentration, $\lambda_0$ 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 ($Kc/R_\theta$ vs. c), respectively.

### 2.6. Sugar analysis

Monosaccharide composition of the crude and protein-free cress seed gums were determined after acid hydrolysis ($4\,\mathrm{g\,L^{-1}}$ substrate, 2 M trifluoroacetic acid, 2 h, 121 °C) and by use of separate recovery factors for each carbohydrate (Arnous & Meyer, 2008). The monosaccharide analysis was performed 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 carried out using a CarboPacTM PA20 (3 mm × 150 mm) analytical column (Dionex Corp., Sunnyvale, CA) and an elution program as described previously by Balaghi, Mohammadifar, Zargaraan, Ahmadi Gavlighi, and Mohammadi (2011).

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