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The physico-chemical properties of chia seed polysaccharide and its microgel dispersion rheology

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ABSTRACT

The polysaccharide gel layer surrounding hydrated chia seeds was extracted using water and isolated by ethanol precipitation. The freeze-dried sample consisted of ~95% non-starch polysaccharides (35% w/w neutral soluble fraction and 65% w/w negatively charged insoluble fraction). The soluble polysaccharide fraction has molar mass, root-mean square radius and intrinsic viscosity of $\sim 5 \times 10^5$ g/mol, 39 nm and 719 mL/g, respectively. The whole polysaccharide (included soluble and insoluble fractions) when dispersed in water showed presence of irregular shape, fibrous microgel particles with an average size ($D_{4,3}$) of ~700 μm. Rheological measurements indicated a 'weak' viscoelastic gel and strong shear dependent properties even at low concentration (0.05% w/w). The viscosity of the dispersion was fairly resistant to variations in temperatures (20–80 °C), pH (4–12), ionic strengths (0.01–0.5 M NaCl) and cation types ($MgCl_2$, $CaCl_2$, NaCl and KCl). The swollen microgel particles dispersed in soluble polysaccharide continuous phase provided complex and potentially useful rheological properties in food systems.

1. Introduction

Chia (*Silva hispanica* L.) is an annual herb of the mint family (*Labiatae* or *Lamiaceae*). It is native to southern Mexico and northern Guatemala (Nieman et al., 2009). Historical records suggested that chia seed was one of the staple foods for the Mayan and Aztec civilization (Chicco, D'Alessandro, Hein, Oliva, & Lombardo, 2009; Peiretti & Gai, 2009). The seeds were either roasted and mixed with water as a gruel or were ground and used as flour for baking (Álvarez-Chávez, Valdivia-López, Aburto-Juarez, & Tecante, 2008). The seeds were also used for medicinal purposes such as for treatment of boils and to relieve pain in the knee and injured feet (Dweck, 2000).

In the early 1990s, a project was launched to develop chia as a commercial crop to help diversify the economy in parts of Latin America. In 1996, the crop was commercially grown in northwestern Argentina (Coates & Ayerza, 1998). Today, chia is also grown in Mexico, Bolivia, Ecuador, Guatemala (Ixtaina, Nolasco, & Tomás, 2008) as well as Western Australia. In fact, the term "chia seed", commonly referred to by many authors, is actually not a seed but a fruitlet of chia (Capitani, Ixtaina, Nolasco, & Tomás, 2013).

The chemical composition of chia seed includes proteins (16–26%), fats (30–34%), carbohydrates (~42%, of which 34% is dietary fibre) and the presence of minerals and micronutrients (Muñoz, Cobos, Diaz, & Aguilera, 2013). The nutritional value of chia seed has drawn much attention in recent years because of its high oil content which consists of a high proportion of alpha-linolenic acid (60%) compared to other oil seeds (Ixtaina et al., 2008). In addition, the seed contains relatively high level of protein compared to the traditional cereals such as wheat, corn, rice, oats and barley (Ayerza & Coates, 2009; Coates & Ayerza, 1996). Another dietary benefit of chia seed is its high fibre content, of which ~6% is the soluble fibre fraction (Coates, 2011; Reyes-Caudillo, Tecante, & Valdivia-López, 2008).

When chia seed are soaked in water, the outer layer of the seeds swells into a firm gel layer which is tightly bound to the seeds (Lin, Daniel, & Whistler, 1994). The appearance of the gel layer surrounding chia seeds resembles those observed in basil seeds, although basil seeds are slightly larger in size (Hosseini-Parvar, Matia-Merino, Goh, Razavi, & Mortazavi, 2010). The morphology and physical appearance of chia seed have been thoroughly described (Capitani et al., 2013; Muñoz, Cobos, Diaz, & Aguilera, 2012). Chia seed polysaccharide is a tetrasaccharide which is made up of β-D-xylopyranosyl, α-Dglucopyranosyl, and 4-*O*-methyl-α-D-glucopyranosyluronic acid presented schematically as:

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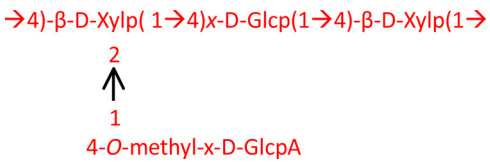

(Lin et al., 1994).

Due to the increasing demand for creating new structures in food products, biopolymers such as polysaccharides are being studied for their contributions to provide unique physical functional properties. Recently, the soluble fraction of chia seed mucilage has been studied in terms of the rheological properties based on different isolation methods (Capitani et al., 2015; Timilsena, Adhikari, Kasapis, & Adhikari, 2015). The authors showed the mucilage possesses pseudoplastic and weak gel characteristics. It is to be noted that the characterization of the mucilage was solely based on the soluble fraction. In our study, we focused largely on the rheological characteristics of the gel layer stripped off from the hydrated chia seeds. To the best of our knowledge, the unique rheological properties of microgel dispersions from chia seeds have not been reported before. Most of the studies reported only on the soluble polysaccharide fraction. In addition, this study characterized to a greater depth the molecular parameters of the soluble polymer fraction (after separating it from the microgel particles) using viscometric and light scattering methods. Understanding the physical functionalities of the entire polysaccharide gel (rather than just the soluble fraction) is necessary when chia seeds or chia flour are added to food products as stabilizers, thickeners or fat replacers, etc.

2. Materials and methods

2.1. Isolation and extraction of chia seed polysaccharide

The chia seeds (consisting of largely greyish white seeds) used in this study were cultivated in Australia. Chia seeds were a gift from Nuchia Foods Corporation, Florida, USA.

In order to hydrate the polysaccharide layer, the seeds (125 g) were dispersed in ultrapure water at a seed to water ratio of 1:20 (w/w) at ~25 °C in a 5 L beaker and gently stirred using an overhead stirrer (IKA, Eurostar Power control-visc, 50 mm propeller stirrer) at 500 rpm for 4 h.

The moderately viscous mixture of seeds and water was centrifuged at 10,000*g* for 30 min at 20 °C (AvantiJ centrifuge and J-LITE JLA-10.500 Rotor, Beckman Coulter, USA). The supernatant which consisted of two layers (see Fig. 1a) was recovered and the pellet which consisted of the seeds was discarded. It was noted that the gum layer surrounding the seeds was not completely removed. As for the supernatant, one volume was mixed with three volumes chilled 99% ethanol (VWR International, Singapore) and stored at 4 °C. The ethanol mixture was centrifuged (5810R centrifuge with A-4-62 rotor, Eppendorf, Hamburg, Germany) at 3150*g* and 20 °C for 15 min. The supernatant was discarded but the pellet was redispersed in approximately ten volumes of ultrapure water and centrifuged again (3150*g*, 20 °C for 15 min) to remove extraneous materials from the seeds. Again, one volume of the supernatant was mixed with three volumes of chilled 99% ethanol and stored overnight at 4 °C. The second ethanol precipitated mixture was centrifuged as described above to obtain the pellet. The pellet was rehydrated in ultrapure water and freeze-dried (Lyophilization Systems Inc., USA) before storing in a desiccator until further analysis. The isolated material is denoted as freeze-dried chia polysaccharide dispersion. A preliminary observation of the freeze-dried dispersion hydrated in water revealed the presence of irregular-shaped gel particles, denoted here as microgel particles (Fig. 1b).

2.2. Microstructure of chia seed polysaccharide microgel particles

For visualization under scanning electron microscope, 0.2% w/w freeze-dried polysaccharide dispersion was hydrated in ultrapure water overnight. The following day, samples were fixed in modified Karnovsky's fixative (3% glutaraldehyde, 2% formaldehyde in 0.1 M phosphate buffer, pH 7.2) for at least 8 h. After rinsing three times in phosphate buffer, the samples were dehydrated in a graded series of ethanol (25%, 50%, 75%, 95%, and 100%) for 15 min each and a final 100% for 1 h. Then, the samples were critical point dried using liquid CO_2 as the transition fluid (Polaron E3000 series II critical point drying apparatus). Once dried, samples were mounted on an aluminum stub, sputter coated with approximately 100 nm of gold (BAL-TEC SCD 005 sputter coater) and viewed in a FEI Quanta 200 scanning electron microscope (FEI Company, USA) at an accelerating voltage of 20 kV.

2.3. Particle size determination of chia seed polysaccharide microgel particles

The analysis of particle size for chia seed polysaccharide microgel dispersions was carried out using a static laser light scattering analyzer (Malvern Mastersizer MS2000; Malvern Instruments, Worcestershire, U.K.). Refractive indices of 1.333 for chia seed polysaccharide microgel dispersion and 1.330 for deionised water were used to determine the particle sizes of the dispersion. Prior to the size measurements, freeze-dried chia seed polysaccharide dispersion (0.2% w/w) was hydrated in ultrapure water for 2 h. The particle sizes measured are reported as volume mean diameters, where n_i is the number of particles with diameter d_i.

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

2.4. Determination of yield and total carbohydrate content of the extracted polysaccharide

The yield of the extracted polysaccharide dispersion was obtained by taking the weight of freeze-dried sample divided by the weight of dry seeds and expressed as% w/w yield.

Total carbohydrate was determined using the phenol sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). 1 mL of 5% w/v phenol (Sigma Aldrich, USA) was added to each diluted sample (1 mL), and mixed vigorously using a vortex mixer for 15 s before 5 mL of concentrated sulphuric acid (BDH, USA) was added. The samples were left to stand for 30 min and thoroughly mixed (using a vortex) again before absorbance readings at 480 nm were taken (UV-1800 Spectrophotometer, Shimadzu Scientific Instruments, Japan). A standard curve was obtained using D-(+) glucose standard (Sigma Aldrich, USA) at 5, 15, 30, 60, 80 and 100 mg/L. The glucose standard was used to determine the amount of total carbohydrate in the sample. All analyses were carried out in duplicates and with two replicates.

2.5. Determination of non-starch polysaccharide (NSP) composition

Freeze-dried sample of chia seed polysaccharide dispersion was analyzed for their NSP composition by Englyst Laboratory (Englyst Carbohydrates Ltd., Southampton Science Park, UK). Determination of the neutral sugar composition and uronic acid was carried out using methods described by Englyst, Quigley, and Hudson (1994). All samples were carried out in two replicates, each in duplicates.