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Original article

In vitro digestion of whole chia seeds (*Salvia hispanica* L.): Nutrient bioaccessibility, structural and functional changes

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Abstract Dietary chia seed (*Salvia hispanica* L.) has significant health-related benefits due to its high nutrient contents. However, few studies reported the fate of whole seeds in the gastrointestinal tract. Herein, we explored the digestive characteristics in terms of hydrolysis of nutrients, structural and functional properties with a static *in vitro* digestion method. After gastrointestinal digestion, the digestibility of lipid and protein was 0.46% and 11.38%, respectively. The release rates of tryptophan, tyrosine and lysine were greater than 20%, whereas the glutamic acid and aspartic acid were less than 5%. The microscopic results (optical microscopy (OM), laser scanning confocal microscopy (LSCM) and scanning electron microscopy (SEM)) demonstrated that the seeds remained intact, and the mucilage adhered tightly to the seed coat during digestion. The water holding capacity and oil holding capacity of seeds accounted for 6.37 and 3.28 g/g after intestinal digestion, which were significantly lower than gastric digestion endpoints ($P < 0.05$). And there were no significant differences in Fourier transform infrared spectroscopy of G-Mucilage and I-Mucilage. In general, preprocessing before being consumed is necessary for chia seeds to take full advantage of rich polyunsaturated fatty acids and proteins.

Keywords chia seed, functional properties, *in vitro* digestion, microstructure, nutrients digestibility.

Introduction

Chia seed (*Salvia hispanica* L.) has gained increasing interest in food science since it was certified as a safe and novel food by FDA in 2005. The seed contains 30%–34% dietary fibre, 25%–40% oil and 18%–24% high-quality protein (Kulczynski *et al*., 2019; Melo *et al*., 2019). It is worth mentioning that α-linolenic acid accounts for 60%, which is higher than flaxseed (Ixtaina *et al*., 2011). Scientifically it was verified that chia seeds will not cause any anti-nutritional allergies and toxic effects on human health (Capitani *et al*., 2013). Based on these attractive nutritional profiles, the seeds have a large potential to be exploited.

The culinary uses of chia seeds were equally diverse and involved whole seeds, seed oil, seed mucilage and seed flour (S. Sivasankari, 2020). As a functional food, chia seeds not only can improve nutritional value but also can act as a thickening agent, hydrocolloid or substitute for egg, fat or gluten. It has been reported that chia seed as a fat or egg substitute in food products does not significantly affect the technological or physical properties (Knez Hrncic *et al*., 2019; Kulczynski *et al*., 2019; Melo *et al*., 2019). A recent study showed that intact chia seeds replaced wheat flour up to 300 g/kg could nutritionally enhance Chinese steamed bread without compromising the eating quality (Zhu & Chan, 2018). The addition of 10% defatted chia flour to cookies could improve the antioxidant quality and not affect the technological or sensorial properties (Lucini Mas *et al*., 2020). Interestingly, chia mucilage is comparable to pork back fat in satiating capacity, which was an effective fat replacer (Câmara *et al*., 2020). However, some important nutrients are easily lost when exposed to high temperature, mechanical crushing and other processing techniques, especially polyunsaturated fatty acids. Thus, the consumption of whole seeds has attained great popularity in recent years.

It is well known that the nutritional value of food not only depends on its composition but also its digestibility (Huang *et al*., 2018). Given the tiny size

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(2.01 ± 0.10 mm length, 1.24 ± 0.08 mm width, 0.83 ± 0.03 mm thickness) of the seeds (Capitani *et al*., 2013), they are rarely chewed when added to yogurt, juices and other drinks. Hence, the structural integrity of seeds could not be destroyed during oral digestion. Recent studies have performed that the cell walls could inhibit macronutrient digestion by acting as steric barriers (McClements, 2021). However, we have not encountered detailed descriptions about the microstructure of chia seeds after digestion, and no information is available about the structural and functional properties of chia seeds in the gastrointestinal tract.

The objective of this study was to investigate the fate of chia seeds in the gastrointestinal tract and to provide valuable information related to the consumption of chia seeds. Hence, we evaluated the digestibility of nutrients using a static *in vitro* digestion method. At the same time, microstructure changes of seeds during digestion were observed by optical microscopy (OM), laser scanning confocal microscopy (LSCM) and scanning electron microscopy (SEM). In addition, the structural and functional properties of chia seeds were also performed to further explore the characteristics of whole seeds in the gastrointestinal tract.

Materials and methods

Materials

Chia seeds were purchased from Benexia Naturkost De Mexico, and packed in hermetic plastic vessels at 4 °C. The enzymes for *in vitro* digestion: Pepsin (EC3.4.23.1, from porcine gastric mucosa) was purchased from Sigma-Aldrich, pancreatin (S10031, from porcine pancreas) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd and bile extract from porcine was purchased from Sinopharm Chemical Reagent Co., Ltd. Reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd.

Chemical composition analysis

Moisture, protein, lipid, dietary fibre and ash contents were determined according to AOAC method. Amino acid content was determined by the method of Sandoval-Oliveros & Paredes-Lopez (2013) using a reversed phase high-performance liquid chromatography. Chemical composition values are expressed as g/100 g dry weight (except for moisture content). All determinations were done in triplicate.

In vitro digestion

The whole chia seeds were subjected to the static *in vitro* digestion method proposed by Minekus *et al*. (2014) with slight modifications. The oral phase was skipped because the seeds were too small to be chewed when added to drinks. Table S1 is the digestion process with 1 g of chia seeds. At the endpoint of gastric and intestinal digestion, the samples were centrifuged at 4000 × ***g*** for 15 min for analytical determinations of digestibility. All the experiments were conducted in triplicate.

Morphology of chia seeds during digestion

To measure the evolution of the seeds during digestion, 6 samples were randomly selected at different time points (1 min, 5 min, 30 min, 60 min, 120 min, 360 min) and blotting paper was used to absorb extra water (Muñoz *et al*., 2012). Water solution was set as control at the same time. Then, we used a vernier caliper to measure the length (L), width (W) and thickness (T). The arithmetic mean diameter (A), geometric mean diameter (D) and the sphericity (ϕ) were calculated as follows (Tunde-Akintunde & Akintunde, 2004):

$$A = (L + W + T)/3. \quad (1)$$

$$D = (LWT)^{1/3}. \quad (2)$$

$$\emptyset = (LWT)^{1/3}/L. \quad (3)$$

Lipid and protein digestibility

The release of free fatty acids (FFA) was measured to quantify lipolysis extent using a free fatty acid colorimetric assay kit (Solarbio BC0595, China) as mentioned by Lamothe *et al*. (2012). And the absorbance was measured at 550 nm with a UV-visible spectrophotometer (Shimadzu UV-3600Plus, Japan). The result was expressed as lipid digestibility (%) (Lamothe *et al*., 2014). Proteolysis was determined by assessing the amino acids by high-performance liquid chromatography (Rayner, 1985). The supernatant was filtered through a 0.22 μm membrane and analysed with an amino acid analyser (Agilent 1100, USA).

Image acquisition and microscopy analysis

To visualise the effects of digestion on the structure of chia seeds, we employed OM, LSCM and SEM. Samples at the endpoint of digestion were collected and fixed with a tissue freezing medium (SAKURA Tissue-Tek® O.C.T. Compound, Order Number 4583) at −20 °C. Then the seeds were cut into slices with a freezing microtome (Leica RM2245, Germany).

The microstructure of chia seeds after digestion was examined using the OM (Olympus CX31, China) at a magnification of 4×. Then the seeds were stained with

Sudan to visualise the fat distribution. LSCM images were captured with Olympus FV3000 LSCM (Japan), according to the method proposed by Câmara *et al.* (2020).

For SEM, all dried samples (intact seed, seeds with mucilage removal, longitudinal section and mucilage) were sputter-coated with gold, and performed on the SEM (Hitachi SU8100, Japan) with an acceleration voltage of 15 kV.

Functional properties

Both WHC and OHC were measured according to the method of Segura-Campos *et al.* (2014). Briefly, 1 g seeds or 0.1 g dried mucilage was added to 20 mL of distilled water or corn oil (0.92 g/mL) for 1 min. The suspensions were then centrifuged at 1600 *g* for 30 min and the supernatant volume was measured.

The freeze-dried mucilage was separated from chia seeds according to Muñoz *et al.* (2012). Mucilage samples were ground using the KBr powders. Then the Nicolet Nexus FT-IR spectrometer (IS10, USA) was used for FT-IR.

The apparent viscosity of the digestion liquid at gastric and intestinal endpoints was measured at 37 °C by a dynamic shear rheometer (Discovery DHR-2, USA) equipped with a 60 mm and 4° cone-plate geometry. The apparent viscosity of the fluid was measured in the shear rate range of 0.01–300 s^{-1}.

Results and discussion

Proximal composition of chia seeds

As described in Table S2, dietary fibre, fat and protein are the major components of chia seeds. The content of dietary fibre is 35.9 g/100 g, and soluble dietary fibre accounts for 10.22%, which is higher than flaxseed and quinoa (NorlailyMohd Ali, 2012; Rahman Ullah *et al.*, 2016). The essential amino acids in chia seeds accounted for 36.56% of the total amino acids, which was higher than soybean and sunflower. Chia seeds are also rich in non-essential amino acids such as glutamate (196.4 g/kg raw protein), arginine (107.2 g/kg raw protein) and aspartic acid (89.8 g/kg raw protein). For details, see Table S3.

Morphological characterisation

When in contact with water, the mucilage appeared immediately which formed a 'protective layer' surrounding it within a short period. From Fig. 1, we could obtain that the length, width, thickness, arithmetic mean diameter, geometric mean diameter and the sphericity reached a maximum at 60 min, meaning that the seeds reached the maximum hydration state. Similar behaviour was observed in water. However, the mucilage formation in the digestive juice was less than that in water mainly due to the presence of ions and digestive enzymes in the system (Calvo-Lerma *et al.*, 2020).

Lipid and protein degradation during digestion

Generally, protein and oil constituents are more important in nutritional studies (Jiménez-Escrig *et al.*, 2010). Therefore, we assessed the fatty acids and amino acids content in the supernatant after digestion. The digestibility of lipid and protein was only 0.10% and 0.65% after gastric digestion. Next, entering intestinal digestion, the digestibility under the action of trypsin and bile salt accounted for 0.46% and 11.38%, respectively. Although pepsin and HCl play an important role in gastric digestion, the final digestion occurs in the small intestine, in which the short peptide fragments into single amino acids or di- and tri-peptides (Boye *et al.*, 2012). Besides, it has been reported that seed mucilage contained 31 g/kg of residual fat and 112 g/kg of protein, which explained protein has higher digestibility than fat (Capitani *et al.*, 2013).

After digestion, the arginine content was the highest, followed by leucine and lysine, which accounted for 32.90% of the total amino acids. In terms of release rates, the tryptophan, tyrosine and lysine were the highest, which were 29.32%, 26.73% and 24.83%, respectively (Fig. 2). However, the release rates of glutamic acid and aspartic acid were relatively low (4.63% and 4.84%, respectively). It has been reported that the C-terminal peptide bonds of arginine and lysine could be hydrolysed specifically by trypsin (Zielińska-Dawidziak *et al.*, 2019). Therefore, the digestive enzymes could only act at specific sites and the hydrolysates existed in the form of oligopeptides and polypeptides. Furthermore, the protein digestibility is also affected by the activity of trypsin inhibitors, fibre content, antinutritional compounds, and even the level of processing (Khattab *et al.*, 2009).

Microscopy analysis

The OM images presented that the bone cell layer was the thickest part of the seed coat (Fig. 3a and b). After staining, we could see cotyledons more clearly, and they were barely destroyed due to the formation of mucilage and the protection of seed coats. It has been reported that the cell wall of wheat, chickpea, pea, almond, mung bean, red kidney bean and sorghum could prevent the diffusion of digestive enzymes (Dhital *et al.*, 2016; Bhattarai *et al.*, 2018; Do *et al.*, 2019; Holland *et al.*, 2020). The LSCM images were shown in Fig. 3c and d. The internal structure consisted of continuous protein chains with larger fat globules, and a portion of proteins was aggregated at the internal

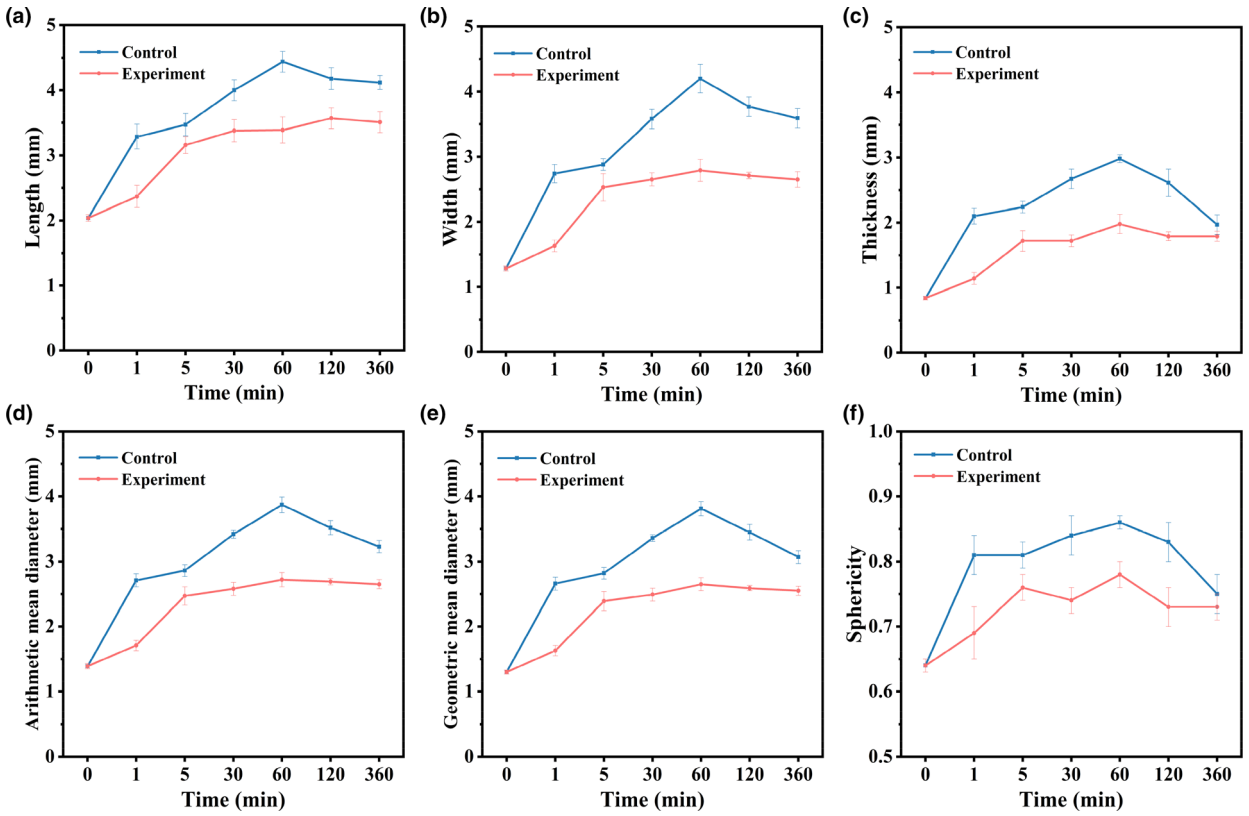

Figure 1 Physical properties of chia seeds during digestion (experiment) and soaking in water (control) for 360 min. (a) length; (b) width; (c) thickness; (d) arithmetic mean diameter; (e) geometric mean diameter; (f) sphericity.

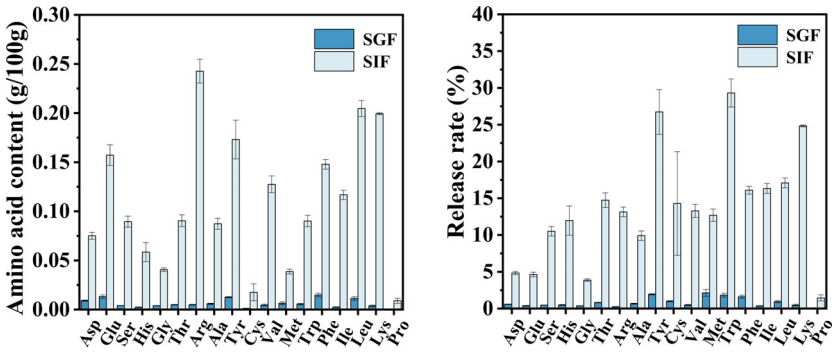

Figure 2 The content and release rate of amino acids after gastric and intestinal digestion.

margin. It was speculated that the imperfection of the internal structure is due to the moisture loss and shrinkage of internal components during freeze-drying.

The SEM images (Fig. 4) showed no significant effects of gastric and intestinal digestion on the structure of chia seeds. However, the network structure of dried mucilage after intestinal digestion was more compact mainly due to the differences between gastric and intestinal digestion conditions, especially the influence of pH value. Noteworthy, the mucilage adhered tightly to the seed coat all the time, and mechanical removal did not remove it completely from the seed coat (Fig. 4e–g, and m–o). In addition, the freeze-dried mucilage with mechanical removal was fragmentary (Fig. 4h and p).

Functional properties

From Fig. 5, we could obtain that dried mucilage powder exhibited a high WHC and OHC, which

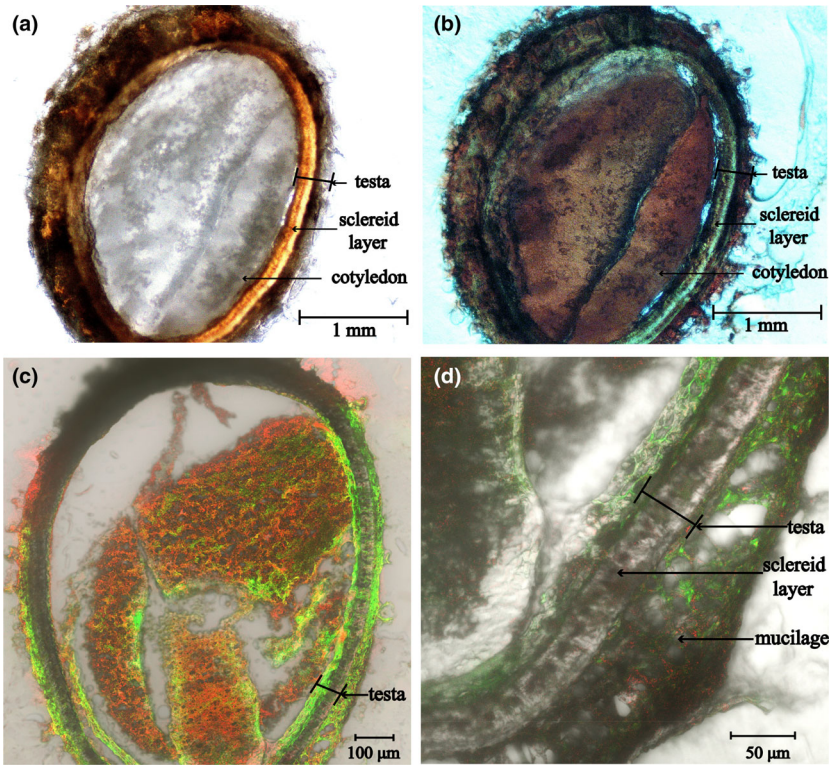

Figure 3 (a, b) OM images of longitudinal section of chia seeds before (a) and after (b) staining at the endpoint of digestion; (c, d) LSCM images of longitudinal section (20 μm) of chia seeds at the endpoint of digestion and partial magnification.

accounted for 26.55 and 8.93 g/g after intestinal digestion. When the mucilage was removed, there was no significant differences in the endpoints of digestion between the two phases, indicating that digestion has little effect on the seeds inside the mucilage. However, due to the effects of enzymes and pH values, the WHC and OHC of seeds decreased significantly ($P < 0.05$) after intestinal digestion (6.37 and 3.28 g/g, respectively). And the mucilage could promote satiety in the gastric phase (Salvador & Sanz, 2020).

FT-IR was carried out to analyse spectroscopic features of the dried mucilage (Fig. 6a). The peak at 2923 cm^{-1} indicates the presence of C–H and CH_2 in mucilage groups (Coates, 2006; Ullah *et al.*, 2017), and 1626 cm^{-1} is the characteristic absorption of C=C, which is the absorption peak of carbohydrate (Chylinska *et al.*, 2016). In addition, the insoluble dietary fibre was degraded into oligosaccharides during digestion, which could be seen from the absorption bands between 1140 and 1050 cm^{-1} (Ullah *et al.*, 2017). 1046 cm^{-1} is the hemicellulose spectrum, which is mainly characterised by the vibration of xylan molecules (Soni *et al.*, 2015). Moreover, mucilage showed a peak of α-pyranose at 835 cm^{-1}, while β structure at 885 cm^{-1}. Taken together, the functional groups are essentially the same in both G-Mucilage and I-Mucilage, indicating that most hydrophilic and other active groups (such as hydroxyl, carboxyl and aldehyde) in mucilage will not be changed during digestion.

Flow curves of chia seeds at digestion fluid revealed the behaviour of a non-Newtonian fluid with shear-thinning characteristics (Fig. 6b). Although there are no significant differences between the two phases, it is visible that the viscosity at the endpoint of gastric digestion was higher than that at the intestinal digestion. Generally, the mechanical strength of mucilage decreases when the water content is high (Xu *et al.*, 2019).

Conclusions

This study showed that both the tough seed coat and the formation of mucilage inhibited the interaction of digestive enzymes with seeds so that the amino acids and fatty acids were barely released throughout digestion. Thus, preprocessing, such as grinding and cooking is indeed essential to obtain high-quality proteins and rich polyunsaturated fatty acids. However, intake of whole seeds could provide benefits in terms of mucilage properties. Overall, these results were based on a simulated *in vitro* digestion model and further researches could be validated in animals whether the whole chia seed is beneficial to human health.

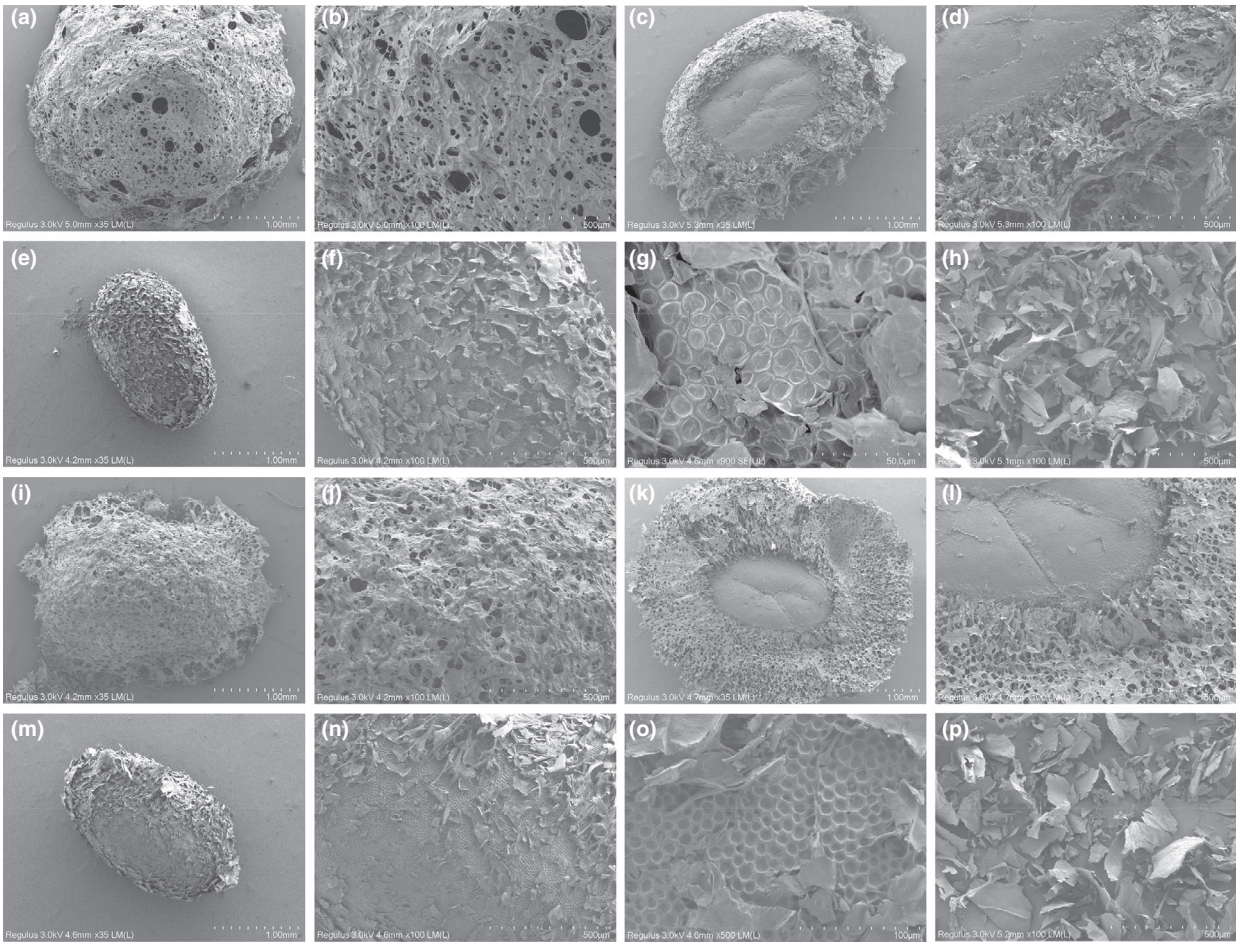

Figure 4 SEM images. (a–h) chia seeds digested by the stomach; (i–p) chia seeds digested by the small intestine; (a, i) the intact seed; (c, k) longitudinal section; (b, j, d, l) partial magnification; (e, m) chia seed removed mucilage; (f, n) partial magnificent; (g, o) seed surface; (h, p) freeze-dried crude mucilage.

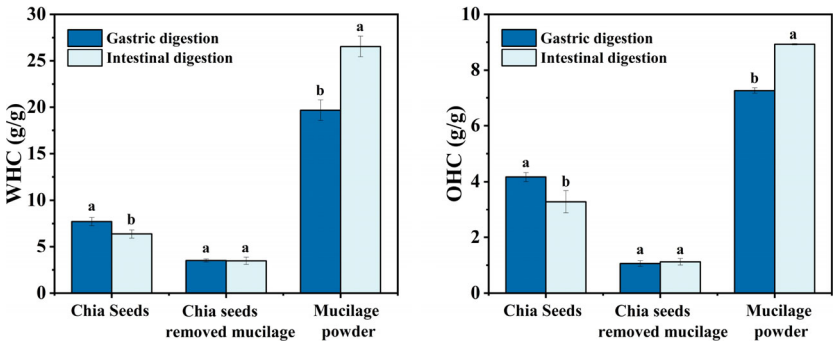

Figure 5 WHC and OHC of samples after gastric and intestinal digestion.

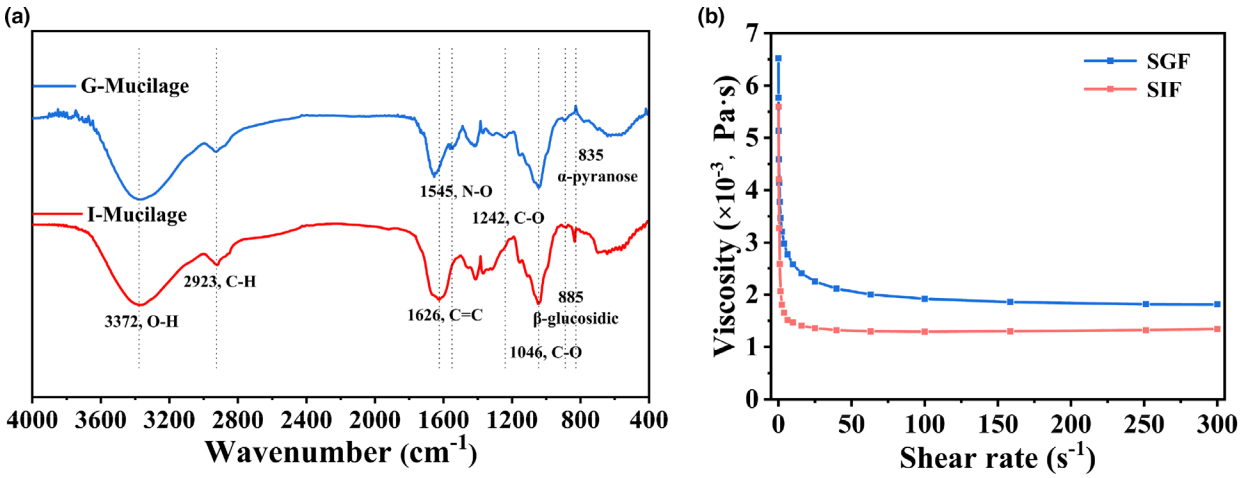

Figure 6 (a) FTIR absorbance spectra of dried mucilage after gastric and intestinal digestion; (b) apparent viscosity of gastric and intestinal fluid.

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Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Author contribution

Jieyu Han: Data curation (lead); Investigation (lead); Methodology (lead); Writing – original draft (lead). **Qiufang Zhang:** Funding acquisition (lead); Supervision (lead); Writing – review & editing (equal). **Wentao Luo:** Writing – review & editing (equal). **Ziyi Wang:** Supervision (equal). **Yuehong Pang:** Validation (equal); Writing – review & editing (equal). **Xiaofang Shen:** Project administration (equal); Writing – review & editing (equal).

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15698.

Data availability statement

Research data are not shared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 *In vitro* digestion with 1 g of chia seeds
Table S2 Proximal composition of chia seeds
Table S3 Amino acid composition of chia seeds