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Research Article

Characterization of Ethanol Precipitated Cress Seed and Flaxseed Mucilages Compared with Commercial Guar Gum

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Abstract

The yield percentage, chemical and physical properties of ethanol precipitated Cress Seed Mucilage (CSM) and flaxseed mucilage (FSM) compared with commercial Guar Gum (GG) were evaluated. Flaxseed or cress seed (100 g) and 900 mL distilled water were stirred for 5 h at a speed of 300 rpm min^{-1} in a 60°C water bath. The filtered extracted mucilage solution was precipitated with 2 V of 95% ethanol and the mucilage was separated by centrifugation at 3000×g for 10 min. The precipitated mucilage was then dried in a hot air oven at 60°C over night. The FSM yield (10.22% w/w) was higher than that CSM (7.29% w/w). Total proteins and ash contents in both FSM and CSM were higher than those in GG. There was no significant difference in Water Holding Capacity (WHC) of starch gel (2.0% starch) containing GG, FSM or CSM at the same concentration (0.1, 0.2 and 0.6%). However, at 0.4% concentration, the WHC of starch gel containing FSM was significantly lower than those containing CSM or GG. All polysaccharides solutions (1.0%) exhibited shear-thinning behavior, which was more pronounced in GG solution. The GG solution had the highest clarity compared with FSM and CSM solutions. However, the lightness and yellowish degrees were the highest, the redness was the lowest in both FSM and CSM solutions compared with GG solution (1.0%). The foaming capacity of FSM and CSM solutions were the highest compared with GG solution (1.0%). The GG solution had the highest foam stability, while the CSM had the lowest foam stability. The antioxidant activity of the CSM solution was the highest followed by FSM and GG solutions (1.0%).

Key words: Cress seed mucilage, flaxseed mucilage, commercial guar gum, physico-chemical properties, WHC

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Polysaccharides are composed of many monosaccharide residues that are joined one to the other by O-glycosidic linkages. The difference in the monosaccharide composition, linkage types, chain shapes and degree of polymerization gives physical properties of polysaccharides. Polysaccharides are used in food as thickeners, stabilizer, gelling agents, emulsifiers or suspension stability and prebiotic (Lucey, 2002; Nikoofar *et al*., 2013). Recently, they have attracted much attention for their function as dietary fiber, which lower cholesterol and blood pressure, thus preventing life style related diseases.

Mucilages are polysaccharides produced within the cell or without injury to the plant (Reddy and Manjunath, 2013). Mucilages are obtained from seeds or other plant parts e.g., in the epidermal cells of leaves, roots, barks and middle lamella (Evans, 2004). Also, they are obtained from marine algae and selected microorganisms (Rangari, 2002). Plant mucilages have been known since ancient times for their medical and pharmaceutical uses (Verma and Razdan, 2003; Murray *et al*., 2006). Mucilages are widely used in the industry as thickeners, water-retention agents, suspending agents, binders and film formers (Kapoor *et al*., 1992; Reid and Edwards, 1995).

Cress seeds (*Lepidium sativum*) contain large amounts of mucilaginous constituents when soaked in water and a transparent gel forms around the whole seed (Karazhiyan *et al*., 2009). Cress Seed Mucilage (CSM) contains L-arabinose, D-xylose, D-galactose, L-rhamnose, D-galacturonicacid and 4-O-methyl-D-glucuronic acid as major components with D-glucose and mannose as trace components. The CSM is widely used in many traditional medicinal preparations, such as cough syrups. It also has antihyperglycaemic properties, which help to control glucose level in diabetics (Behrouzian *et al*., 2014).

Flaxseed (*Linum usitatissimum* L.) is unique among oil seeds due to its rich in dairy fibre (soluble and insoluble), which reducing the diabetes, contrary heart diseases, colon cancer and incidence obesity (Franklin, 2009; Singer *et al*., 2011). Hijova *et al*. (2011) reported that the health benefit of flaxseed related to many components, such as lignans, α-linolenic acid and soluble dietary fibre or mucilage. Flaxseed mucilage (FSM) of consists of two polysaccharide components, acidic and neutral, at a ratio of 2:l (Fedeniuk and Billiardis, 1994). The neutral fraction contains L-arabinose, D-xylose and D-galactose. The acidic fraction contains L-rhamnose, L-fucose, L-galactose and D-galactouronic acid. Shan *et al*. (2000) reported that flaxseed gum had good formability, stability, emulsibility, salt resistance and has the same rheology as non-Newtonian flow.

Guar Gum (GG) is extracted from the endosperm of the seeds of the guar plant (*Cyamopsis tetragonolobus*), or cluster bean (Clarke, 2004; Bahramparvar and Tehrani, 2011). It's a yellow to light grey powder with a slight smell and taste of beans (Kilara and Chandan, 2008). Chemically, GG is a polysaccharide composed of the D-galactose and mannose in a molecular ratio of 1:1.6 (Stephen *et al*., 2006). The backbone is a linear chain of β 1, 4-linked mannose residues, to which galactose residues are 1, 6-linked at every second mannose, forming short side-branches (Sujitha *et al*., 2013). The GG is largely used in the powder form as an additive in food, pharmaceuticals, paper, textile and cosmetics industry. Industrial applications of GG are possible because of its ability to form hydrogen bonding with water molecules. Thus, it is chiefly used as thickener and stabilizer. It is also beneficial in the control of many health problems like diabetes, bowel movements, heart disease and colon cancer (Mudgil *et al*., 2014). Physico-chemical properties of flaxseeds and cress seeds mucilage's were widely studied (Lin *et al*., 2005; Chen *et al*., 2006; Karazhiyan *et al*., 2009, 2011b; Wang *et al*., 2011; Naji *et al*., 2013). Therefore, the aim of this study was the chemical and physical properties of ethanol precipitated cress seed and flaxseed mucilages compared with commercial guar gum.

MATERIALS AND METHODS

Materials: Flaxseeds (*Linum usitatissimum*) and cress seeds (*Lepidium sativum*) were purchased from local market (Cairo, Egypt) with moisture content of 6.64 and 6.84%, respectively. Commercial guar gum powder was purchased from Sigma (Sigma Aldrich Co., St., Louis, MO, USA). Corn starch was obtained from National Co. for Maize Products, 10th of Ramadan City, Egypt.

Flaxseed or cress seed mucilage extraction: Flaxseed or cress seed (100 g) were washed in water for 1 min to remove the surface dust and then mixed with 900 mL distilled water. The seeds and water were then stirred for 5 h at a speed of 300 rpm min^{-1} in a 60°C water bath, according to the method of Cui (2001). The extracted mucilage solution was filtered through 40-mesh screen and precipitated with 2 V of 95% ethanol. The mucilage was separated by centrifugation at 3000×g for 10 min. The precipitated mucilage was then dried in a hot air oven at 60°C overnight.

Mucilage yield: Mucilage yield was calculated according to the following equation as described by Kadivar (2001).

$$\text{Mucilage yield} = \frac{W_{rm}(1\text{-}M_{rm}/100)}{W_{os}(1\text{-}M_{os}/100)} \times 100$$

where, W_{rm} is weight of the recovered mucilage, M_{rm} is moisture content of the recovered mucilage (%), W_{os} is weight of the original sample and M_{os} is the moisture content of the original sample (%).

Composition of mucilage powder: Total solids, Total Nitrogen (TN) and ash contents of dried mucilage were determined according to AOAC (2007). The protein content was obtained by multiplying the percentage of TN by 6.25.

Preparation of polysaccharides solutions: The dried ethanol precipitated flaxseed mucilage (FSM) and Cress Seed Mucilage (CSM) and commercial Guar Gum (GG) were dissolved in distilled water by stirring the powder at 25°C for 30 min to make the 1.0% (w/w) polysaccharides solutions. The solutions were kept at 5±2°C overnight to achieve complete hydration.

Clarity: The FSM, CSM and GG solutions (1.0% w/w) were diluted with distilled water to obtained 0.25% (w/w) polysaccharides solutions. The absorbance (OD) of diluted polysaccharides solutions were measured at 500 nm using an UV-visible spectrophotometer (Schimadzu spectrophotometer 1201, Japan), according to the method of Che *et al.* (2009). The clarity of the polysaccharides were represented by the absorbance (OD) of their solutions. The distilled water was used as blank.

Color measurements: The color of FSM, CSM and GG solutions (1.0% w/w) were measured using a Spectro-colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE-Reston VA, USA) in the reflection mode. The color was expressed in terms of L, a and b; where, L is value represents darkness from black (0) to white (100), a is value represents color ranging from red (+) to green (-) and b is value represents yellow (+) to blue (-).

Foaming properties: Foaming capacity and stability of FSM, CSM and GG solutions (1.0% w/w) were determined using the methods described by Wang *et al.* (2010). Solution of 100 mL was whipped at 15,000 rpm for 2 min with a high-speed homogenizer (T25, IKA Laboratory Technology, Staufen, Germany), the total volume was measured every minute after whipping. Foaming capacity was expressed as foam expansion immediately after whipping, while foaming stability was expressed as the time required for the foam volume to decrease to its half at 25±2°C.

Water holding capacity: Starch-polysaccharides suspension (2.0% starch and 0.1, 0.2, 0.4 and 0.6% polysaccharides) were put into tubes with coated screw-caps and heated in a boiling water bath for 10 min. A starch-water mixture, similarly heated, served as a control. After cooling to 5±2°C for 24 h, the Water Holding Capacity (WHC) was measured as the volume of water (mL) adsorbed by 1 g starch after centrifugation of the gel at 5000×g at room temperature for 20 min (Chen *et al.*, 2006).

Apparent viscosity: Apparent viscosity of FSM, CSM and GG solutions (1.0% w/w) were determined using a Brookfield Synchro-lectric viscometer (Model LVT; Brookfield Engineering Inc. Stoughton, MA). Readings were taken at the speed of 4-60 rpm using spindle-4 at 7±1°C for upward curve. Apparent viscosity was expressed as Pascal (Pa sec).

Antioxidant activity: The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging activity of FSM, CSM and GG solutions) were measured according to the method of Blois (1958). Each 0.3 mL of mucilage 1.0% solution was added to 0.1 mL of 1 M tris-HCl buffer (pH 7.9) and then mixed with 0.6 mL of 100 μM DPPH in methanol for 20 min under light protection at room temperature. After brief centrifugation at 12000×g for 10 min, the absorbance at 517 nm was measured. Deionized water was used as a blank. The scavenging activity of DPPH radicals (%) was calculated following the equation:

$$\text{Antioxidant activity} = \frac{A517_{blank}\text{-}A517_{sample}}{A517_{blank}} \times 100$$

Statistical analysis: Statistical analysis was performed using the GLM procedure with SAS Institute Inc. (2004) software. Analysis of variance (ANOVA) and Duncan's multiple comparison procedure were used to compare the means. A probability of $p \leq 0.05$ was used to establish statistical significance.

RESULTS AND DISCUSSION

Mucilage yield and composition: Yield and composition of mucilage extracted from flaxseed (FSM) and cress seeds (CSM) are shown in Table 1. The yield (w/w) of FSM was higher than that of CSM ($p<0.05$). The yield of FSM and CSM were 10.22 and 7.29%, respectively. The FSM yield was similar to that found earlier by Maherani *et al.* (2005) (10.3% mucilage). Cui *et al.* (1994) reported that yields of mucilage from flaxseeds ranged from 3.6-9.4%. The CSM yield was agreement

Table 1: Yield and composition of ethanol precipitated flaxseed and cress seed mucilage compared with commercial guar gum

	Dried polysaccharide		
Items	FSM	CSM	GG*
Yield (g/100 g seeds)	10.22±0.52[a]	7.29±0.59[b]	--
Composition (%)			
Moisture	3.76±0.30[a]	5.30±0.69[a]	≤12.0
Proteins	12.17±0.41[a]	13.97±0.95[a]	≤4.6
Ash	6.97±0.55[b]	9.78±0.75[a]	≤02.5

Mean±SE (n = 3) with the same letters in the same row are not significantly different at p≤0.05, FSM: Ethanol precipitated flaxseed mucilage, CSM: Ethanol precipitated cress seed mucilage, GG: Commercial guar gum and *: Composition as data of the supplier

Table 2: Clarity and color parameters of ethanol precipitated flaxseed and cress seed mucilage compared with commercial guar gum solutions

		Color parameters		
Solution types	Clarity (OD)	L	a	b
FSM	0.508±0.03[a]	13.91±0.72[a]	-0.30±0.06[b]	-1.84±0.11[b]
CSM	0.364±0.03[b]	12.87±0.41[a]	-0.20±0.01[b]	-1.60±0.08[b]
GG	0.109±0.02[c]	8.58±0.31[b]	0.05±0.04[a]	-0.62±0.09[a]

Mean±SE (n = 3) with the same letter in the same column are not significantly different at p≤0.05, FSM: Ethanol precipitated flaxseed mucilage, CSM: Ethanol precipitated cress seed mucilage, GG: Commercial guar gum, OD: Optical density, L: Darkness from black (0) to white (100), a: Color red (+) to green (-) and b: Color yellow (+) to blue (-)

with that found by Karazhiyan *et al*. (2011a) (6.46%), but it's lower than that reported by Varsha *et al*. (2010) (14%). The lower CSM yield could be attributed to mucilage in cress seed was separated hardly and residue of mucilage has been stayed on seeds. Behrouzian *et al*. (2014) reported that cress seeds contain large amounts of mucilaginous constituents (6.5-15.0%) when soaked in water and a transparent gel forms around the whole seed.

Moisture, proteins and ash contents (Table 1) were lower in FSM than in CSM, the difference was significant only in ash content ($p<0.05$). Similar observations in proteins content were found by Kadivar (2001) and Fekri *et al*. (2008). However, proteins and ash contents in both FSM and CSM were lower than in commercial GG and those found by Singer *et al*. (2011) in FSM extracted using boiling water. The ash content of FSM was 6.97% that is higher than found by Fekri *et al*. (2008) (5.8%). The variations in mucilage composition could be attributed to the condition, in which the analyses were done.

Clarity and color of polysaccharides solutions: The clarity and color degrees of the extracted FSM and CSM solutions compared with the GG solution were presented in Table 2. The clarity of the polysaccharides was represented by the absorbance (OD) of their solutions. The absorbance of GG solution was significantly lower than that of both FSM and CSM solutions ($p<0.05$). Also, the absorbance of CSM solution was significantly lower than that of FSM solution ($p<0.05$). In particular, the absorbance of the GG, CSM and FSM solutions were 0.109, 0.364 and 0.508 OD, respectively. These results reflected that the clarity of the polysaccharides solutions followed the trend: Commercial GG>extracted CSM>extracted FSM ($p<0.05$), clarity negatively correlated with OD. Wang *et al*. (2011) have reported that the absorbance of 1.0% un-homogenized FSM was ~0.7 OD. However, there are no references for the clarity of CSM and GG.

On the other hand, the lightness and yellowish degrees of both FSM and CSM solutions (1.0% w/w) were significantly higher ($p<0.05$) than those of GG solution (Table 2). The redness degree was lower in both FSM and CSM solutions than in GG solution ($p<0.05$). The higher lightness and lower redness indicating that extracts have a good quality in terms of color and potentially good applicability (Wang *et al*., 2010). However, there was no significant difference in the lightness, redness and yellowish degrees of FSM solution compared with CSM solution ($p>0.05$). Koochecki *et al*. (2009) suggested that the color developed by the extracted mucilage may be due to the passage of pigments or tannic substances from the tegument.

Foaming capacity and stability: Foaming capacity and stability of FSM and CSM solutions compared with the GG solution are given in Table 3. In general, the foaming capacity was influenced by type of polysaccharides. In particular, both FSM and CSM showed a significant increase in the foaming capacity after 1 and 2 min ($p<0.05$) as a compared to GG solution. The foaming capacity of 1.0% GG solution was 19.91% that is lower than that reported by Sciarini *et al*. (2009) in 0.5% GG solution (27.0%). The difference could be attributed to the variation in GG concentration, hence the difference in viscosity. The foaming capacity in FSM was higher than that in CSM solution, the difference was not significant ($p>0.05$). The foaming capacity of FSM solutions in this study (38.85%) was higher than that found by Wang *et al*. (2010) in ethanol precipitation of FSM (~28%). However, the foaming capacity of 1.0% CSM was similar that measured at 25°C by Naji *et al*. (2013). Also, as shown in Table 3, the CSM solution had the lowest foam stability ($p<0.05$), while GG solution had the highest foam stability. Adapa *et al*. (2000) reported that high viscous systems do not favor foaming capacity but do favor foam stability. However, there was no difference in foam stability formed by FSM and GG solutions ($p>0.05$). The foam stability of FSM solutions (30.41 min) was higher than that found by Wang *et al*. (2010) in ethanol precipitation of FSM (~14 min).

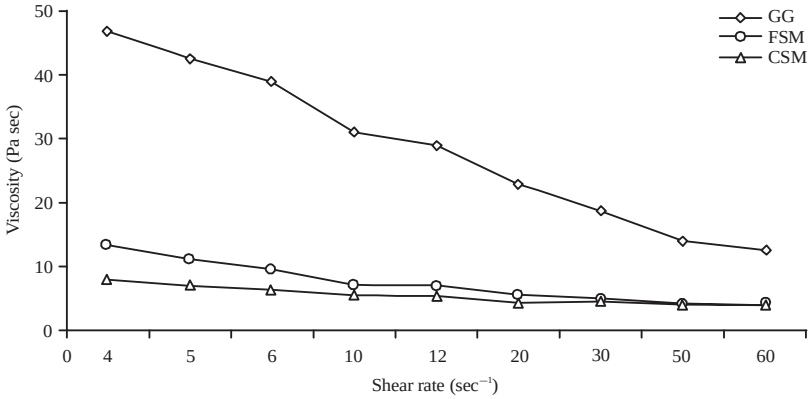

Fig. 1: Viscosity of flaxseed mucilage (FSM), Cress Seed Mucilage (CSM) compared with commercial Guar Gum (GG) solutions

Table 3: Foaming capacity and stability of ethanol precipitated flaxseed and cress seed mucilage compared with commercial guar gum solutions

	Foaming capacity (mL/100 mL)		
Solution types	1 min	2 min	Faming stability (min)
FSM	28.96±3.61^{a}	38.85±2.42^{a}	30.41±0.35^{a}
CSM	25.28±0.83^{a}	35.46±2.12^{a}	2.50±0.50^{b}
GG	9.07±2.53^{b}	19.91±1.26^{b}	32.50±2.50^{a}

Mean±SE (n = 3) with the same letter in the same column are not significantly different at p≤0.05, FSM: Ethanol precipitated flaxseed mucilage, CSM: Ethanol precipitated cress seed mucilage and GG: Commercial guar gum

Table 4: Effect of polysaccharide types and concentrations on the water holding capacity of starch solution

Solution types	Polysaccharides concentration (%)	Water holding capacity (mL g^{-1} starch)
Starch (2.0% w/v)	0.0	12.18±0.31^{e}
Starch with FSM	0.1	14.45±2.16^{de}
	0.2	22.48±1.44^{c}
	0.4	37.12±2.98^{b}
	0.6	44.78±2.88^{a}
Starch with CSM	0.1	13.96±0.24^{de}
	0.2	23.15±1.23^{c}
	0.4	42.43±1.51^{a}
	0.6	42.39±0.95^{ab}
Starch with GG	0.1	16.38±1.01^{de}
	0.2	21.20±1.04^{c}
	0.4	43.45±1.05^{a}
	0.6	44.80±1.27^{a}

Mean±SE (n = 3) with the same letter in the same column are not significantly different at p≤0.05, FSM: Ethanol precipitated flaxseed mucilage, CSM: Ethanol precipitated cress seed mucilage and GG: Commercial guar gum

Water holding capacity: The effect of polysaccharide types and concentrations on the Water Holding Capacity (WHC) of starch-polysaccharides solutions are shown in Table 4. In general, starch control (dissolved in water) showed a lower WHC than that dissolved in polysaccharides solutions. The lower WHC in starch gel is due to increased molecular association between starch chains at reduced temperature, excluding water from the gel structure (Liu *et al*., 1999). The WHC of the starch gel containing GG, FSM or CSM was increased along with the increase of the polysaccharides concentrations. A similar observation was found by Liu *et al*. (2006) in buckwheat and pea starches dissolved in yellow mustard mucilage solution. Shi and BeMiller (2002) stated that interactions between certain leached molecules, primarily amylose and certain gums were responsible for the viscosity increased. However, the WHC of starch gel containing 0.1% FSM, CSM or GG did not show a great difference compared with the starch control (p>0.05), which reflected that addition of ≤0.1% polysaccharide had no significant effect on WHC of starch solution. Thereafter, starch gel containing ≥0.2% FSM, CSM or GG showed a significant increase in the WHC (p<0.05) compared with both starch control and that containing 0.1% FSM, CSM or GG. However, the WHC of starch gel containing 0.4% FSM was significantly lower than those containing 0.4% CSM or GG (p<0.05). The WHC of starch gels containing 0.6% FSM, CSM or GG did not show significant difference among each other in Duncan test (p>0.05).

Apparent viscosity: Apparent viscosity of the FSM, CSM and GG solutions (1.0% w/w) is depicted in Fig. 1. The appearance viscosity of all the polysaccharide solutions was gradually decreased along with the increase of the shear rate, which reflected that all the polysaccharide solutions show shear-thinning behavior. Similar observations were found by Wang *et al*. (2011) in 1.0% (w/w) homogenized flaxseed gum solution and by Wu *et al*. (2009a) in yellow mustard mucilage solution. Juszczak *et al*. (2004) reported that commercial mustard is a pseudoplastic fluid exhibiting a yield stress, thixotropy and viscoelastic properties. However, the apparent viscosity of GG solution was higher than that of both FSM and CSM solutions (p<0.05). This is similar to the variation observed by Wang *et al*. (2011) between GG and

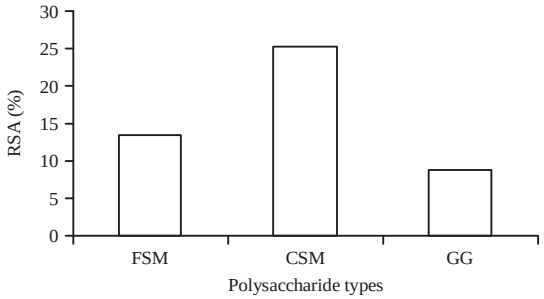

Fig. 2: Radical-scavenging activity (RSA) of flaxseed mucilage (FSM), Cress Seed Mucilage (CSM) compared with commercial Guar Gum (GG) solutions

FSM solutions. Some researchers observed intrinsic viscosity followed the trend: Guar gum>fenugreek gum>tara gum>locust bean gum (Wu *et al*., 2009b). Also, the apparent viscosity of FSM solution was higher than that of CSM, the difference was not significantly at shear rat ≥30 rpm. On the other hand, more pronounced shear thinning behavior was observed in GG solution compared with FSM and CSM solutions. The shear thinning behavior of polysaccharides solutions followed the trend: Guar gum>FSM>CSM solutions. Karazhiyan *et al*. (2011a) have reported the cress seed extract shows a Newtonian flow behavior at dilute concentrations below 0.1%. However, at higher concentrations there is a pronounced shear thinning behavior in steady shear measurements and viscoelastic and weak gel type behaviors in dynamic tests.

Antioxidant activity: Figure 2 shows the DPPH-scavenging activity of the FSM, CSM and GG solutions (1.0% w/w). The antioxidant activity of polysaccharides solutions was influenced by the source of polysaccharides. In particular, the antioxidant activity was significantly ($p<0.05$) higher in CSM and FSM solutions than in GG solution and significantly higher in CSM solution than in FSM solution. The antioxidant activity of the CSM, FSM and GG solutions were 25.43±0.6, 13.57±0.8 and 8.75±0.4, respectively. These results are agreement with Xie *et al*. (2010), they found that a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) exerted significant scavenging effects on DPPH radicals compared to the reference controls of Butylated Hydroxyl Toluene (BHT) and ascorbic acid. The variation in the antioxidant capacity between polysaccharides solution could be attributed to the variation in glucuronic acid March 18, 2016 content. King *et al*. (2000) reported that glucuronic acid is often linked to the substances such as drugs, pollutants, bilirubin and bile acids.

CONCLUSION

The functional properties of polysaccharides in the aqueous phase depend on the type and concentration of polysaccharides as well as type of other ingredients in aqueous phase. The hydration rate (viscosity) of GG was higher than both ethanol precipitated FSM and CSM; however, there was no significant difference in water holding capacity among GG, FSM and CSM when added to starch suspension. Therefore, the technical characteristics of mucilage in the food system that contains proteins, fats, carbohydrates and electrolytes may be required for further studies.

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