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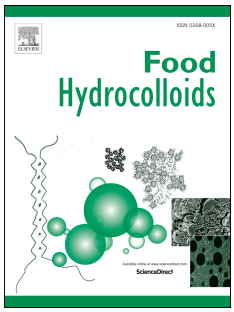

Composition, morphology and pasting properties of *Orchis anatolica* tuber gum

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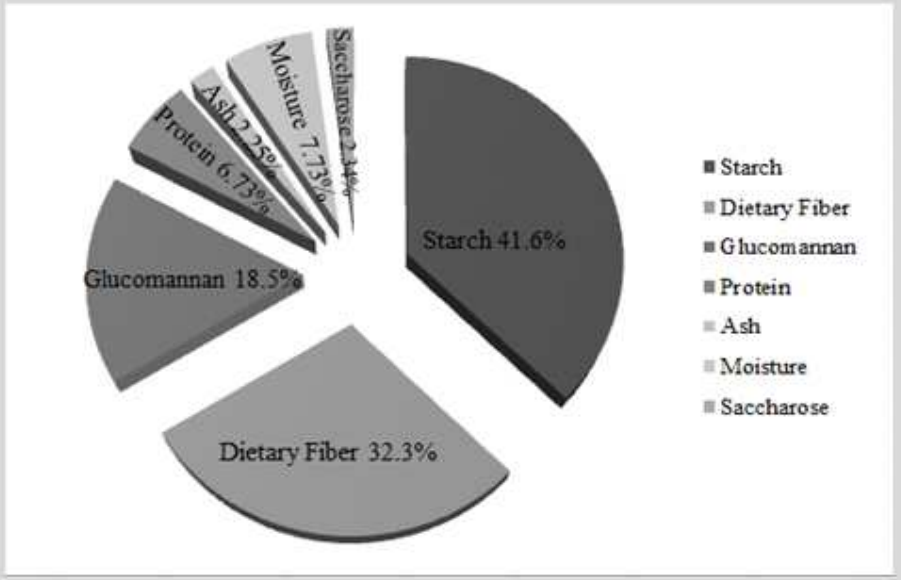
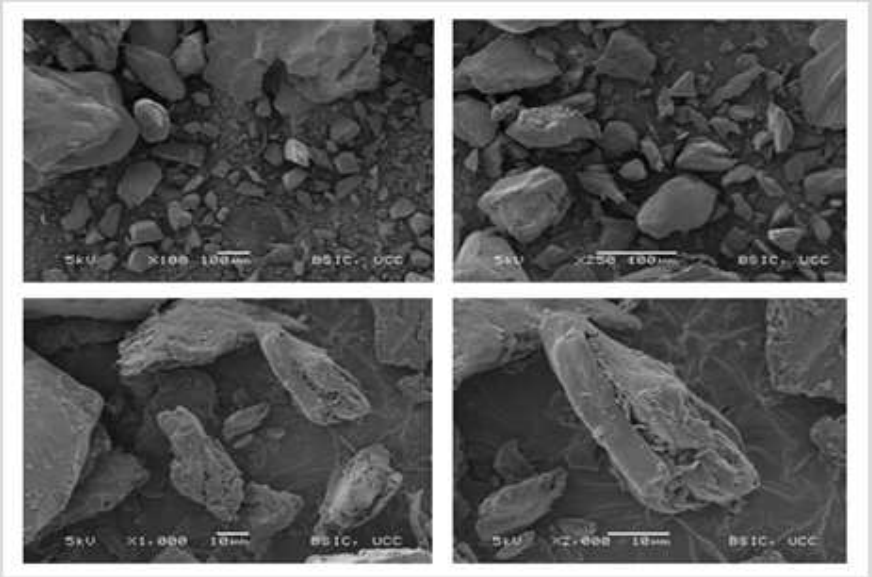
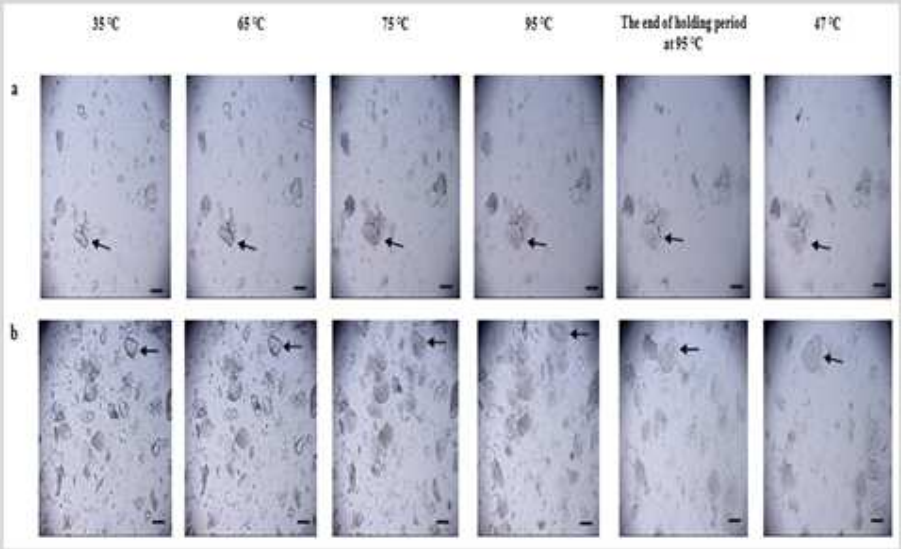


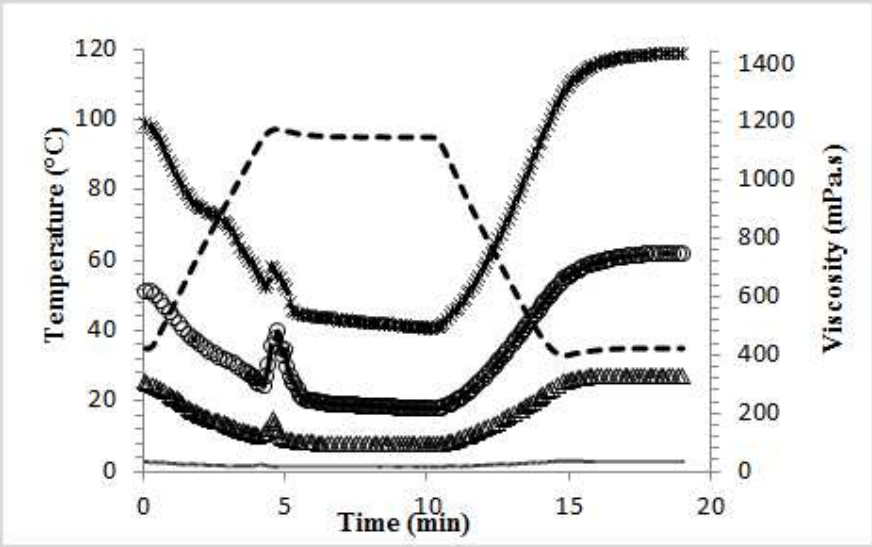
Chemical compounds of *Orchis anatolica* tuber gum



Scanning electron micrographs of native *Orchis anatolica* tuber gum's powder particles



Light microscopy micrographs of *Orchis anatolica* tuber gum All: (a) 2.0% *Oa*G; (b) 2.5% *Oa*G. Left-hand column at 35 °C, middle-columns from 55 to 95 °C on heating, right-hand column at 47 °C on cooling. Scale bar 200 μm.



Pasting curves for *Orchis anatolica* gum at concentrations of 0.5% (—), 1.5% (Δ), 2% (o) and 2.5% (*)

Composition, Morphology and Pasting Properties of *Orchis anatolica* Tuber Gum

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Abstract

Orchis anatolica (*O. anatolica*) tuber is commonly used in the production of Salep gum or *O. anatolica* tuber gum (*Oa*G) for use as a thickener, flavouring agent, gelling agent, film former and emulsifier in the food industry. The aim of this study was to investigate the chemical composition, physical, morphological and pasting properties of *Oa*G. Physical and morphological analyses, and pasting properties of *Oa*G were analysed using static light scattering, scanning electron microscopy, light microscopy and rotational rheometry, respectively. Volume-weighted mean particle diameter (D [4,3]) value of *Oa*G was 180±1.25 μm. *Oa*G was composed mainly of starch (41.6%), dietary fiber (32.3%) and glucomannan (18.5%). The powder of *Oa*G had irregular shaped particles with smooth surfaces and round edges. After pasting treatment, the initial and final viscosity values of the *Oa*G dispersions at a concentration of 0.5% *Oa*G were 33.7±0.24 and 34.3±0.45 mPa.s, whereas, the corresponding values at a concentration of 2.5% *Oa*G were 1193±92.0 and 1437±83.3 mPa.s, respectively. The glucomannan and dietary fiber components and their possible interactions with starch, in *Oa*G appear to have influenced the peak temperature and viscosity on pasting, due to limitation of the leaching of amylose and amylopectin from starch granules. Therefore, *O. anatolica* tuber gum, a complex biopolymer, can provide interesting and unique functionality to the food industry in the development of novel food structures.

Keywords: Gelatinisation, Gum, *Orchis anatolica,* Orchidaceae, Pasting, Salep.

1.0. Introduction

Orchis anatolica (*O. anatolica*) is one of the terrestrial species of the *Orchis* genus in the Orchidaceae family, which has a larger number of species than any other family of flowering plants. *O. anatolica* generally grows in pine or macchie forests from the Southern Aegean Islands to Iran, especially in parts of Anatolia. Salep gum or *Oa*G is obtained from dried tubers of terrestrial orchids. It is used either as a powder or in the form of a hot traditional beverage with pleasant taste since the time of the Ottoman Empire (Tamer, Karaman, & Copur, 2006; Hossain, 2011; Georgiadis et al., 2012). Salep gum has also been used in medicines and ice-creams (Lange, 1998).

Salep gum is a non-toxic, water-soluble and multi-component polysaccharide (e.g., glucomannan, starch) with a high molecular weight (Pourjavadi, Doulabi, Soleyman, Sharif, & Eghtesadi, 2012) compared with other polysaccharides, such as high-amylose starch (Salemis & Rinaudo, 1984), chitosan and alginate (Harding, Abdelhameed, & Morris, 2010). It contains starch, mucilage, sugar, nitrogenous substances, ash, particularly calcium, potassium, iron, chlorides and phosphates, and some trace levels of volatile oils (Hossain, 2011; Lalika et al., 2013). It is a good source of glucomannan that is considered a dietary fiber (Tekinsen & Guner, 2010; Nikjooy, Joo, & Jahanshahi, 2014). The composition of orchid tuber also varies considerably, depending on botanical origin, environmental and biological factors, such as the species, the development state of the perennial plant, the stage of flowering, the time of harvesting, season and the site composition (Tekinsen & Guner, 2010; Lalika et al., 2013).

Salep gum also confers many important functional properties such as thickening, stabilizing, flavouring and gelling properties in food formulations, as well as medical formulations. It is one

of the most thermally-stable hydrocolloids and produces hydrogels for biomedical applications due to its high capacity for water storage. In addition, Salep biopolymer modified by graft copolymerization have higher swelling rates, which makes it a good candidate for drug delivery systems (Bardajee, Hooshyar, & Kabiri, 2012; Pourjavadi et al., 2012). Therefore, Salep gum has attracted increasing research interest in the last decade, because of its unique functional properties and nutritional value in food and medical formulations (Alonso-Sande, Teijeiro-Osorio, Remunan-Lopez, & Alonso, 2009; Bardajee, Hooshyar, Asli, Shahidi, & Dianatnejad, 2013).

Starch, which abounds in nature as an energy source in tubers, roots, cereal grains and legumes, is widely used in the food industry. The starch content of plants is variable depending on botanical origin, environmental and biological factors, such as the species, site composition, harvesting etc. It consists primarily of two polysaccharides; amylose (AM) and amylopectin (AP). These polysaccharides are homopolymers of α-D-glucose, and occur together in compact assemblies declared as starch granules (Miles, Morris, & Ring, 1985; Kett et al., 2013). AM is basically a linear polymer, with the formation of uncharged coaxial double helices being promoted by its (1→4)-axial-equatorial linkage geometry. On the other hand, the α-D-glucose residues in AP are also extensively (1→4)-linked, but with branches through 1→6 linkages (Considine et al., 2011).

When starch granules are heated in excess water, they hydrate and swell, because hydrogen bonds in amorphous regions are disrupted, leading to absorption of water which acts as a plasticizer. Greater hydration and swelling occur in amorphous regions, pulling apart crystallites, with these regions ultimately undergoing hydration and melting (Considine et al., 2011). This process is known as gelatinisation and is characterised by changes in viscosity. The gelatinisation

process includes “pasting temperature” which is the temperature coinciding with the rapid increase in viscosity which ensues the onset of gelatinisation, and the “peak viscosity”, which is the maximum viscosity value during pasting (Ratnayake & Jackson, 2009; Kett et al., 2013). After the process of gelatinisation or pasting, the resultant viscoelastic mass called a ‘‘paste’’ consists of a continuous phase with a molecular dispersion containing dissolved starch polymer molecules forming a network, and discontinuous phase of swollen granules, granule ghosts and granule fragments, depending on the origin of starches (Atkin, Abeysekera, & Robards, 1998; Baldwin, 2001; Debet & Gidley, 2006; Kett et al., 2013).

Sezik (1967) and Tekinsen and Guner (2010) in previous works also researched the compositional properties of *Oa*G which would be expected to vary significantly due to biological and environmental factors. Moreover, there is not much information in literature about the chemical properties of *Oa*G. Nevertheless, the physical, morphology and specifically pasting properties of *Oa*G have not been determined to date yet. There are also no recorded physical, morphology and gelatinisation data available in literature about these properties of *Oa*G. Therefore, the objective in this study was to determine the chemical composition, physical, morphological and pasting properties of *Oa*G to better understand and predict its physicochemical properties to aid its use as a functional ingredient in the food industry.

2.0. Materials and Methods

2.1. Materials

O. anatolica, which is a delicate perennial plant with pink to violet flowers, grows mostly in lightly-shaded pine forests or mountains in several parts of Anatolia, such as in the Taurus Mountains (Fig. 1 a, b). *O. anatolica* is similar to *O. quadripunctata* but spike lax of *O. anatolica* has 5-10 large flowers with bracts. The colour of *O. anatolica*'s flowers is rose purple and/or seldom white, with purple dots and lines in centre of labellum. Sepal of *O. anatolica*, with spreading or ± reflexed ovate-obtuse, is 7-11 x 3.5-4 mm. Labellum ovate of *O. anatolica* is also nearly 10-15 mm with 3-lobed. Flowering time of *O. anatolica* is between March and May (Altundag et al. 2012).

In this study, the fully formed orchid tubers, at the end of the flowering stage, were harvested from several defined locations in the Taurus Mountains near Tatlicak, Konya (Turkey) between June 1st and July 7th of 2013. This harvesting period was selected because the seed vessels of *O. anatolica* would be fully formed at the end of flowering stage, and a randomized sampling technique was used for tuber harvesting. The orchid plants at the flowering stage were identified as being *O. anatolica* by the Herbarium Laboratory in the Biology Department at Selcuk University, Konya, Turkey, as described by Altundag et al. (2012). All chemicals, reagents and solvents used were of analytical grade and purchased from Sigma-Aldrich (Vale Road, Arklow, Wicklow, Ireland).

2.2. Powder preparation

The round-edged appearances of *O. anatolica* freshly harvested from Taurus Mountains are shown in Fig. 1 (c). After harvesting, *O. anatolica* terrestrial tubers were washed, followed by poaching in boiling water for 12 min to remove the bitterness of their fresh state, with their epidermis removed, after which they were dried in the shade for 7 d (Fig. 1 d). They were milled

114 at 600 rpm using a rotary mill (Break Mill SM 3, Brabender, Germany) until a fine powder was
115 finally obtained as described by Bulut-Solak and O'Mahony (2015).
116
117 *2.3. Preparation of dispersions*
118 Dispersions of *O. anatolica* tuber gum (*Oa*G) were prepared at concentrations of 0.5%, 1.5%,
119 2.0% and 2.5% w/v by dispersing finely-ground *Oa*G gum in deionised water, and stirred at low
120 speed using a magnetic stirrer for 2 h at 22°C until completely dispersed. All dispersions were
121 held for 18 h at 4°C to ensure complete hydration prior to assessment. Each dispersion was
122 removed from storage at 4°C and equilibrated at room temperature (22^{o}C) for 15 min before the
123 pasting treatment.
124
125 *2.4. Analytical determinations*
126 *2.4.1. Particle size distribution*
127 The particle size distribution of the milled powders (Section 2.2) was analysed using a Malvern
128 Mastersizer 3000 with Aero S dry dispersion unit (Malvern Instruments, Worcestershire, UK) as
129 described by Amagliani, O'Regan, Kelly and O'Mahony (2016).
130
131 *2.4.2. Chemical composition*
132 Moisture, ash, fat, protein and crude fiber contents of the samples were determined according to
133 the standard methods of the Association of Analytical Chemists (AOAC, 2010). The samples
134 were analysed for moisture and ash contents using Gravimetric methods, fat content using the
135 Soxhlet method, and protein content using the Kjeldahl method using a total nitrogen to protein

conversion factor of 6.25 (AOAC, 2010). Total dietary fibre content was determined using a commercial test kit from Megazyme International (K-TDRF-12/15, Bray, Ireland) based on AOAC Method 991.43 and AACC Method 32-07.01. Samples were enzymatically treated with heat-stable α-amylase, protease and amyloglucosidase, followed by a treatment with four volumes of 95% ethanol to precipitate the fibre and remove depolymerised protein and glucose from starch. The residue was filtered, washed, dried overnight and weighed (Megazyme International Ireland Limited, 2015). Total reducing sugars (glucose, fructose and saccharose) were analyzed with HPLC as described by Makila et al. (2014). pH values of the *Oa*G dispersions were measured at ambient temperature using a WTW digital pH meter equipped with a glass electrode (Hach, H-Series H260G Bench-top pH and ISE Meter, Canada).

2.4.3. Starch content

The samples were prepared according to the AOAC procedure specified for total starch (AOAC Method 996.11 and AACC Method 76-13.01) using the enzyme-based assay kit from Megazyme International (Catalogue Number: AMG/AA, 05/16, K-TSTA-50A/K-TSTA-100A). The absorbance of all samples and standard solutions was determined at 510 nm (Megazyme International Ireland Limited, 2016) and the starch content of the samples was estimated using the following formula:

Starch = $\Delta A \times (F/W) \times FV \times 0.9$ [g/100 g]

Δ**A** = Absorbance (reaction) read against the reagent blank

F = 100 (μg of D-glucose control) /absorbance value for 100 μg glucose (Conversion from absorbance to μg)

W= weight of the sample (100 mg)

159 **FV**= Final volume

160

161 *2.4.4. Glucomannan content*

162 The glucomannan content of *Oa*G was determined using the method of Chua et al. (2012). The
163 *Oa*G was processed with physical procedures, such as removing epidermis, in order to reduce the
164 levels of impurities prior to drying (Bulut-Solak and O'Mahony 2015). A sample of *Oa*G (200
165 mg wet basis) was weighed and added to formic acid-sodium hydroxide buffer (0.1 mol/L; 50
166 mL) and stirred magnetically at pH 3.13 for 4 h at 22 °C in order to separate the free sugars of the
167 *Oa*G sample. The mixture was diluted with deionised water to 100 mL and then centrifuged at
168 4500 g for 40 min at 25°C. One sample was prepared as a blank for the tuber glucomannan
169 dispersion extract (TGE). Sulphuric acid (3 mol/L; 2.5 mL) was added to a flask containing 5 mL
170 of supernatant of TGE. The resultant dispersions were stirred and hydrolyzed for 90 min in a
171 boiling water bath, and cooled quickly to 22°C, and sodium hydroxide (6 mol/L; 2.5 mL) was
172 added. The solution was made up to 25 mL with deionised water to form the TGE hydrolysate
173 (TGEH). One sample was prepared as a blank for the TGEH. 3,5-dinitro salicylic acid (3,5-DNS;
174 1.5 mL of a 1% w/w reagent) was added to TGE and TGEH solutions (2 mL) and heated in a
175 boiling water bath for 5 min before being cooled quickly to 22°C. Deionised water was added to
176 dilute the samples to a volume of 25 mL. The absorbances of the TGE and the TGEH samples
177 were measured at 550 nm with deionised water used as a blank (Chua et al. 2012). D-glucose and
178 D-mannose stock solutions (1 mg/mL; 0.4 mL, 0.8 mL, 1.2 mL, 1.6 mL, 2.0 mL) were
179 transferred into eleven 25 mL volumetric flasks, as well as 2 mL of deionised water as a blank.
180 Deionised water was added to a volume of 2 mL, followed by the addition of 3,5-DNS (1% w/w;
181 1.5 mL) to each volumetric flask. This mixture was heated for 5 min in a boiling water bath and

cooled to 22°C quickly before diluting to 25 mL with deionised water. The absorbance of the standard solutions was read at 550 nm (Chua et al. 2012). A D-mannose standard curve was constructed using the procedure as described for glucose, and used as a correction factor in Eq. 1. The glucomannan content of the samples was determined according to Eq. (1),

$$\text{Glucomannan (\%)} = \frac{5000\ f \times (5T - T_0)}{m} \quad \textbf{(Eq. 1)}$$

f is the correction factor obtained from the glucose and mannose standards

T is the glucose content (mg) in the tuber glucomannan dispersion extract hydrolysate (TGEH) obtained from the standard curve

T_0 is the glucose content (mg) in the tuber glucomannan dispersion extract (TGE) obtained from the standard curve

m is the mass of the *Oa*G sample (200 mg wet basis).

2.4.5. Pasting behaviour

The pasting properties of all *Oa*G dispersions were determined using an AR-G2 controlled-stress rheometer equipped with a starch pasting cell (AR-G2; TA Instruments Ltd., Waters LLC, Leatherhead, Surrey, UK); the internal diameter of the cell was 36.0 mm, the diameter of the rotor was 32.4 mm, and the gap between the two elements at the base of the geometry was 0.55 mm. All measurements of viscosity were carried out at a fixed shear rate of 16.8 s^{-1}. Analysis was conducted as described by Kett et al. (2013), whereby, after loading onto the rheometer, the sample was equilibrated at 35°C for 1 min, heated from 35 to 95°C over 4 min, held at 95°C for 6 min, cooled from 95 to 35°C within 4 min and held for 5 min at 35°C.

2.4.6. Microstructural analyses

2.4.6.1. Scanning electron microscopy

Scanning electron microscopy (SEM) analysis was carried out in the Biosciences Imaging Centre, Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland. *Oa*G powder was mounted on aluminium stubs using double-sided adhesive carbon tape, and sputter coated with a 5 nm layer of gold/palladium (Au:Pd = 80:20) using a Quorum Q150R ES Sputter Coating Unit (Quorum Technologies Ltd., Sussex, U.K.) to prevent surface charging by the electron beam. Subsequently, the *Oa*G sample was loaded into a sample tube and then examined using a JSM-5510 scanning electron microscope (JEOL Ltd, Tokyo, Japan), operated at an accelerating voltage of 5 kV.

2.4.6.2. Light microscopy

The *Oa*G dispersions were monitored using polarised light on an Olympus B×51 light microscope (LM) (Olympus Corporation, Tokyo, Japan) fitted with a heating stage (CO 102; Linkam Scientific Instruments, Tadworth, Surrey, UK). After placing on the microscope, all samples were equilibrated at 35°C for 2 min, heated from 35 to 95°C at 10^{o}C min^{-1} and tempered at 95°C over a period of 6 min before cooling from 95 to 35°C for 5 min. A magnification of 10× was used for microscopy analysis.

2.5. Statistical data analysis

All samples were prepared freshly on three separate occasions, with all analytical measurements conducted in triplicate. The results are given as mean values ± standard deviations. One-way

225 analysis of variance (ANOVA) was performed for statistical analysis. SAS 9.1.3 was used for all
226 analyses (SAS Institute Inc., Cary, NC, USA). Duncan's multiple comparison test was used to
227 determine significant differences between the various treatments and results with $P < 0.05$ were
228 considered significantly different.

229

230 **3.0. Results and Discussion**

231

232 *3.1. Physical and chemical properties*

233 The volume mean diameter (D [4,3]) of the milled *OaG* powder was 180 ±1.25μm. While 10% of
234 the powder particles (Dv [10]) had diameter less than 18.6 ± 0.22 μm, 90% of the powder
235 particles (Dv [90]) had diameter less than 406 ± 1.63 μm. Surface weighted mean particle
236 diameter of *OaG* powder (D[3,2]) was 37±0.76 μm. Specific surface area (SSA) of the *OaG* was
237 also 161.8±0.00 $(m^2kg^{-1})^g$. The chemical composition of *Oa*G is presented in Table 1. *Oa*G
238 contained 41.6% starch, 32.3% dietary fiber, 18.5% glucomannan, 1.52% crude fiber, 0.19%
239 glucose, 2.34% saccharose, 6.73% protein, 7.73% moisture, 2.25% ash and 0.28% fat; no
240 fructose was found in *Oa*G. The mean pH value of the *Oa*G dispersions was 6.11±0.02. The other
241 undetermined compounds in *Oa*G would possibly be other minor reducing sugars which are
242 related to the raffinose series frequently found in photosynthetic tissues (Avigad & Dey, 1997).
243 These compounds also represent low molecular weight heteropolymers associated with the
244 metabolism of mucilaginous polysaccharides, as noted in some temperate orchid species
245 (Buchala, Franz, & Meier, 1974; Franz, 1979; Meier & Reid, 1982).

246

247 The compositional profile of *Oa*G is similar to some previous results published by Farhoosh and
248 Rizai (2007), and Tekinsen and Guner (2010). Farhoosh and Rizai (2007) reported that round-
249 edged tuber gum contained 19.3% glucomannan, 6.85% starch, 13.2% moisture, 7.35% protein
250 and 2.8% ash whereas, Tekinsen and Guner (2010) reported that *Oa*G contained 43.5%
251 glucomannan, 14.7% starch, 10.7% moisture, 3.20% protein, 1.94% ash and had a pH of 5.71.
252 Lalika et al. (2013) reported that edible orchids had 5.36% protein, 2.7% crude fiber, 2.2% ash,
253 1.57% fat and 0.09 mg/100g vitamin C. Moreover, Tekinsen and Guner (2010) also reported that
254 the mean values of compositional contents of Salep gum, depending on the different species in
255 the Orchidaceae family, were 17.7-54.6% glucomannan, 5.4-38.7% starch, 0.95-2.83% ash, 9.35-
256 12.4% moisture with pH ranging from 5.61-6.20.

257 Starch serves as an energy reserve in tubers (Buckeridge, 2010). When orchid tubers are fully
258 formed, they contain maximal levels of starchy matter to supply energy to the perennial plant
259 during winter (Hossain, 2011). As indicated in Table 1, the main component of *Oa*G was starch.
260 Additionally, the high level of starch (41.6%) in the *Oa*G could be possibly due to the fact that
261 the plants were harvested in the period June-July. *Oa*G had high levels of dietary fiber (32.3%),
262 glucomannan (18.5%) and crude fiber (1.52%), which are considered to have positive health
263 benefits (Nishinari, 2000). Previous work has also shown that Turkish orchid tubers generally
264 had total dietary fiber content ranging from 11.6-40.1% (Gumus, 2009). When compared to some
265 previous results, if the tubers are harvested early at the flowering stage, the tubers contain less
266 starch and higher glucomannan, because the formation of starch in the tubers takes place mainly
267 between March to July (Buchala et al., 1974); it should be noted that there is a negative
268 correlation between starch and glucomannan contents in *Oa*G (Tekinsen and Guner 2010).

*Oa*G had relatively high protein content (6.73%), but there is a considerable variation for protein values reported previously. For example, Tekinsen and Guner (2010) reported that the protein content in *Oa*G was 3.20%. The low protein content reported in previous study may arise from using the generic nitrogen to protein conversion factor of 5.70 (Tkachuk, 1969). Additionally, *Oa*G had 7.73% moisture and 2.25% ash (Table 1). Even if depending on the period of the drying process during summer, the ash and moisture contents were similar to other previous results because all dried tubers were held until their milky appearance changed to almost a semi-transparent yellowish rough state. Sezik (1967) also reported that if the moisture content of Salep gum was lower than 10%, it could result in enhanced stability and a longer shelf life. Moreover, *Oa*G contained trace levels of fat. However, Citil and Tekinsen (2011) also reported that the mean fat content in Salep gum was 2.02%, but this could be due to boiling the tubers in milk before drying, and the presence of milk fat may possibly have increased the fat content in Salep gum.

3.2. Morphological structures of native OaG

The morphological properties of native *Oa*G milled powder particles are presented in Fig. 2. It was found that the *Oa*G particles had irregular shapes. Some fracture shapes of the *Oa*G particles were probably caused by mechanical breakdown of *Oa*G during milling. When the surface of the *Oa*G particles was focussed and magnified at the highest level, multilayered surfaces of the *Oa*G were not well observed. Moreover, the smooth surface of *Oa*G with round edges was clearly observed in Fig. 2, even if a disadvantage of SEM for the sample preparation, especially drying and metal coating, could slightly limit the visualisation to the *Oa*G particles in their original environment. However, some irregular shaped particles with partly roughened surfaces were also

observed at the highest magnification level. Rough surfaces of the particles may have been generated by partial gelatinisation of the starch granules/containing particles of *Oa*G during pre-treatment (i.e., poaching in water prior to drying) of the tubers. Similarly, lamellar surfaces of konjac glucomannan have also been reported by Cheng, Abd Karim and Seow (2011). Razavi, Nyamathulla, Karimian and Noordin (2014) also observed the morphological properties of palmate tubers of *Orchis morio var mascula*, which were reported to be irregular rod-shaped particles with roughened surfaces.

*3.3. Pasting properties of Oa*G

The pasting treatment was applied to the dispersions of *Oa*G at varying concentrations. The mean values of the initial viscosity, peak viscosity, and viscosity recorded at the end of the holding period at 95°C, on completion of cooling to 35°C, and at the end of the final holding period at 35°C are presented in Table 2. The pasting curves obtained from the dispersions of *Oa*G are also shown in Fig. 3. The pH values of all dispersions were similar (6.11±0.01) ($P > 0.05$).

As seen in Table 2 and Fig 3, the values of initial viscosity of the dispersions at varying concentrations ranged from 33.7±0.24 to 1193±92.0 mPa.s, whereas the values of peak viscosity ranged from 23.0±0.68 to 702±59.1 mPa.s ($P < 0.05$). After the cooling period at 35°C, the final viscosity values of the *Oa*G dispersions ranged from 34.3±0.45 to 1437±83.3 mPa.s, depending on the concentration of the dispersions (Table 2). At the end of the starch pasting treatment, the viscosity values of the final pastes at 35°C were higher compared to the initial viscosity values (Table 2 and Fig. 3). These higher viscosity values were due to the swelling of starch granules during pasting. It should also be noted that while fresh orchid tubers were being poached in

boiling water, partial, limited gelatinisation/pasting of starch may have taken place as part of this pre-treatment process.

Moreover, the temperatures of the peak viscosity of the dispersions ranged from 94.2±0.64°C to 97.4±0.35°C. At the lowest *Oa*G concentration (0.5%), the starch granules were more free to expand during the gelatinisation process, so that the peak viscosity was seen at a lower temperature (94.2±0.64°C) while the temperature of the peak viscosity of the dispersion at the highest concentration (2.5%) occurred at a significantly higher temperature (95.4±0.32°C) ($P < 0.05$). For the *Oa*G dispersion at 2.5%, a possible reason for lower peak temperature compared to the 1.5 and 2.0% *Oa*G samples could also be an increase in the effective concentration of dietary fiber and glucomannan which has a relatively high water holding capacity, which could limit the swelling of starch granules because of reducing amount of water available for gelatinisation. It could likely prevent both dissolution and association of primarily AM, as well as AP. These findings hypothesise that paste characteristics of *Oa*G could be affected by glucomannan in the continuous phase. Interactions between starch and glucomannan, as well as dietary fiber could also possibly have influenced pasting properties, with respect to changes in paste viscosity and gelatinisation temperature. Moreover, a delay starch gelatinization could be also due to the interaction between dietary fiber and starch, because dietary fiber with the presence of high amount of hydroxyl groups has a great water binding capacity, and can be attributed to a reduction in water availability which causes partial gelatinization of crystalline regions in the starch granules (Funami et al., 2005). This interaction could also lower viscosity and reduce pasting. Jiang and Ramsden also reported that effect of this interaction is dependent upon starch and dietary fiber concentration (1999). Nagano et al. (2008) reported that guar gum in a maize starch suspension (5%) inhibited starch components from leaching out of the granules to the

continuous phase of starch pastes during gelatinisation, to an extent dependant on the concentration of added guar gum. However, Tester and Sommerville (2003) reported that the presence of polymers and/or other compounds in the liquid phase has no effect on swelling volume until the amount of available water becomes a limiting factor. The swelling properties of the starch granules in *Oa*G were probably offset to higher temperatures as a result of the chemical composition of *Oa*G.

*3.4. Visual assessment of pasting properties of Oa*G *using light microscopy*

The effect of gelatinisation on heating of starch granules in *Oa*G was explored further using a polarised LM fitted with a heating stage. Several micrographs of the dispersions of *Oa*G prepared freshly at varying concentrations from 0.5 to 2.5% were taken at different temperatures using LM and these micrographs were recorded from the middle of the sample in each slide (Fig. 4). The arrows in the each micrograph clearly show selected starch granules/particles in the *Oa*G dispersions during pasting (Fig. 4).

As seen in the micrographs, starch granules in *Oa*G particles began to swell gradually during heating and the swelling of the starch granules greatly increased between 65 and 75ºC. Moreover, the changes in the size of *Oa*G particles increased when the temperature reached 65ºC, demonstrating that gelatinisation had already begun. However, the remaining intact granule ghosts in *Oa*G were further swollen at the end of the holding period of the 95ºC, when compared to 75ºC (Fig. 4). Other important factors affecting the gelatinisation could be the amount of available water in the dispersions, the nature of starch and the starch: glucomannan ratio in *Oa*G as they could affect the relative importance of swelling of starch granules in the discontinuous and continuous phases of pastes/gels.

In addition, lipids and proteins in plants, as well as tubers, are known to be associated with both the surface layer and the interior of starch granules. The protein and lipid in the surface layer of starch granules also prevent the swelling of starch granules, due to the fracturing of a restricting layer of lipid and protein at the surface of the starch granules (Debet & Gidley, 2007). The protein content of starch granules in the *Oa*G particles could also slightly influence the swelling property of the granules during gelatinisation.

4.0. Conclusion

*Oa*G has a unique and interesting chemical composition and is mainly composed of starch, dietary fiber and glucomannan. The pasting behaviour of *Oa*G appears to be influenced by its chemical composition, in particular the content of the high molecular weight polysaccharide, glucomannan and dietary fiber. These glucomannan and dietary fiber components, and their possible interactions with starch, in *Oa*G may have resulted in higher peak pasting temperatures and lower peak viscosity, due to limitation of the leaching of AM and AP from starch granules. These findings provide novel practical information on the role and potential usefulness of *Oa*G as an ingredient in controlling rheology and modifying texture of foodstuffs. Consequently, *Oa*G-based ingredients may be used for enhancing the functional properties of food formulations, which may provide interesting opportunities to the food industry in the development of new product structures.

5.0. Acknowledgements

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563 **Figure Legends**

564

565 **Figure 1 (a-d).** Photographs of *Orchis anatolica* harvested from the Taurus Mountains
566 All : (a-b) *Orchis anatolica*; (c) fresh round-edged tubers of *O. antolica*; dried tubers of *O.*
567 *antolica*.
568 **Figure 2.** Scanning electron micrographs of native *Oa*G granules at magnifications of
569 100 x (a), 250 x (b), 1000 x (c) and 2000 x (d).
570
571 **Figure 3.** Pasting curves for *Orchis anatolica* gum at concentrations of 0.5 % (—),1.5% (Δ), 2%
572 (o) and 2.5% (*) at pH 6.11 ±0.01. The dotted lines without symbols show temperature.
573
574 **Figure 4** The micrographs of *Oa*G at varying concentrations. All: (a) 2.0% *Oa*G; (b) 2.5% *Oa*G;
575 at different temperatures viewed under polarised light. Left-hand column at 35 ºC, middle-
576 columns from 55 to 95 ºC on heating, right-hand column at 47 ºC on cooling. Magnification 10×.
577 Scale bar 200 μm.

Table 1. Chemical composition and pH value of *Orchis anatolica* tuber gum

Composition	***O. anatolica* tuber gum** (g/100 g)	**Range***
Starch	41.6±1.52	5.40-38.7
Total dietary fibre	32.3±1.44	11.6-40.1
Glucomannan	18.5±0.33	17.7-54.6
Crude fibre	1.52±0.03	2.70
Protein	6.73±0.22	3.20-7.35
Ash	2.25±0.04	0.95-2.83
Fat	0.28±0.08	2.02
Moisture	7.73±0.51	9.35-13.2
Glucose	0.19±0.01	-
Saccharose	2.34±0.02	-
Fructose	Not founded	-
pH	6.11±0.02	5.71-6.20

Data presented are means of three replicates ± the standard deviations.
*Range values based on previous published information from Farhoosh and Rizai (2007), Gumus (2009), Tekinsen and Guner (2010) and Lalika et al. (2013).

Table 2. Viscosity (mPa.s) of *O. anatolica* tuber gum dispersions at various stages of the pasting regime

Stage of pasting	**0.5%**	**1.5%**	**2.0%**	**2.5%**
Initial viscosity (mPa.s)	33.7±0.24^{d}	306±8.91^{c}	622±40.5^{b}	1193±92.0^{a}
Peak viscosity (mPa.s)	23.0±0.68^{c}	177±97.5^{c}	481±178^{b}	702±59.1^{a}
End of holding at 95 °C (mPa.s)	16.4±0.09^{d}	93.0±4.12^{c}	217±24.5^{b}	494±47.8^{a}
End of cooling to 35 °C (mPa.s)	32.0±0.38^{d}	256±11.6^{c}	566±43.9^{b}	1130±72.9^{a}
Final paste at 35 °C (mPa.s)	34.3±0.45^{d}	328±13.5^{c}	751±58.1^{b}	1437±83.3^{a}
Temperature of peak viscosity (°C)	94.2 ±0.64^{c}	97.4±0.35^{a}	96.8±0.14^{a}	95.4±0.32^{b}

Data presented are means of three replicates ± the standard deviations.
Means (n=3) within the same column with different superscript letters differ significantly ($P < 0.05$).

Figure 1 (a-d).

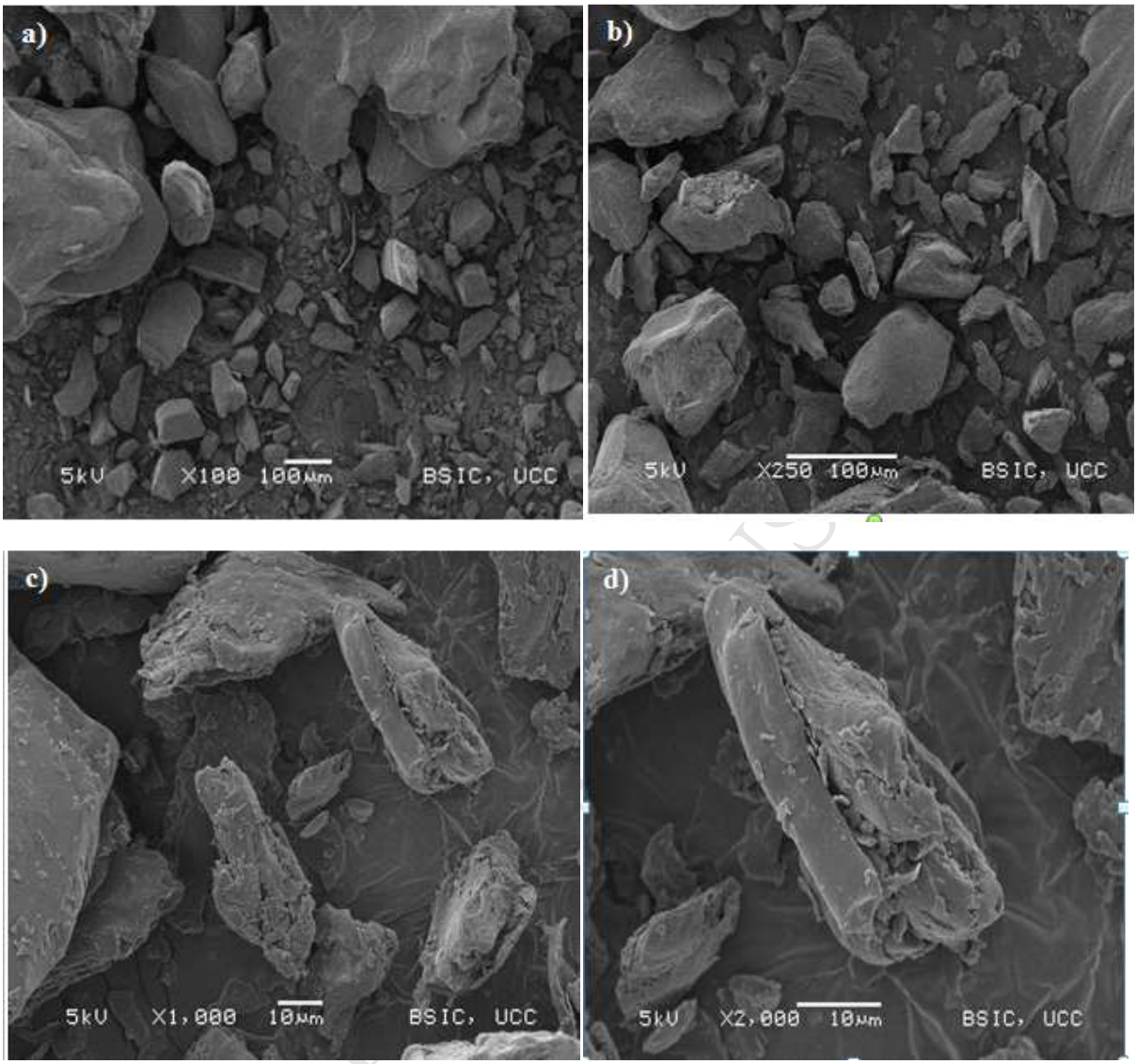
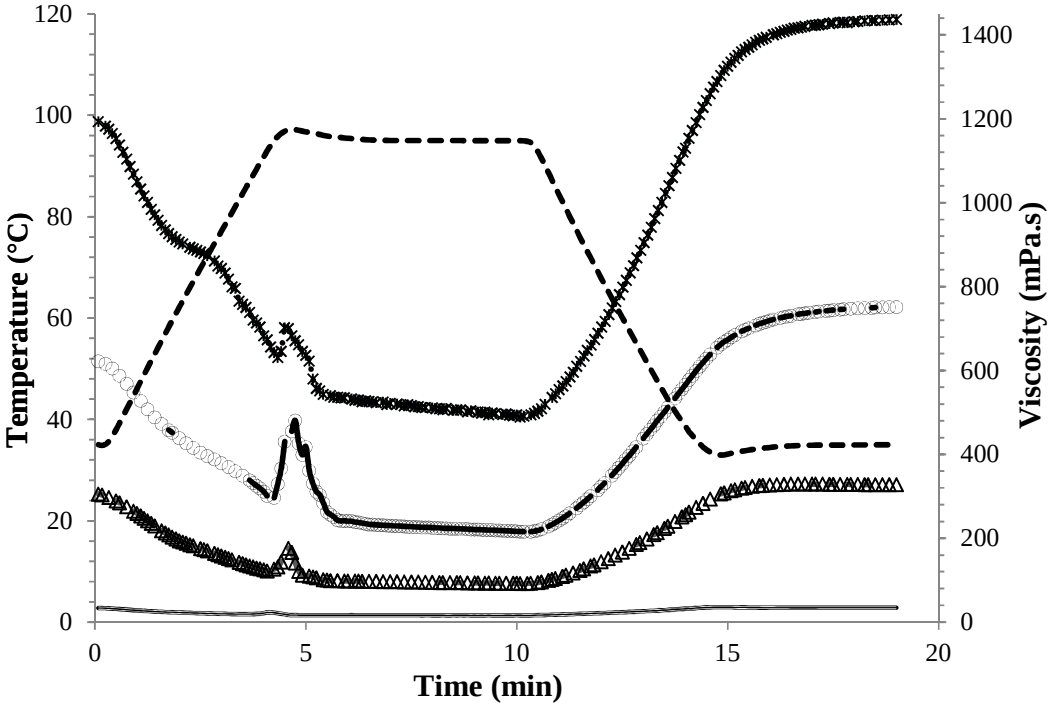


Figure 2.

28



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30 **Figure 3.**

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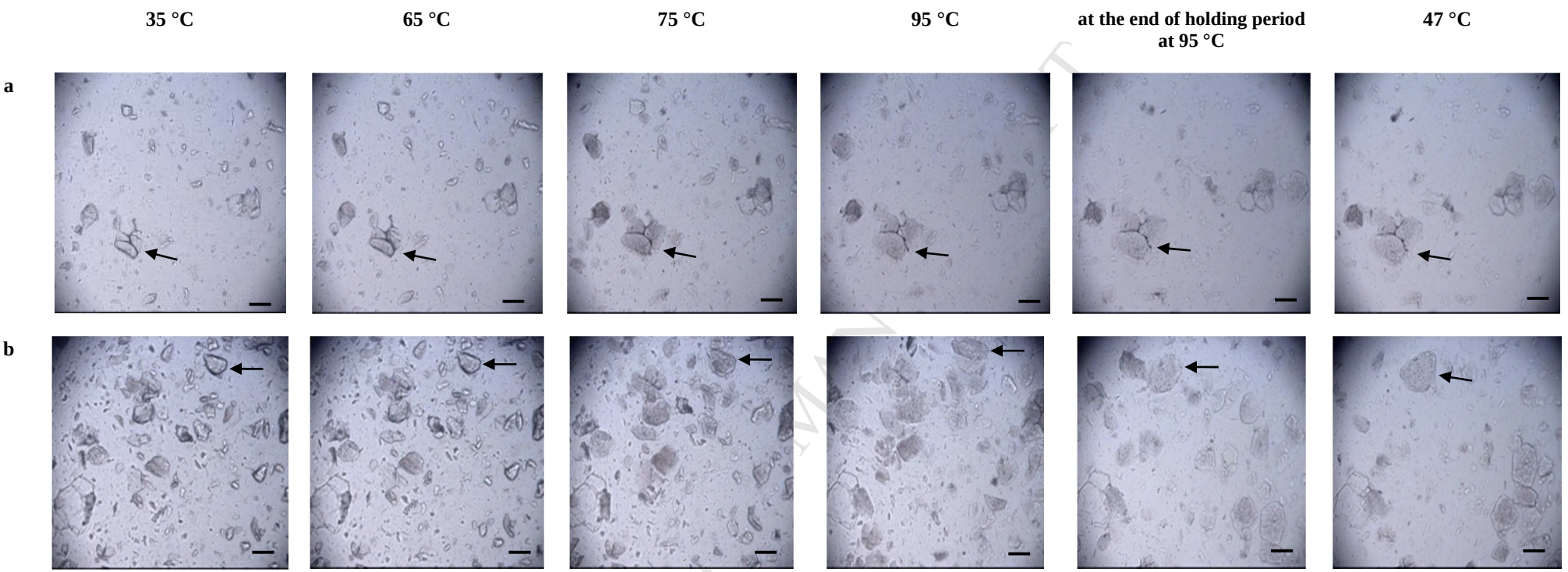

35

36 **Figure 4.**

37

Research Highlights

- Milled *Oa*G had irregular shaped particles with round edges
- The main components of *Oa*G were starch, dietary fiber and glucomannan
- The starch pasting behaviour of *Oa*G was influenced by the glucomannan and dietary fiber components
- OaG is a potent viscosity modifier and thickener for food applications